

## IIT JAM - BIOLOGICAL SCIENCE

### SAMPLE THEORY

- TRANSLATION
- INITIATION OF POLYPEPTIDES
- INITIATION IN PROKARYOTES
- INITIATION IN EUKARYOTES
- ELONGATION OF POLYPEPTIDE
- GENE REGULATION
- LIPIDS ATTACHMENT
- PROTEIN DEGRADATION

# VPM CLASSES

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## Synthesis of proteins

### TRANSLATION

- The translation of protein synthesis involves translation of the language of nucleic acid into language of proteins. This is much more complex than transcription and involves initiation, elongation and termination of polypeptide chain.
- Initiation and elongation of polypeptide is the formation of aminoacyl transfer RNA's.

### FORMATION OF AMINOACYL t - RNA's

- a) The reaction is brought about by the binding of an amino acid with ATP. This step is mediated by an enzyme called as **aminoacyl-tRNA synthetase**.
- This reaction between amino acid and adenosine triphosphate is mediated by specific enzyme a complex is formed.
- b) **TRANSFER OF AMINO ACID TO t RNA**
  - The aminoacyl AMP enzyme complex reacts with a particular t-RNA and transfer the amino acid to the t-RNA.
  - This reaction is accompanied by the liberation of AMP and pyrophosphate. It is said that a particular amino acid will require particular enzyme and particular species of t RNA.

### INITIATION OF POLYPEPTIDES

- The initiation of polypeptide chain is always brought about by the amino acid methionine which is regularly coded by **AUG** but rarely also by **GUA** as **initiation codon**.
- In E.coli there are two t-RNA for methionine **t-RNA<sup>met</sup> [non formylated]** and **t RNA<sup>met</sup> [formylated]**.
- In prokaryotes formulation of initiating amino acid is methionine and is the essential requirement so that t-RNA<sup>met</sup> is meant for depositing methionine as a first amino acid.

- The presence of t- RNA<sup>met</sup> is only at intercalary position. In eukaryotes formulation of methionine does not occurs due to absence of t RNA<sup>met</sup> in plants and also due to the absence of enzyme **transformylase** in animals.
- In case of **prokaryotes** ribosomes enter the m- RNA at AUG codon or at nearly **Shine Dalgarno** site while in case of eukaryotes ribosomes enter at the capped 5' end of m -RNA and then advances to AUG codon by linear scanning.
- Small ribosomal subunit (30s) can engage the m- RNA before binding of initiator met- t RNA<sup>met</sup> while in case of eukaryotes small ribosomal subunit 40s binds stably to m- RNA only after initiation met -RNA has bound.

## INITIATION IN PROKARYOTES

- The initiation codon always function in combination with a Shine **Dalgarno** sequence. This has homology with the 3'end of 16s RNA found in 30s subunit of ribosomes.
  - Shine Dalgarno region is a part of ribosomal binding site [ RBS ] which is itself a part of a longer region called **translation initiation region** or **TIR**.
  - The TIR helps in binding with ribosomes and also accepts the formylated methionyl t RNA at the initiating codon.
- a) FORMATION OF FORMYL METHIONYL t RNA<sub>f</sub><sup>Met</sup> [f-Met - t RNA<sub>f</sub><sup>Met</sup>]**
- The binding of methionine with t RNA<sub>f</sub><sup>Met</sup> takes place in the manner outlined earlier for other amino acids.
  - The met - t RNA<sup>Met</sup> undergoes the process of formylation in the presence of enzyme **transformylase** in the presence of formate source as **formyl tetrahydrofolic acid**.
- b) GENERATION OF 30s SUBUNIT AND BINDING OF IF - 3**
- The ribosomes are released out from m RNA in the form of 70 - s ribosomes particles. There is need to dissociate these units as the 2 subunits ( 30S, 50S ) with the help of protein called as **initiation factor 3 (IF - 3)**.
  - IF - 3 then binds to the 30s subunit so as to stabilize it.

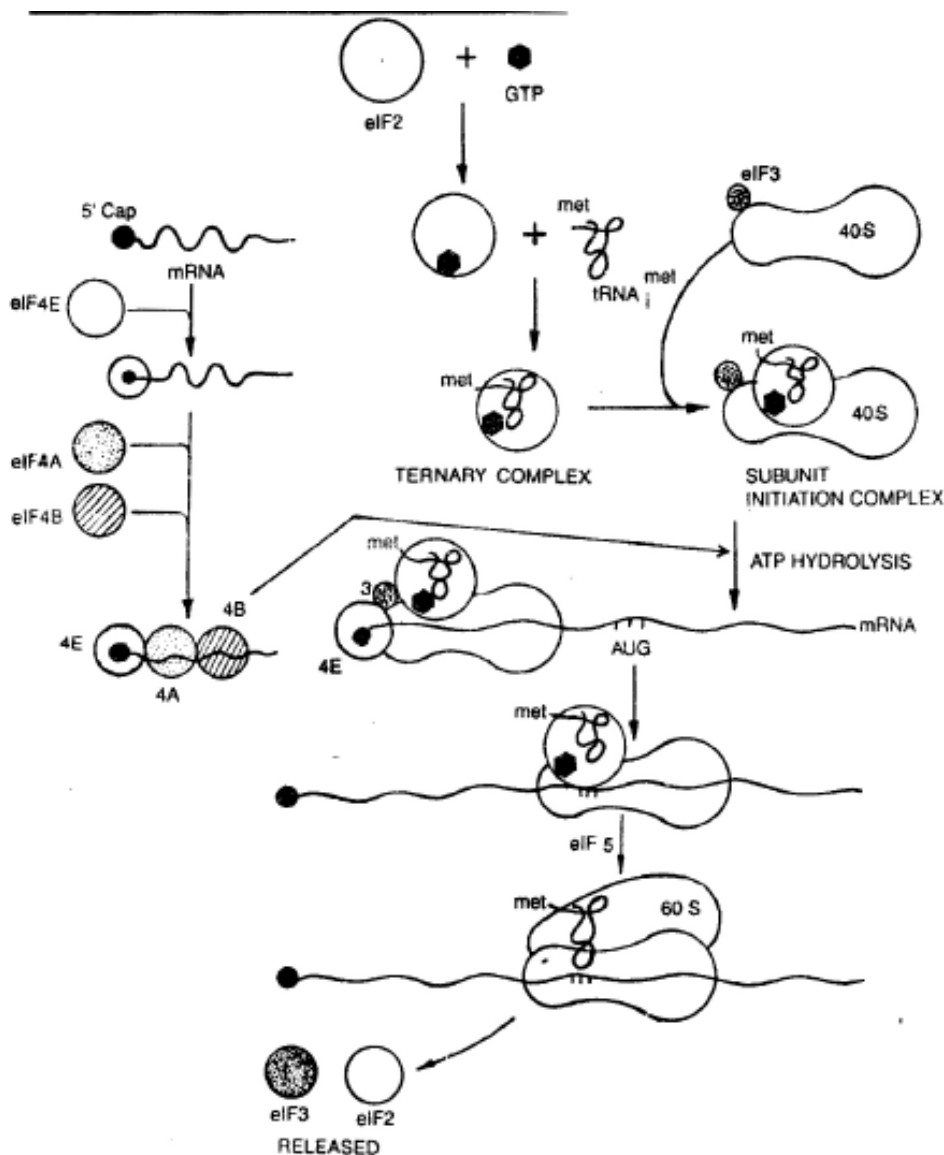
## FORMATION OF 70 - S INITIATION COMPLEX

- (i) 30S and 50S ribosomal subunit.
  - (ii) The initiator t - RNA.
  - (iii) The TIR of the m- RNA .
  - (iv) The 3 initiator factor [ IF 1, IF -2 IF - 3] and
  - (v) A GTP molecule
- The 30 -S subunit of ribosomes is completed with either one of the initiation factor and then binds and randomly order the RNA and f- met - RNA<sup>Met</sup>.
  - This leads to the formation of a preternary complex which undergoes the process of rearrangement to form a bonafide 30-S complex.
  - The 30S and 50S associate is rendered irreversible by the initial injection of IF 1 and IF 3 and subsequent release of IF 2 after hydrolysis of a GTP molecule.
  - After association of 30S and 50S the f met t RNA<sup>met</sup> get's associated on to the P site of ribosomal. It is so that it can form a bond with incoming amino acyl t RNA encoded by the second m - RNA codon on the ribosomal A site.

## INITIATION IN EUKARYOTES

- It is similar to that of elongation in prokaryotes excepts the following differences:
- i) In eukaryotes the initiating factors are more and at least 10 of them is discovered in RBC.
    - They are named by putting suffix “ e ” to their **eukaryotic origin** these can be named as follows i.e. eIF1, eIF-2, eIF-3, eIF-4A, eIF4B, eIF4C, eIF4D, eIFDF, eIF 5, and eIF 6.
    - The eIF1, eIF-2, eIF-3, eIF-4A, eIF, 4F contains multiple polypeptide chains but other are single polypeptide. eIF2 and eIF3 are analogous to IF2 and IF3 of prokaryotes.
  - ii) In eukaryotes formylation of methionine does not take place.
  - iii) In eukaryotes smaller subunit (40 -s) associate with initiator t-RNA known as t RNA<sup>met<sub>f</sub></sup> without the help of mRNA, while in prokaryotes generally the 30 -s m RNA complex is first formed which then associate with f - met -t RNA<sup>met<sub>f</sub></sup>.
    - Following steps are involved in the initiation of eukaryotic polypeptide synthesis.
    - GTP binds to eIF-2 which increase it's affinity for Met -t RNA<sub>i</sub><sup>Met</sup>.

- The Met - t RNA<sub>i</sub><sup>Met</sup> associate with eIF -2 - GTP complex forming a ternary complex Met - t - RNA<sub>i</sub><sup>Met</sup> - eIF2- GTP.
- The ternary complex associates with 40-s subunit to form 43 s initiation complex.
- The eIF - 2 also consist of three subunits i.e. of (35,000)  $\beta$  (38,000),  $\gamma$  (55,000).
- eIF2  $\alpha$  binds to GTP, eIF2  $\gamma$  binds Met - t - RNA and eIF2  $\beta$  may be a recycling factor.
- The m RNA with it's 5' end binds to the 43 - S initiation complex.
- The reaction depends on eIF - 3 and the binding of m RNA is assisted by eIF 4F, eIF 4A and eIF 4B and the high energy bond of ATP.
- After it's association at 5' end the initiation complex moves towards 3' end in search of initiation codon AUG and also associate with 60 - S subunit.
- This association requires eIF - 5 because it helps in releasing eIF - 2 and eIF - 3.
- eIF 2 releases as a binary complex eIF 2 - GDP.
- The 40-S - 60-S joining reaction really depends on eIF 4C and the GTP of the initiation complex is hydrolyzed when 60 -S subunit joins.



**Fig: 1** Translation initiation in eukaryotes. For simplicity, only some of the initiation factors are depicted.

### ELONGATION OF POLYPEPTIDE

- After the formation of 70 - S - m RNA - f met -t RNAs met complex elongation of polypeptide chain is brought about by the regular addition of amino acids.
- The following 3 steps are needed in the elongation process.

## BINDING OF AA - t RNA AT SITE 'A' OF RIBOSOMES

- Each ribosome has two cavities in which t-RNA can be inserted. These are 'P' and 'A' site. It was however not known that whether the f-Met - t RNA met comes on P or A but it has to be present on 'P' site so that the 'A' site must be made available to the incoming amino acyl t RNA.
- Another site called as 'R' site located on smaller subunit of ribosomes has also been proposed. The presence of R is necessary because it plays an important role in improving the accuracy of translation.
- It is postulated that amino acyl t RNA first binds with R site with codon anticodon pairing and then it flips to A site using the energy of GTP.
- After formation of 70-S initiation complex the first amino acyl t RNA enter A sites. Factor responsible for this entry includes the elongation factor EF - Tu (u means unstable on heating) and EF - Ts (stable when heated).
- The proper placing of AA - t RNA takes place in presence of EF Tu factor which takes it to 'A' site.
- The elongation factor EF - Tu first combines with GTP and changes to an active binary complex this binds to AA -T - RNA form a ternary complex.

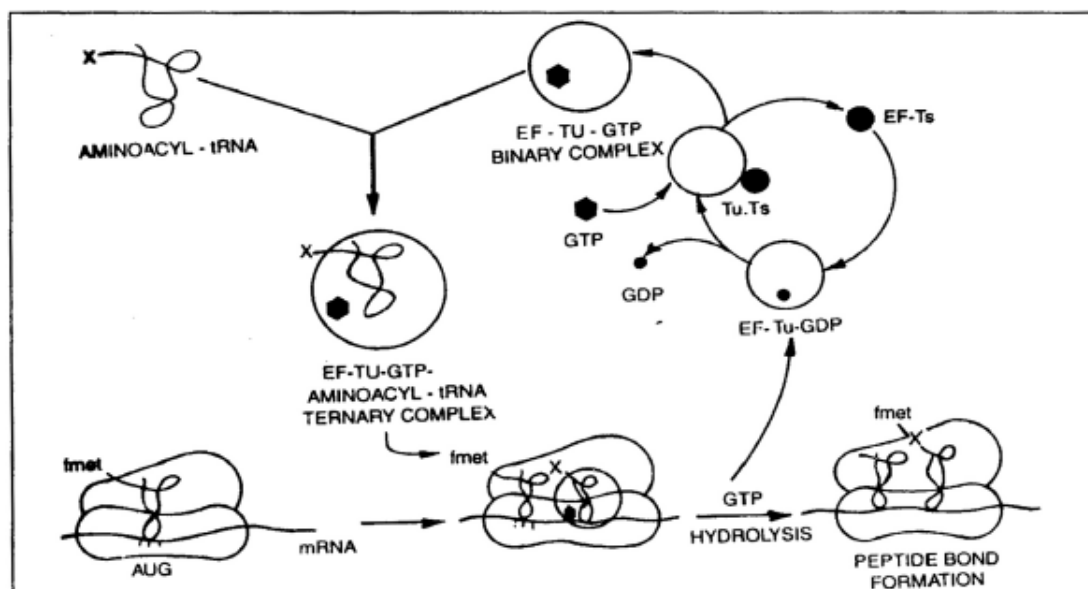


Fig :2 Addition of aminoacyl- tRNA at A site of ribosome mediated by EF-Tu (in bacteria).

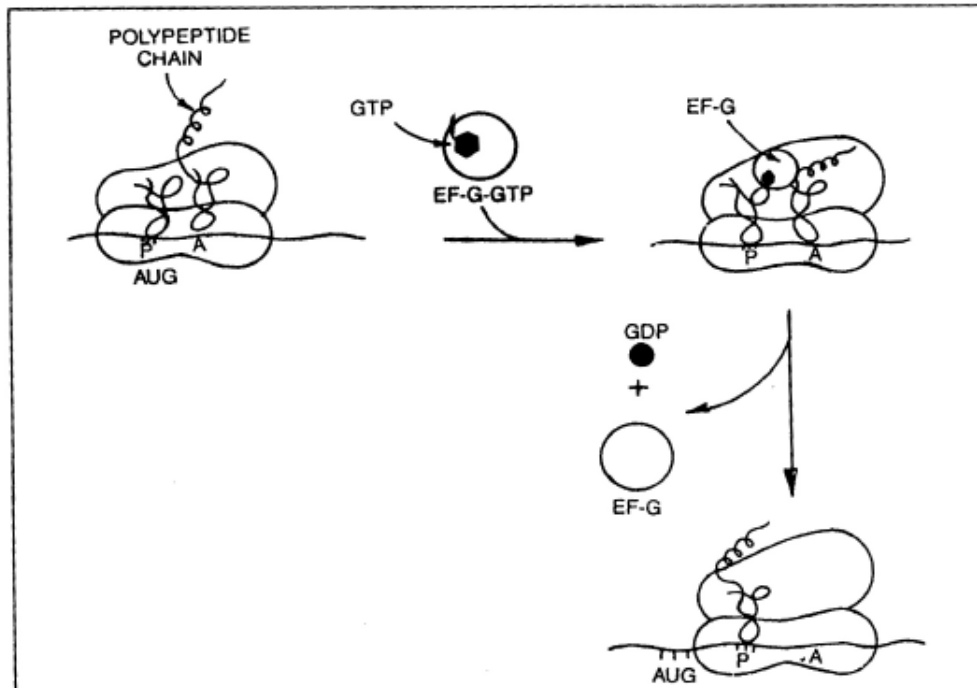
## FORMATION OF PEPTIDE BOND

- This is catalytic reaction during which a peptide bond is formed between the free carboxyl group of the peptidyl t-RNA at the 'P' site and the free amino group present with amino acyl t RNA at the 'A' site.
- The enzyme involved in a reaction is **peptidyl transferase** and is an integral part of 50 - S ribosomal unit.
- After formation of peptide bond the t RNA at 'P' site is deacylated and the t RNA at 'A' now carries the polypeptide.

## TRANSLATION OF PEPTIDE t- RNA FROM 'A' TO 'P' SITE

- The peptidyl t RNA present at A site is now translocated at 'P' site
- For the translocation two models are available:
  - i) According to two site (A,P) model deacylated t RNA is liberated from 'P' site and with the help of one GTP molecule and elongation factor EF - G. Thus according to this model t RNA is entirely in the A site or entirely in the P site.
  - ii) Acc. to newer three sites (A,P and E) model initially the amino acyl end of t RNA bound to A site moves to P site on 50 - S subunit at the time of peptide transfer but only later during translocation the anticodon end of this t RNA moves from A to P site on 30-S subunit.
- The movement of the deacylated t RNA from P to E site also occurs in two steps:
  - i) The aminoacyl end moves to E site from P during peptide bond formation.
  - ii) The anticodon end leaves the P site only during translocation.
- The requirement of EF-G and GTP for translocation was reduced by the use of **antibiotic puromycin** which closely resemble AA tRNA in structure and can therefore occupy 'A' site and form peptidyl puromycin.
- This puromycin reaction depends on EF-G and GTP presence. In eukaryotes the elongation factor needed for translocation is called as **eEF - 2**.
- The formation of one peptide bond requires the presence of one ATP molecule and 2 GTP molecule.
- However in 1993 the evidences are made available where there is a prominent requirement of 3 or even more GTP molecules.





**Fig :3** Translocation mediated by EF-G, P and A denote the P and A sites of ribosome.

### TERMINATION OF POLYPEPTIDES

- Termination of polypeptide chain is brought about by the presence of any one of the three termination codons mainly **UAA**, **UAG** and **UGA**.
- These termination codons are recognized by the two release factor i.e. RF<sub>1</sub> and RF<sub>2</sub>. Of these two RF<sub>1</sub> recognizes UAA and UAG and RF<sub>2</sub> recognizes **UGA**.
- They help the ribosomes to recognize their triplet. These release factors get bound to the A site. Other release factor RF-3 seems to stimulate the action of RF1 and RF2.
- For release reaction, the polypeptide tRNA must be present on 'P' site and the release factor helps in splitting of the carboxyl group between the polypeptide and the last tRNA carrying this chain.

### GENE REGULATION

- Regulatory proteins bind to specific DNA sequences.

- Their affinity for these target sequences is roughly  $10^4$  to  $10^6$  times higher than for any other sequences.
  - To interact with bases in major groove of DNA, a protein requires a smaller structure that can protrude from protein surface.
  - Such DNA binding sites are inverted repeats of short DNA sequence.
- 1) **Helix turn helix**
    - The helix-turn-helix motif comprises about 20 amino acids in two short  $\alpha$ -helical segments, each seven to nine amino acid residues long, separated by a  $\beta$  turn.
    - One of the two  $\alpha$ -helical segments is called the **recognition helix**, because it interacts with the DNA in a sequence-specific way.
  - 2) **Zinc finger**
    - In a zinc finger, about 30 amino acid residues form an elongated loop held together at the base by a single  $Zn^{2+}$  ion, which is coordinated to four of the residues (four Cys, or two Cys and two His).
    - Several hydrophobic side chains in the core of the structure also lend stability.
  - 3) **Homeodomain**
    - This domain of 60 amino acids-called the **homeodomain**, because it was discovered in homeotic genes (genes that regulate the development of body patterns).
    - It is highly conserved and has now been identified in proteins from a wide variety of organisms, including humans.
    - The DNA sequence that encodes this domain is known as the **homeobox**.
  - 4) **Leucine Zipper**
    - This motif is an amphipathic  $\alpha$  helix with a series of hydrophobic amino acid residues concentrated on one side.
    - A striking feature of these  $\alpha$  helices is the occurrence of Leu residues at every seventh position, forming a straight line along the hydrophobic surface.
  - 5) **Basic Helix-Loop-Helix**
    - Occurs in some eukaryotic regulatory proteins implicated in the control of gene expression during the development of multicellular organisms.

- This region can form two short amphipathic  $\alpha$  helices linked by a loop of variable length, the helix-loop-helix.

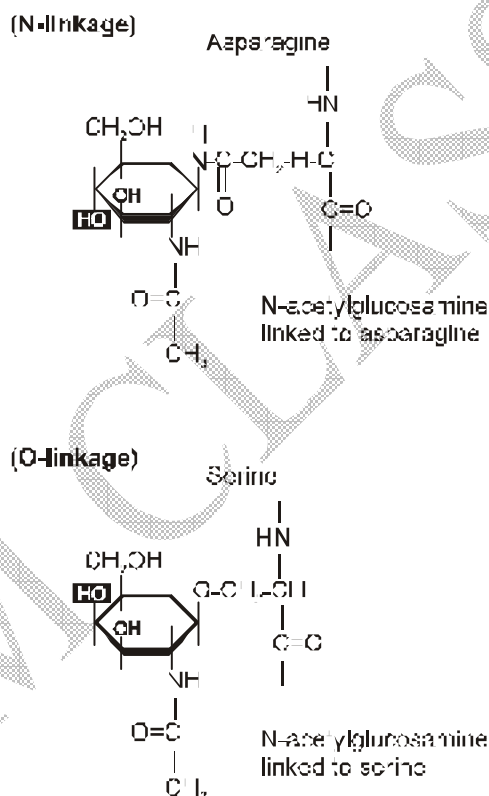
## 6) Protein Folding

- To be useful, polypeptides must fold into distinct three-dimensional conformations.
- Many proteins undergo further modifications, including cleavage and the covalent attachment of carbohydrates and within the cell. Proteins that facilitate the folding of other proteins are called molecular **chaperones**. The term "chaperone" was first used by **Ron Laskey**.
- A protein (nucleoplasmin) that is required for the assembly of nucleosomes from histones and DNA.
- The binding of chaperons stabilizes these unfolded polypeptides, thereby preventing incorrect folding or aggregation and allowing the polypeptide chain to fold into its correct conformation.

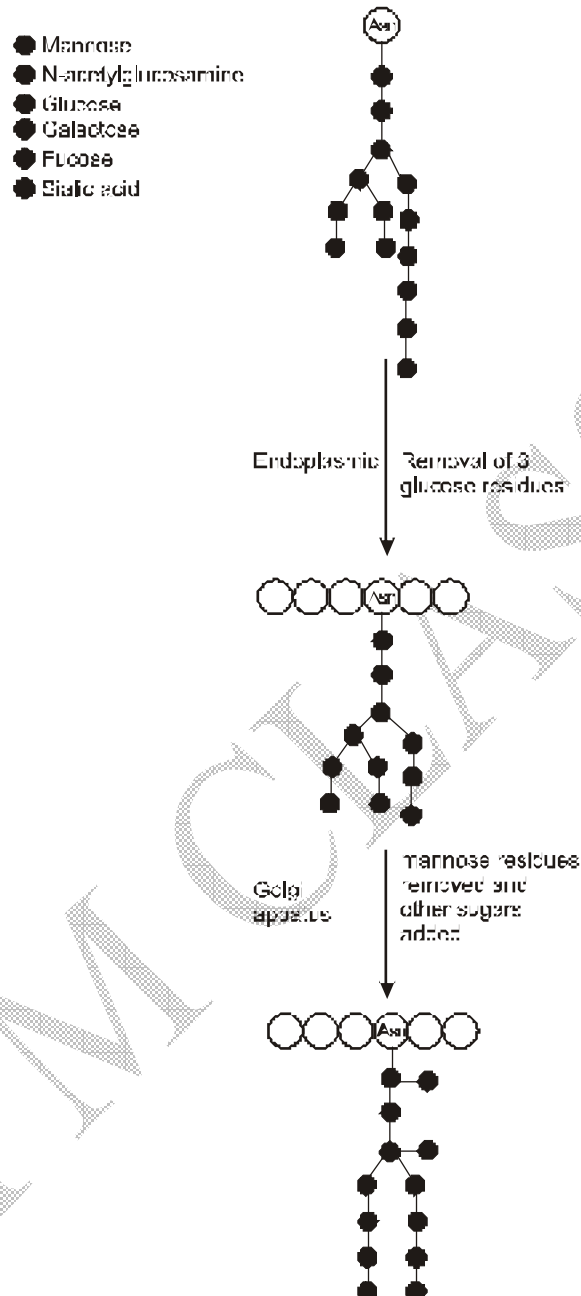
## 7) Glycosylation

- Many proteins, particularly in eukaryotic cells, are modified by the addition of carbohydrates, a process called **glycosylation**.
- The proteins to which carbohydrate chains have been added (called **glycoproteins**) are usually secreted or localized to the cell surface.
- Many nuclear and cytosolic proteins are also glycosylated. The carbohydrate moieties of glycoproteins play important roles in protein folding in the endoplasmic reticulum, in the targeting of proteins for delivery to the appropriate intracellular compartments, and as recognition sites in cell-cell interactions.
- Glycoproteins are classified as either **N-linked** or **O-linked**, depending on the site of attachment of the carbohydrate side chain.
- In N-linked glycoproteins, the carbohydrate is attached to the nitrogen atom in the side chain of asparagine.
- In O-linked glycoproteins, the oxygen atom in the side chain of **serine** or **threonine** is the site of carbohydrate attachment.

- The sugars directly attached to these positions are usually either N-acetyl-glucosamine or N-acetylgalactosamine, respectively.
- The oligosaccharide is assembled within the endoplasmic reticulum on a lipid carrier (**dolichol phosphate**).
- It is then transferred as an intact unit to an acceptor **asparagine (Asn)** residue within the sequence **Asn-X-Ser or Asn-X-Thr** (where X is any amino acid other than proline).
- The oligosaccharide is then further modified in the Golgi apparatus, to which glycoproteins are transferred from the endoplasmic reticulum.

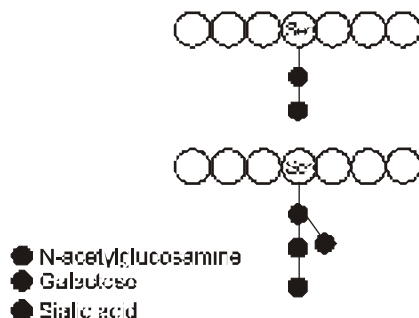


**Figure :4 Linkage of carbohydrate side chains to glycoproteins** The carbohydrate chains of N-linked glycoproteins are attached to either serine (shown) or threonine. The sugars joined to the amino acids are usually either N-acetylglucosamine (N-linked) or N-acetylgalactosamine (O-linked).



**Figure: 5 Processing of N-linked oligosaccharides** Various oligosaccharides form further modifications of the common 14-sugar unit initially added in the endoplasmic reticulum. Three glucose residues are removed in the endoplasmic reticulum. The

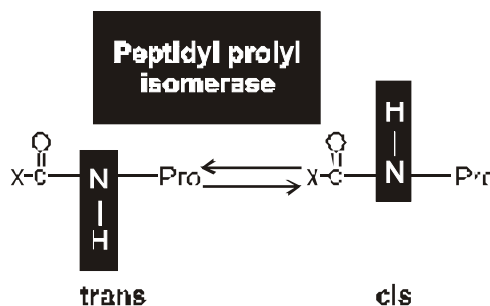
glycoprotein is then transferred to the Golgi apparatus in which mannose residues are removed and other sugars are added. The structure shown is a representative example.



**Figure 6 Examples of O-linked oligosaccharides** O-linked oligosaccharides usually consist of only a few carbohydrate residues, which are added one sugar at a time.

## 9) Protein Disulphide Isomerase

- Protein disulfide isomerase (PDI), which as discovered by **Christian Anfinsen** in 1963, catalyzes disulfides.
- For proteins that contain multiple cysteine residue, PDI plays an important role by importing rapid exchanges between paired disulfides.
- Disulfide bonds are generally restricted to secreted proteins and some membrane proteins because the cytosol contains reducing agents that maintain cysteine residues in their reduced (-SH) form.
- In eukaryotic cells, disulfide bonds form in the endoplasmic reticulum in which an oxidizing environment is maintained.
- PDI is a **critical chaperone** and catalyst of protein folding in the endoplasmic reticulum and is one of the most abundant proteins in that organelle.
- **Proline** is an unusual amino acid in that the equilibrium between other amino acids the cis and trans conformations of peptide bonds that precede proline residues.
- Peptide bonds between other amino acids are almost always in the trans form.
- Catalyzed by the enzyme **peptidyl prolyl isomerase**. This enzyme is widely distributed in both prokaryotic and eukaryotic cells and plays an important role in the folding.

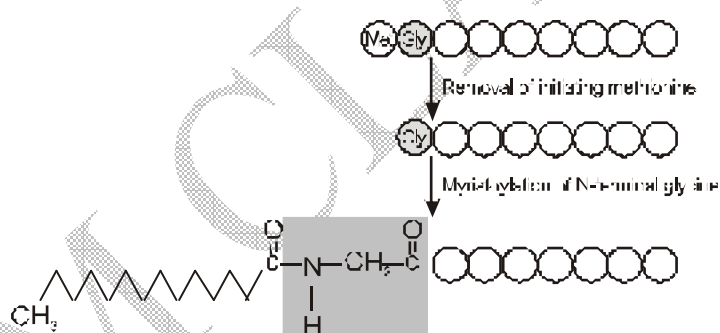


**Figure :7 The action of peptidyl prolyl isomerase** Peptidyl prolyl isomerase catalyze the isomerization of peptide bonds that involve proline between the cis and trans conformations.

### LIPID ATTACHMENT

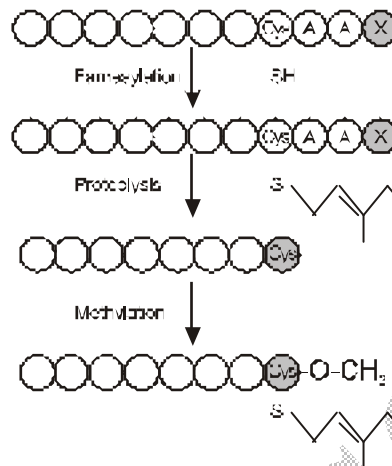
- Some proteins in eukaryotic cells are modified by the attachment of lipids to the polypeptide chain.
- Such modifications frequently target and anchor these proteins to the plasma membrane, with which the hydrophobic lipid is able to interact.
- Three general types of lipid additions-**N-myristoylation, prenylation, and palmitoylation**-are common.
- A fourth type of modification, in the addition of **glycolipids**, plays an important role in enclosing some cell surface proteins to the extracellular face of the plasma membrane.
- In **N-myristoylation**, myristic acid is attached to an N-terminal glycine residue.
- The glycine is usually the second amino acid incorporated into the polypeptide chain.
- Proteins that are modified by N-myristoylation are associated with the inner face of the plasma.
- Lipids can also be attached to the side chains of cytosine, serine, and threonine residues.
- **Prenylation** in which specific types of lipids (**prenyl groups**) are attached to the sulfur atoms in the side chains of cysteine residues located near the C terminus of the polypeptide chain.
- Plasma membrane associated proteins involved in the control of cell growth and differentiation are modified in this way, including the Ras oncogene proteins.

- **Farnesylation** is a relatively rare modification of cellular proteins.
- Inhibitors of the key enzyme (**farnesyl transferase**) might prove useful as drugs for the treatment of cancers that involve Ras proteins.
- In the third type of modification, **palmitoylation**, palmitic acid (a 16-carbon fatty acid) is added to sulfur atoms of the side chains of internal cysteine residues.
- Palmitoylation plays an important role in the association of some proteins with the cytosolic face of the plasma membrane.
- Lipids linked to oligosaccharides (**glycolipids**) are added to the C-terminal carboxyl groups of some proteins.
- They serve as anchor that attach the proteins to the external face of the plasma membrane.
- Glycolipids attached to these proteins contain phosphatidylinositol, they are usually called **glycosylphosphatidylinositol**, or **GPI anchors**.
- The GPI anchors are synthesized and added to proteins as a preassembled unit within the endoplasmic reticulum.

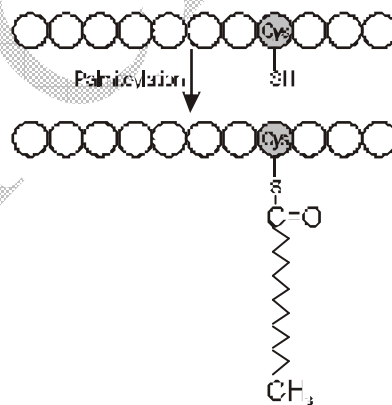


**Figure:8 Addition of a fatty acid by –myristoylation**  
The initiating methionine is removed, leaving glycine at the N terminus of the polypeptide chain. Myristic acid (a 14-carbon fatty acid) is then added.





**Figure: 9 Prenylation of a C-terminal cysteine residue** The type of prenylation shown affects Ras proteins and proteins of the nuclear envelope (nuclear lamins). These proteins terminate with a cysteine residue (Cys) followed by two aliphatic amino acids (A) and any other amino acid (X) at the C terminus. The first step in their modification is addition of the 15-carbon farnesyl group to the side chain of cysteine (farnesylation). This step is followed by proteolytic removal of the three C-terminal amino acids and methylation of the cysteine, which is now at the C terminus.



**Figure :10 Palmitoylation** palmitate (a 16-carbon fatty acid) is added to the side chain of an internal cysteine residue.

## Protein Degradation

- Many rapidly degraded proteins function as regulatory molecules, such as transcription factors.
- Other proteins are rapidly degraded in response to specific signals, providing another mechanism for the regulation of intracellular enzyme activity.

### Ubiquitin-Proteasome Pathway

- The major pathway of selective protein degradation in eukaryotic cells uses ubiquitin as a marker that targets cytosolic and nuclear proteins for rapid proteolysis.
- Ubiquitin is a 76-amino-acid polypeptide that is highly conserved in all eukaryotes (yeasts, animals, and plants).
- Proteins are marked for degradation by the attachment of ubiquitin to the amino group of the side chain of a lysine residue.
- Polyubiquitinated proteins are recognized and degraded by a large, multisubunit protease complex, called the **proteasome**.
- Ubiquitin is released in the process, so it can be reused in another cycle.
- Both the attachment of ubiquitin and the degradation of marked proteins require energy in the form of ATP.
- Proteins that control gene expression and cell proliferation, are targets for regulated ubiquitination and proteolysis.

### Inhibitors and Modifiers of Protein synthesis

- Many inhibitors of protein synthesis are known.
- Many antibacterial agents (**antibiotics**) have been isolated from fungi. They have been widely used both clinically and as reagents for unraveling the details of protein synthesis and RNA and DNA synthesis.
- **Puromycin** is chemically similar to the aminoacyl part of a charged tRNA molecule.
- It competes effectively with charged tRNA molecules for the A site.

- Puromycin has an  $\alpha$ -amino group that can form a peptide bond with the carboxyl group of a growing peptide chain in a reaction catalyzed by peptidyl transferase.
- When this bond forms, the polypeptide is cleaved from the tRNA in the P site and the puromycin leaves the A site.
- **Streptomycin** interferes with the binding of tRNA<sup>fMet</sup> to the P site and thereby inhibits the initiation of synthesis of a polypeptide chain.
- Streptomycin alters codon-anticodon recognition -that is, it induces misreading of the code.

### Antibiotic inhibitors of protein synthesis in prokaryotes

Antibiotic	Action
Streptomycin	Binds to the S12 protein of the 30S ribosomal subunit and thereby inhibits binding of tRNA <sup>fMet</sup> to the P site. Also causes misreading in a system that is in the act of synthesis.
Neomycin, kanamycin	Same as streptomycin
Chloramphenicol (also called chloromycetin)	Inhibits peptidyl transferase of 70S ribosome
Tetracycline	Inhibits binding of charged tRNA to 30S particle
Erythromycin	Binds to free 50S particle and prevents formation of the 70S ribosome. Has no effect on an active 70S ribosome.
Puromycin	Causes premature chain termination by acting as an analogue of charged tRNA
Fusidic acid	Binds to EF-G. ATP is still hydrolyzed and translocation occurs. However, EF-G and GDP are not released from ribosome, so the ribosome cannot bind another aminoacyl-tRNA
Kasugamycin	Inhibits binding of tRNA <sup>fMet</sup>
Lincomycin	Inhibits peptidyl transferase complex
Kirromycin	Binds to EF-Tu; stimulates formation of (EF-Tu)-GTP

and binding of ternary complex to ribosome; and inhibits release of EF-Tu

Thiostrepton

Prevents translocation by inhibits EF-G

### Inhibitors of Protein Synthesis in Eukaryotes

- The nontoxic antibiotics that have no effect on eukaryotes are nontoxic to them either because they fail to penetrate the eukaryotic cell membrane or because they do not bind to eukaryotic ribosomes.
- Some antibiotics are active against both bacterial and mammalian cells.
- **Chloramphenicol (also called chloromycetin)**, which inhibits peptidyl transferase in both bacterial and mitochondrial ribosomes, though normal cytoplasmic ribosomes are unaffected. many drugs that act either mainly or significantly on eukaryotes.
- Some of these are toxins of bacterial origin while others are synthetic.
- Toxin produced by **Corynebacterium diphtheriae**.
- This toxin is an enzyme that causes covalent modification of the eukaryotic elongation factor need for translocation and thereby inhibits that step.
- **Cycloheximide** is a chemical inhibitor of the peptidyl bond.
- Substances like cycloheximide are commonly used in cancer chemotherapy.

### Inhibitors of protein synthesis in eukaryotes

Inhibitor	Action
Abrin, ricin Diphtheria toxin	Inhibits binding of aminoacyl tRNA Enzymatic catalysis of a reaction between NAD <sup>+</sup> and eEF2 to yield an inactive factor. Inhibits translocation.
Chloramphenicol (also called chloromycetin)	Inhibits peptidyl transferase of mitochondrial ribosomes. Inactive against cytoplasmic ribosomes
Puromycin	Causes premature chain termination by acting as an

	anabgue of charged tRNA
Fusidic acid	Inhibits translocation by altering an elongation factor
Cycloheximide (also called actidione)	Inhibits peptidyl transferase
Pactamycin	Inhibits positioning of tRNA <sup>Met</sup> on the 40S ribosome
Show domycin	Inhibits formation of the eF2-tRNA <sup>Met</sup> - GTP complex
Sparsomycin	Inhibits translocation

### EXAMPLE QUESTIONS

- Enhancer element are segments of DNA that
  - Are required for the transcription of certain genes
  - Activate transcription of a genes irrespective of their relative position in the DNA chain
  - Have no polarity (i.e., they can be inverted)
  - Are nonspecific for tissue or species
- (A)** Enhancers stimulate transcription of DNA sequences irrespective of its position.
- Cycloheximide inhibit protein synthesis in?
  - Prokaryotes
  - Eukaryotes
  - Both
  - Don't inhibit protein synthesis
- (B)** Cycloheximide inhibit protein synthesis in eukaryotes.