Indira Gandhi National
Open University
School of Sciences

BBCCT - 103

CELL BIOLOGY

Block

3

PROTEIN TRANSPORT

BLOCK 3 : PROTEIN TRANSPORT

In BBCCT-101 course (Molecules of life), you have studied about the protein (Polymer of amino acids) is one of the major biomolecule essential for cell structure and function. It serves as enzymes, hormones and receptor in biological process. Have you ever thought how do cells transport proteins within their compartment (subcellular organelles) and surface (plasma membrane) when they are synthesized in the cell?

Therefore, Block 3 is devoted exclusively to the protein transport.

There are three units (8-10) in this block on Protein Trafficking.

Unit 8 Protein Trafficking I discusses the protein transport to the ER lumen and across the ER membrane and role of Endoplasmic reticulum in the protein trafficking. This unit explains you about signal hypothesis and protein transport pathway.

Unit 9 Proteins Trafficking II explains the role of Golgi Body in the protein transport. It describes how secretory proteins are transported from the ER to the Golgi body or their final destination. The vesicular pathway and proteins modification are also discussed.

Unit 10 Protein Trafficking III deals with the protein transport to the subcellular organelles: nucleus, mitochondria, chloroplast and peroxisomes.

Each unit covers essential components of the trafficking machinery and the accompanying modifications as the proteins moves from one compartment to the next.

Expected Learning Outcomes

After studying this block, you should be able to:

- define the role of ER and Golgi complex in protein transport;
- explain the signal hypothesis and components of protein translocation pathways;
- describe the steps of co-and post-translational protein translocation into the ER lumen and across the ER membrane;
- highlight the importance of co-translational proteins modification, protein folding and quality control in the ER;
- illustrate the proteins sorting and secretory pathway;
- explain the vesicular transport models and vesicular fusion;
- describe the targeting signal and protein trafficking pathway to Nucleus, Mitochondria, Chloroplast and Peroxisomes.

UNIT 8

PROTEIN TRAFFICKING-I

Structure

8.1 INTRODUCTION

Recall from Block 2, unit 5 the organisation of a eukaryotic cell. You would have noticed that the internal membrane system divides the cell into many subcellular compartments like the nucleus, ER, etc. Each organelle requires a unique set of proteins, which have to be transported selectively from the site of synthesis to the appropriate destination. The endomembrane system acts like a traffic barrier to transport of proteins, lipid and other components between intracellular compartments.

In a typical mammalian cell, several thousand proteins are synthesized either on membrane bound ribosomes (Rough ER) or on free ribosomes in the cytosol. **You will study about the protein synthesis in the 5th Semester course: Gene Expression and Regulation (BBCCT-123).** For protein trafficking along the secretory route the ER and Golgi body play a crucial role in processing, folding, quality control and finally sorting proteins into separate vesicles for transport to their final subcellular compartment.

This unit deals with the role of ER in protein synthesis, translocation, processing and trafficking. We will also discuss the role of ER in protein folding and quality control.

Expected Learning Outcomes

After studying this unit, you should be able to:

- explain the role of Endoplasmic reticulum in protein trafficking;
- describe the signal hypothesis;
- explain the steps of co- and post-translational protein translocation;
- explain the mechanism of protein insertion into the ER membrane;
- point out the co- & post-translational modifications in proteins; and
- highlight the relevance of protein folding and quality control in the ER.

8.2 ROLE OF ENDOPLASMIC RETICULUM IN PROTEIN TRAFFICKING

Recall the structure and function of endoplasmic reticulum from Unit 5, Block 2. You know that the ER is a network of flattened tube-like structure present in almost all eukaryotic cells that closely works with the Golgi apparatus (Fig.8.1). The rough ER is the site of protein synthesis for most proteins that fall along the secretory route. It is also the site for N-glycosylation, protein folding, quality control, disulfide bond formation and assembly of multisubunit proteins.

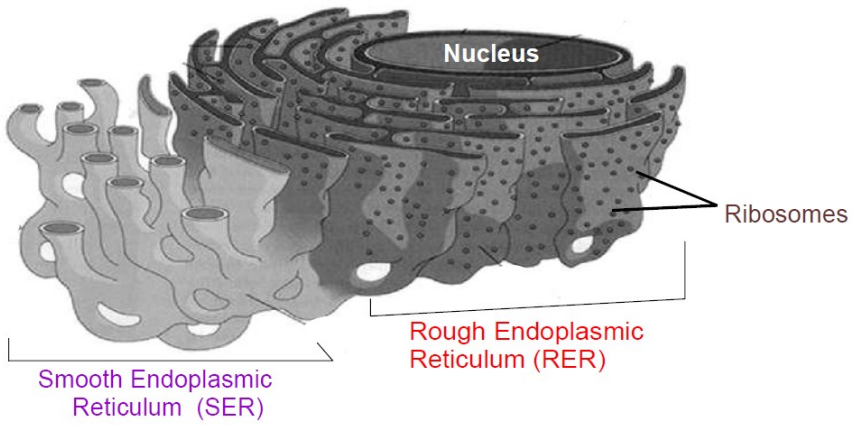


Fig. 8.1: Structure of Endoplasmic reticulum.

In the 1960s **George Emil Palade** and co-workers described the role of ER in protein synthesis and transport. They studied the fate of newly synthesised protein in pancreatic cells (a secretory cell) by labelling them with radioactive amino acids. They also showed in a **pulse –chase experiment** that radio labelled proteins was first detected in Rough ER. Subsequently radioactivity could be tracked in the Golgi apparatus and secretory vesicles prior to secretion.

Rough ER ➡ Golgi Complex ➡ Secretory vesicle ➡ Cell surface

In eukaryotic cells, rough ER is the primary site of protein synthesis that is destined for secretion or incorporation into the ER; Golgi body; lysosome and plasma membrane. Most proteins are translocated into the ER as they are translated on membrane bound ribosomes (**co translational protein translocation).** On the other hand, proteins that are destined to the nucleus, mitochondria chloroplast and peroxisomes are imported post translationally (Fig. 8.2). The last unit of block 3 covers **post translational protein translocation** to these organelles.

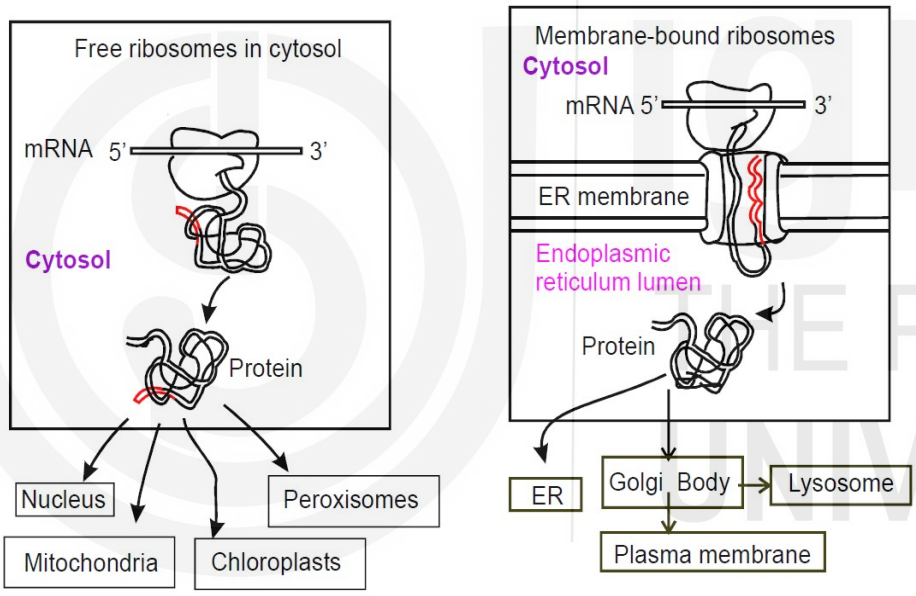


Fig. 8.2: Road map of protein targeting pathways in eukaryotes

George E. Palade

(1912- 2008)

Palade's scientific contributions spanned nearly 50 years that led to fundamental understanding of the structure and function of cells. He is best known for his pioneering work on the pathway for synthesis and vectorial transport of proteins along the secretory pathway. He shared the 1974 Nobel Prize in Physiology or Medicine with Albert Claude and Christian De Duve. The Nobel Committee said of Palade, "He added important methodological improvements both to the differential centrifugation and to the electron microscopy in order to obtain biologically basic information.

8.3 THE SIGNAL HYPOTHESIS

The synthesis of all proteins with the exception of those made in the mitochondria and chloroplast is initiated in the cytosol. The proteins may either be completely translated by cytosolic ribosomes or they complete their synthesis on rough ER. The basic question that had to be addressed was how to select the mRNA for translation between the two locations? This is essential as most proteins are not efficiently imported into the ER lumen once they fold and co translational translocation is a feasible alternative. The proteins made on RER are destined along the secretory route as shown by Palade and his group. On the other hand, the trafficking of proteins synthesised in the cytosol is undertaken post translationally and they also have to be correctly targeted.

The other question therefore is who directs newly synthesized proteins to their final destination?

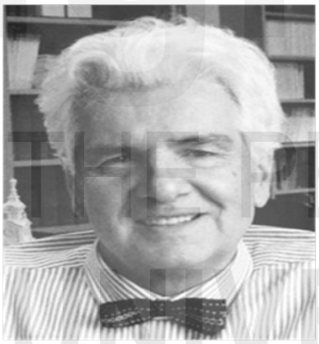
In order to resolve this problem, **Günter Blobel and David D Sabatini (1971)** proposed the **signal hypothesis**. They said that the selection of mRNA to the ER membrane is not by direct binding of mRNA. Instead all mRNAs for the secretory proteins code for an additional N terminal sequence (**signal sequence**) that is recognised by a soluble factor.

This in turn will guide the nascent chain- ribosome complex to the ER membrane. The tag may be transient. Finally, ribosomes whether free or ER bound is indistinguishable.

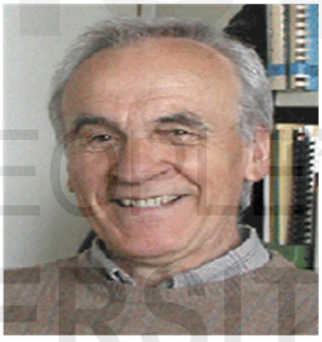
The presence of a signal peptide was confirmed by in vitro translation of immunoglobulin light chains (a secreted protein) on free ribosomes initially by Cesar Milstein and George Brownlee. In 1975, **Bernhard Dobberstein and G. Blobel** succeeded in demonstrating the difference in the size of light chains synthesised on free Vs membrane bound ribosomes.

They went ahead to expand their original hypothesis and included the idea of a protein conducting channel in microsome membranes (ER) through which the growing polypeptide is translocated.

David D. Sabatini



Günter Blobel



Bernhard Dobberstein

Later work by other investigators has identified the key components of the membrane insertion and translocation machinery. The concept of having tags for the selective transfer across membranes is essential for maintaining the identity of organelles.

Therefore, the impact of this hypothesis is beyond the ER. It applies to all types of trafficking pathways operative in cells. In recognition for the discovery that protein transport and localisation in the cell is governed by intrinsic signals or “zip codes” in the protein, **Günter Blobel** was awarded the 1999 Nobel Prize for physiology or medicine.

Once the hypothesis was supported experimentally by different laboratories, the emphasis shifted towards finding the molecular components of the machinery and to analyse the nature of the intrinsic signals that govern their transport. The sequence of signal peptides of representative proteins is given in Table 8.1.

Table 8.1 N-terminal sequences of some eukaryotic proteins.

Eukaryotic nascent proteins	Signal sequences
Human influenza virus A	**Met** *Lys* **Ala** *Lys* **Leu Leu Val Leu Leu Tyr Ala Phe Val Ala** Gly || Asp Gln…
Human preproinsulin	Met Ala Leu Trp Met *Arg* Leu Leu Pro **Leu Leu Ala Leu Leu Ala Leu Trp** Gly Pro Asp Pro Ala Ala Ala || Phe Val…
Bovine Growth Hormone	Met Met Ala Ala Gly Pro *Arg* Thr Ser **Leu Leu Leu Ala Phe Ala Leu Leu Cys Leu Pro Trp** Thr Gln Val Val Gly || Ala Phe…
Bee Promellitin	Met *Lys* Phe Leu Val Asn **Val Ala Leu Val Phe Met Val Val Try Ile** Ser Tyr Ile Tyr Ala || Ala Pro….
Drosophila glue protein	Met *Lys* **Leu Leu Val Val Ala Val Ile Ala** Cys **Met Leu Ile** Gly **Phe Ala** Asp Pro Ala Ser Gly || Cys Lys.....
||- cleavage site	

The common characteristics shared by all the signal sequences are:

- **The length of the signal sequence ranges from 13-36 amino acids.**
- **The amino terminal part of the signal contains one or more positively charged amino acid residues (italics), preceding the hydrophobic sequence.**
- **A hydrophobic stretch (bold) of 10-15 amino acid residues long form the centre of the signal sequence. Amino acids like alanine, valine leucine, phenylalanine and Isoleucine are common in this region.**
- **At the carboxyl terminal, the amino acid residues having small, neutral side chain (mostly alanine) predominate at positions closest to the cleavage site.**

The signal sequence is not always situated at the N-terminus. Certain secretory and plasma membrane proteins have an **internal signal sequence**. The signal hypothesis is thus universal, meaning the process is similar for plants, yeasts, animal cells and even prokaryotes. The signal sequence is both necessary and sufficient to bring about the initial membrane attachment. In most cases it is cleaved as it is exposed on the ER luminal side.

8.3.1 Components of protein translocation

Now we will proceed to study the basic components of proteins translocation across ER membrane and indicate their role(s).

1. Signal sequence: The signal sequences comprise of 13-36 amino acids, generally at N-terminal side of a nascent protein. As the signal sequence emerges from the ribosomes, it is bound by a signal recognition particle that guides it to the ER membrane. The affinity of the signal sequence for SRP decreases with chain length.

2. Signal recognition particle (SRP): G. Blobel and P. Walter showed that the adaptor that links the protein synthesising machinery in the cytosol to protein translocating machinery in the ER is **SRP**. It is an 11S conserved cytosolic ribonucleoprotein (RNP) particle consisting of 7S RNA and six different proteins (Fig.8.3). As the name suggests it detects signal sequences as they emerge from ribosomes. The SRP carries the ternary complex (SRP-ribosome- nascent polypeptide) to the ER membrane where it binds both to the **SRP receptor** and the translocon via the ribosomes. Both SRP and its receptor work catalytically to transfer a ribosome with a growing chain to the membrane for further synthesis and translocation. The binding of SRP to the signal sequence prevents premature elongation and folding of the nascent chain.

Kilodalton (kD) is a unit of an atomic mass equal to 1,000 daltons (abbreviated Da). One dalton is equivalent to one-twelfth the mass of carbon-12; a kilodalton (kDa) is 1,000 daltons;
It is usually used to express the molecular mass of large molecules such as proteins.

The SRP54 has two domains, namely the methionine rich M domain that binds signal sequence and a GTP binding G domain. The 68-72 kD (in kilodalton) dimer binds to the central region of the RNA and is required for recognising the receptor and for translocation through the membrane. The 9-14 kD dimer is responsible for elongation arrest. The non-coding RNA provides the structural backbone to the particle.

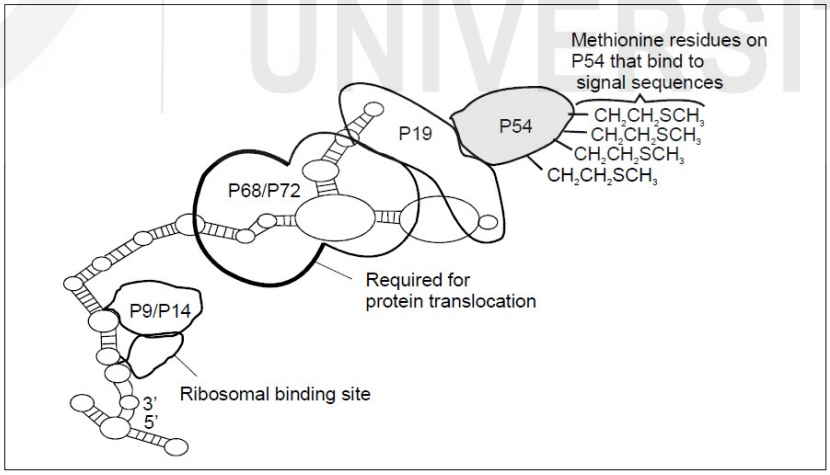


Fig. 8.3: Signal Recognition particle.

3. SRP receptor (docking protein) is a heterodimer of alpha and beta subunits, attached to the ER translocon. The larger subunit is a peripheral membrane protein that protrudes into the cytosol while the smaller subunit is an integral membrane protein. Like SRP, SRP receptor also binds and

hydrolyses GTP. It recognises SRP and also regulates the transport of SRP-ribosome-nascent polypeptide complexes to the ER translocon.

4. Translocon is a protein-lined channel or translocator embedded in the ER membrane. The ER translocon is Sec61/62/63 trimeric protein complex in eukaryotes through which the secretory protein in translocated across the ER membrane. The translocon attaches firmly to ribosome-nascent protein chain complex after dissociation of the SRP. It also plays an important role in insertion of membrane proteins in the correct orientation in the membrane.

SAQ 1

(a) Fill in the blanks:

i. The process of sorting and transporting newly synthesized proteins to their correct destination in a cell is called ……..
ii. The role of ER in the protein processing and sorting was recognised by .....
iii. The signal sequences are ……amino acids long and are generally located at ……
iv. The signal hypothesis was originally proposed by ……
v. The signal recognition particle is a.....
vi. Zip codes are ……

(b) Write the function(s) of the following in protein targeting:

i. Signal sequence …….....
ii. SRP ……
iii. SRP receptor ……...
iv. Translocon ……...

8.4 PROTEIN TRANSLOCATION ACROSS ROUGH ER MEMBRANE

In this section you will learn how secretory proteins are synthesised and transported into the ER during or after their synthesis is completed. Protein translocation is the first decisive step in the trafficking of proteins to their site of action. Broadly there are two kinds of proteins that are translocated to the ER. They are either soluble or membrane bound proteins.

1. **Soluble proteins** are completely translocated into the ER lumen where they are either retained or moved through vesicular transport to their final destination such as the lysosome or secreted.

2. **Membrane proteins** are not completely translocated to the ER lumen. They carry sequences that guide their insertion into the ER membrane. Once inserted the protein they may either be retained in the ER membrane or become part of other membranes along the secretory route.

In the next section you will now study the process of protein translocation across the ER membrane. The co-translational pathway is best understood in mammals and will be dealt with first followed by post translational translocation.

8.4.1 Co-translational translocation

In co-translational translocation the growing nascent protein chain is driven into the ER lumen as it is synthesised on membrane bound ribosomes. It is an evolutionarily conserved mechanism to target proteins into the secretary pathway. It was demonstrated by **George Palade** in 1975. **The pathway is explained below in steps and schematically depicted in Fig.8.4.**

Step 1: The synthesis of secretory proteins is initiated by cytosolic free ribosomes. Once the signal sequence / peptide emerge from the ribosomes, it is bound by the signal recognition particle present in the cytosol. This binding impedes protein synthesis.

Step 2: SRP-54 undergoes GDP-GTP exchange**.** The ribosomes function as a GTP exchange factor.

Step 3: SRP delivers ribosome-nascent chain complex to the SRP receptor located near to translocon on the ER membrane. The role of the receptor is transient.

Step 4: SRP is released from both the SRP receptor and the ribosome-nascent protein once it dissociates from translocon. The SRP and SR receptor proteins both hydrolyse their bound GTP to GDP. GTP hydrolysis drives the unidirectional transport of protein across the ER membrane.

As a result, the protein-ribosome subunits are attached to the translocon channel on ER membrane. SRP is recycled.

Step 5: As the ribosome is bound to the translocon, the channel opens and protein synthesis commences again with simultaneous translocation.

This process continues until the protein is completely translated. The protein conducting channel opens towards the lumen and the protein is translocated in an unfolded state.

Step 6: Some modifications occur co translationally. These include N-glycosylation and removal of the signal peptide. They will be discussed in the later sections.

Step 7: Once the protein is completely translated the ribosome subunits are released from the translocon and at the same time, the remainder of nascent protein is released through the translocon into the ER lumen.

Step 8: The nascent protein chain is modified and folded to its native conformation.

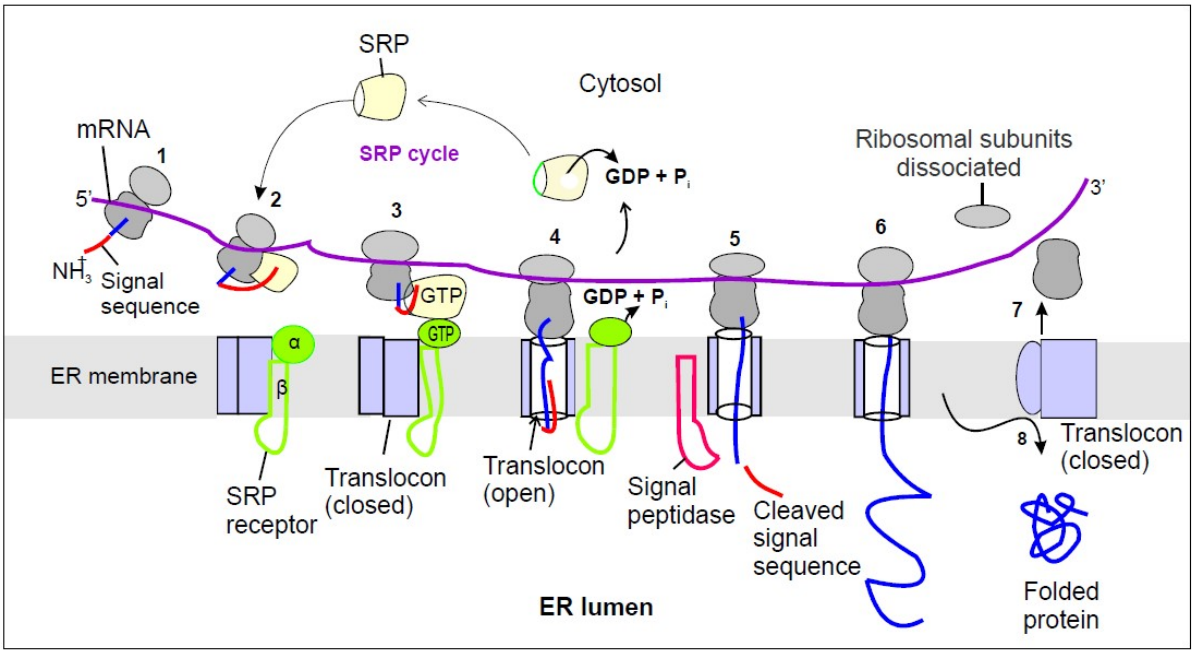


Fig.8.4: Co-translational translocation of nascent protein across the ER.

(Modified Image, Source: Lodish: Molecular Cell Biology)

8.4.2 Post-translational translocation

Most secretory proteins in a yeast cell and probably few secretory proteins in mammalian cells are transported into the ER after translation on free cytosolic ribosomes. Unlike Co translational translocation, post translational translocation in eukaryotes requires special proteins **Sec62/63 complex** and the chaperone (BiP). The process is best studied in *Saccharomyces cerevisiae.* The currently acceptable model for post translational translocation is given in Fig. 8.5.

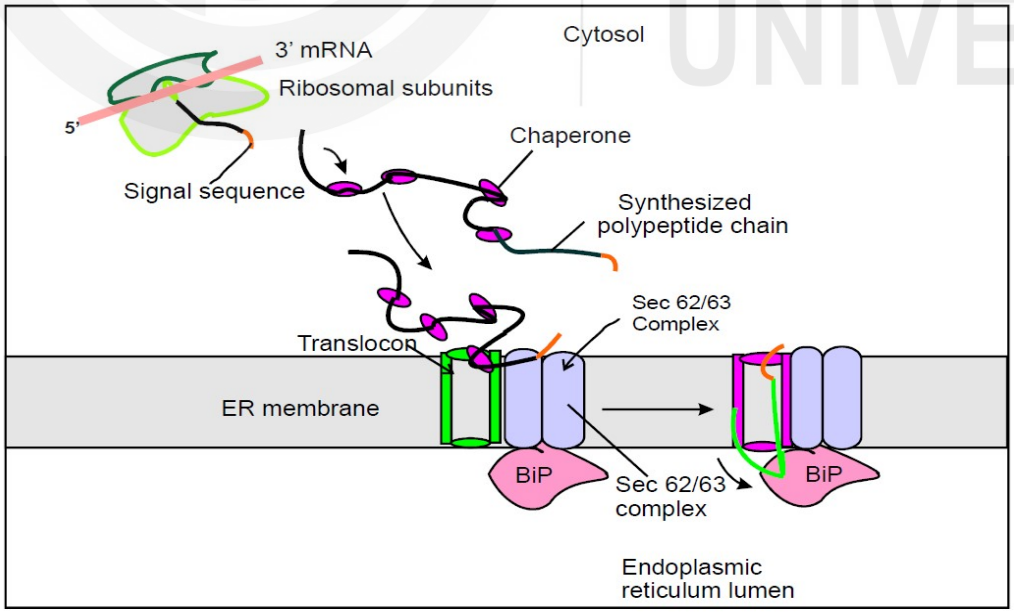


Fig. 8.5: Post translational translocation pathway in eukaryotes.

The completely synthesized nascent protein is seen bound by the cytosolic chaperone, Hsp 70 (**Heat shock protein-70)** that maintains it in an unfolded

state. As the protein moves across the ER membrane its signal sequence is recognized by **Sec62/63 complex** that in turn is associated with another chaperone **BiP** on the luminal side of ER membrane. The energy for post translational translocation is derived from the ATPase activity of the BiP protein.

The imported proteins may either be retained in the ER or they move further to their final destination by vesicular transport (discussed in the next unit 9). The proteins can also be directed to the cytosol (retrograde movement) for degradation by the proteasomes.

SAQ 2

(a) Fill in the blanks:

(i) Most secretory proteins are synthesized on ……
(ii) Most secretory proteins are transported across the ER by…….
(iii) The fully synthesized proteins in cytosol is transported across the ER by ……..pathway.
(iv) The source of energy that drives unidirectional transfer of proteins across the ER membrane is .....
(v) The N-terminal signal sequence is recognised by ……
(vi) The signal sequence is cleaved by ……

(b) Indicate true or false in the boxes provided:

(i) Signal peptides are permanent structural parts of a protein. [ ]
(ii) The translocon attaches firmly with ribosome-nascent protein chain complex after dissociation of the SRP. [ ]
(iii) Signal Recognition Particle (SRP) binds to the SRP receptor. [ ]
(iv) Most lysosomal, plasma membrane or secreted proteins have an N terminal signal sequence that marks them for synthesis on RER. [ ]
(v) ER translocon opens to translocate an elongating protein chain into the ER lumen. [ ]
(vi) All newly synthesised proteins on RER have same signal peptide sequence at their N-terminus. [ ]

(c) Differentiate between the co-translational and post translational protein translocation.

8.5 INSERTION OF PROTEINS INTO THE ER MEMBRANE

In section 8.4 you have learnt about protein translocation across the ER. Now we will discuss the example of membrane proteins which are imported co-translationally into ER membrane. The proteins destined for incorporation into

membranes of ER, Golgi, lysosomes, storage vesicles or plasma membrane all begin their journey by inserting in the right orientation in the ER membrane. These proteins carry specific signal sequence known as **topogenic sequence** that guides their insertion.

A topogenic sequence contains a single α-helical region of 20–25 hydrophobic amino acid residues near the N-terminus signal sequence of the nascent polypeptide. This sequence acts as a "**Stop transfer anchor sequence"** that stops the transfer of nascent proteins into ER lumen through the translocon. It is a non cleavable membrane spanning domain that also establishes the orientation of a transmembrane protein in the lipid bilayer. The anchor sequence is stabilised through multiple interactions with other proteins and hydrophobic parts of membrane lipids.

There are four types of transmembrane (TM) proteins (Fig. 8.6) of which type I, II, and III have single membrane spanning alpha-helical segment while type IV are multiple membranes spanning segment proteins. The topogenic sequence of all four types is shown in red. You will notice that the single pass TM proteins differ in their orientation and whether the signal sequence is cleaved or not. The lumen of the ER is topologically equivalent to the cell exterior.

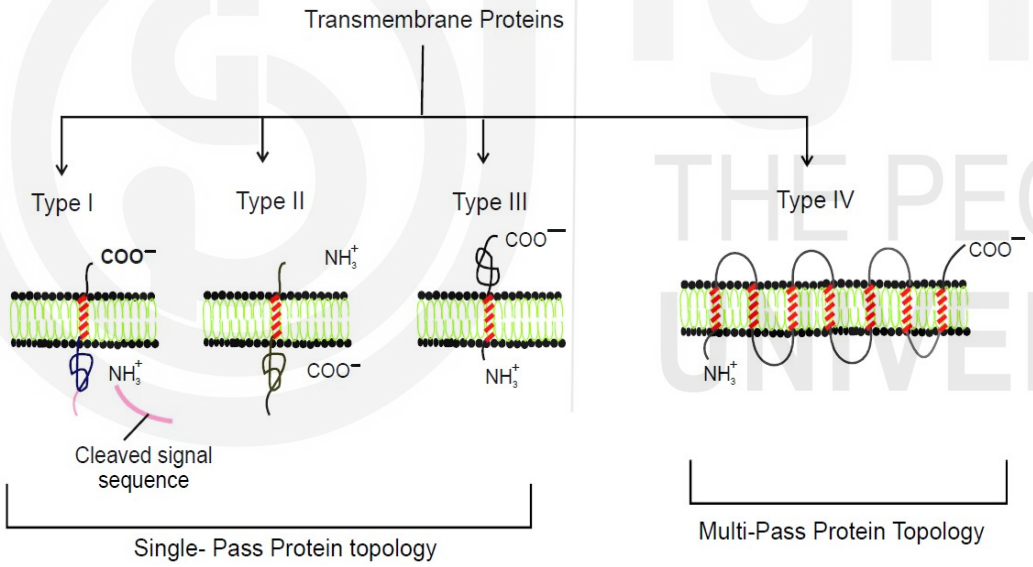


Fig. 8.6: Types of transmembrane proteins.

Let us understand how a growing protein chain with a type I topological sequence inserts into the ER membrane. A type I integral membrane protein may contain both a signal peptide and stop transfer sequence. The synthesis of a TM protein begins like a soluble / secreted protein and it is co-translationally translocated through the ER lumen. The difference comes when the stop transfer sequence is translated. The insertion of type I proteins is the simplest case since their carboxyl terminal is exposed to the cytosol.

The process is described in steps and schematically depicted in Fig.8.7.

Step1: As the nascent polypeptide-ribosome complex binds to the ER translocon (section 8.4.1) and resumes translation, the N-terminal signal sequence is cleaved by luminal signal peptidase once it becomes accessible.

Step2: The nascent polypeptide chain is elongated and translocated until the stop transfer anchor sequence is synthesised.

Step 3: The stop transfer anchor sequence signals the closure of the translocon thereby stopping further translocation into the ER lumen.

Step 4: At this point, the stop-transfer anchor sequence exits the translocon and moves laterally into bilayer membrane where it is anchored.

Step 5: After this the ribosome continues to synthesize the remaining protein and the elongating protein chain may loop out into the cytosol through the small space between the ribosome and translocon.

Step 6: As the synthesis is completed, the ribosomal subunits are released from translocon into the cytosol and the transmembrane protein is inserted correctly into the ER membrane with C-terminal end facing the cytosol and N-terminus in the ER lumen.

Some proteins have a non cleavable signal sequence that is recognised by SRP and brought to the ER and they also function as a TM domain sequence that exit the translocon and anchor to the membrane.

On the other hand, proteins that possess multiple TM domains have alternating internal signal sequence and stop transfer sequences.

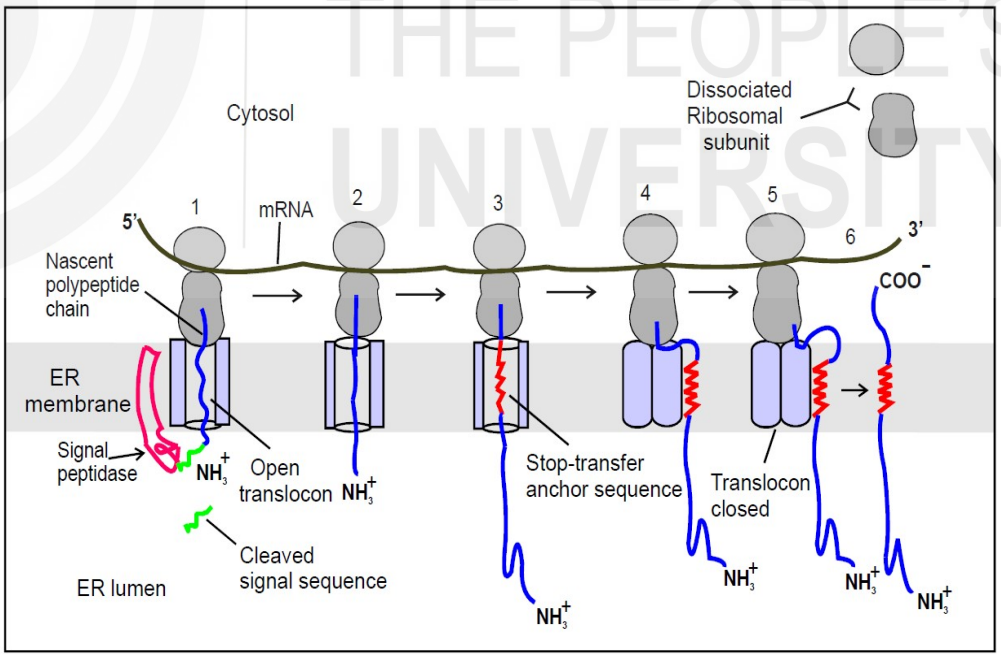


Fig. 8.7: Synthesis & insertion of type I single pass integral membrane protein.
(Modified image, Source: Lodish: Molecular Cell Biology)

SAQ 3

(a) What is meant by topogenic sequences?

(b) Match the items in Column I with that in Column II.

Column I	Column II
(i) Type I topogenic sequence possess	(a) protein lined aqueous channel
(ii) Translocon is a	(b) protein into the ER membranes
(iii) Class IV TM proteins	(c)signal peptide sequences and stop transfer sequence
(iv) Topogenic sequences direct	(d) Contain seven / multiple topological sequences

8.6 CO-TRANSLATIONAL MODIFICATION OF PROTEINS

The modifications in the nascent polypeptide chain begin even before the completion of protein synthesis. As the proteins are co-translationally translocated into the ER lumen it becomes accessible to the **processing enzymes** that are present either in the ER lumen or is membrane bound with their active sites facing the lumen. The removal of the signal sequence and N-glycosylation occur co-translationally. Both signal **peptidase** and **oligosaccharyl transferase** is integral membrane enzymes, present close to the translocon. Both enzymes are as abundant as their substrates. The other modifications like trimming reactions; addition of GPI anchors; protein folding and quality control are post translational events. All these changes are essential for generating a functional protein.

The process of addition of carbohydrates to a protein is called **glycosylation** and the resultant proteins are glycoproteins. These proteins are either soluble proteins / enzymes or localized to the membrane of organelles and plasma membrane. Many nuclear and cytosolic proteins are also O-glycosylated to some extent by cytosolic sugar transferases.

In this section you will learn about N-glycosylation. The process is initiated co-translationally in the ER but it continues by way of trimming and addition reactions both in the ER and Golgi apparatus. The addition of a readymade oligosaccharide (14 sugars) is a common feature for almost all proteins entering the ER lumen. It is the subsequent modifications that confer a unique signature to glycoproteins. In N-glycosylation the oligosaccharide is attached to the **asparagine residue** present around the sequence, Asn-X-Ser or Thr in the protein.

8.6.1 Synthesis & transfer of core oligosaccharide to the growing protein chain

The process of N-glycosylation can be divided into two phases, namely synthesis of the core oligosaccharide and it's transfer to the Asn residue of the growing polypeptide (Fig. 8.8).

The synthesis of oligosaccharide is a **multistep process**. It starts on the cytosolic side of the ER and continues on the luminal side following flipping of the lipid bound intermediate. It requires an activated sugar donor; a hydrophobic sugar carrier **dolichol pyrophosphate** and sugar transferases. The branched oligosaccharide is assembled step by step on the sugar carrier as described below:

1. The first step is the generation of dolichol phosphate (activated form) by a phosphoryl group transfer from ATP to dolichol. Dolichol is an isoprenoid compound, firmly embedded in the ER membrane.

2. The next two steps is the transfer of **N-acetyl glucosamine** from UDP-GlcNAc to dolichol phosphate. In the first transfer a sugar phosphate is transferred forming dolichol pyrophosphate. The oligosaccharide will be linked to dolichol by a pyrophosphate linkage.

3. Five residues of **mannose** are then transferred step by step from GDP-mannose to the growing sugar chain.

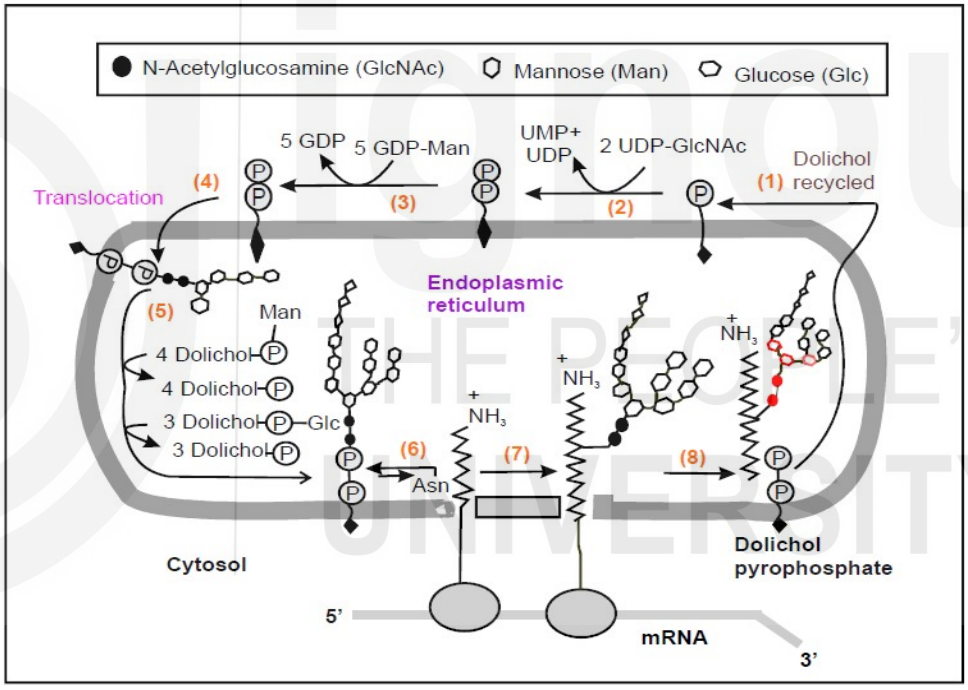


Fig. 8.8: The synthesis and transfer of core oligosaccharide to the growing protein chain in ER lumen. (Source: Nelson & Cox, Lehninger Principles of Biochemistry)

4. The lipid carrier with the incomplete oligosaccharide flips in the membrane and then the resumes synthesis.

5. Additional units of mannose (4 residues) and glucose (3 residues) are transferred from dolichol phosphate linked sugars. Glucose units are transferred from UDP-glucose and mannose units come from GDP-mannose.

6. The 14 residue **branched oligosaccharide** chain is then transferred to an asparagine (Asn) residue from dolichol pyrophosphate in the ER lumen by oligosaccharyl transferase (OST).

7. Dolichol pyrophosphate is released and recycled after hydrolysis of a phosphate by phosphatase.

8. All further modifications of the oligosaccharide in ER and Golgi apparatus will be discussed later in this and the next unit.

8.6.2 N-Linked Glycosylation

N-Linked Glycosylation covers more than 90% of the glycosylation that occurs in proteins. The N-Linked oligosaccharides that are attached co-translationally consist of 14 sugars (Fig. 8.9). The reaction is driven by dolichol lipid which is linked to the precursor oligosaccharide via high energy pyrophosphate bond.

N-glycosylation can be inhibited by antibiotics that block any of the steps of this process. Two well studied antibiotics are **bacitracin and tunicamycin**. The former blocks the recycling of the sugar carrier by inhibiting the phosphatase enzyme while the latter is a hydrophobic analog of UDP-N-acetylglucosamine and it blocks the addition of the first sugar.

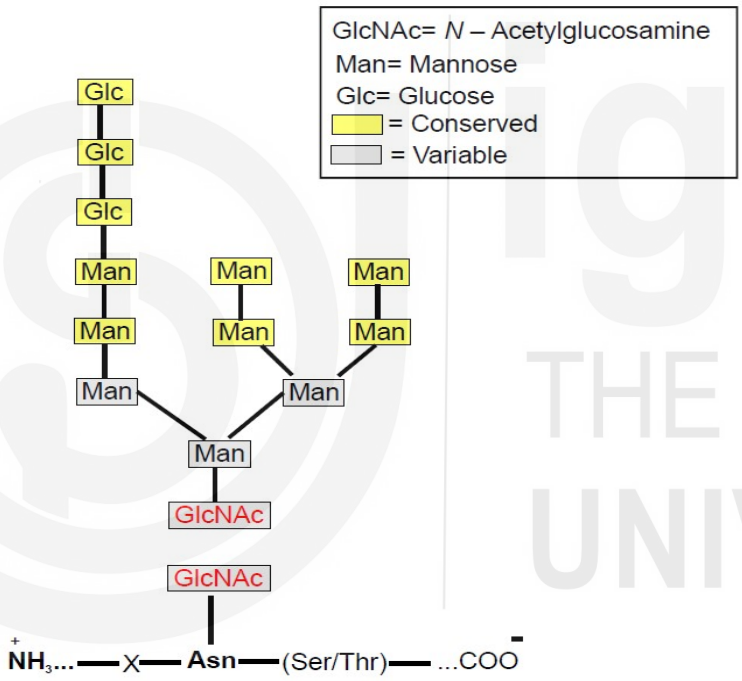


Fig. 8.9: N-Linked core oligosaccharide. (Credit: Lodish, Molecular Cell Biology)

8.6.3 Trimming reactions

The trimming of the oligosaccharide begins in the ER. The removal of sugars takes place in a fixed order. The three glucose residues are removed by **glucosidase I and II**. In mammals mannose residues are added only in the ER and trimmed by an **ER mannosidase**. The number of mannose residues removed is variable as the enzyme attacks the first mannose with ease and the next three much more slowly.

8.6.4 Glycosylation is a key to proteome diversity

Glycosylation is one of the most common modifications that increase proteome diversity. Structurally, glycosylation is highly complex due to micro heterogeneity in the site of glycan attachment or glycan structure. These

variations include glycan length, glycan structure (unbranched or branched chains), glycan composition (the types of sugars) and glycosidic bond.

Significance of Glycosylation

We are only beginning to understand the importance of extensive glycosylation. Our current understanding of the sugar code is limited. Some of the ways by which glycosylation may be useful include:

- Glycosylation prevents aggregation of nascent proteins since attachment of glycan increases solubility. Some proteins such as hemagglutinin precursor require N-linked oligosaccharide to fold properly.
- They confer targeting signal to some proteins like addition of mannose 6 phosphate residues serves as a lysosomal targeting signal. Similarly GPI anchors are apical targeting signals.
- The removal of terminal sialic acid residues from the circulating plasma glycoproteins (asialoglycoproteins) is a signal for their removal by the liver and subsequent degradation. Even aged RBC's are removed from blood by detecting such glycoproteins on their surface.
- The presence of specific arrangement of sugars on proteins facilitates cell-cell adhesion such as between selectins and mucin family glycoproteins. Lectins are primitive proteins that read the sugar code.
- Quality control: The misfolded proteins are distinguished from properly folded proteins by the presence of glucose in the former.
- Some membrane proteins are anchored to the plasma membrane by glycolipids (glycosyl phosphatidylinositol).
- The oligosaccharides on proteins may even promote their stability as in case of fibronectin. The non-glycosylated version of fibronectin is rapidly degraded by tissue proteases.

8.6.5 Removal of signal peptide

The signal peptidase cleaves the N-terminal signal sequence on nascent secretory proteins. In mammals, this enzyme is a complex of five proteins and the catalytic site includes an active serine residue. It cleaves at a site that has small aliphatic residues at position -1 and – 3 and normally 4-6 residues from the end of the hydrophobic core.

8.7 PROTEIN FOLDING AND QUALITY CONTROL IN THE ER

Protein folding is the most important function of ER. It has an assorted combination of enzymes that assist in folding and assembly of multi protein complexes. The process is very efficient and it takes less than 3-4min to fold a 30Kd protein. The ER lumen has a more oxidizing environment as compared to cytosol that helps to maintain disulphide bonds. The main players involved in folding are molecular chaperones (BiP and many others), protein disulphide isomerase (PDI), peptidyl prolyl cis-trans isomerase and foldase. These enzymes will be covered in detail in core course Proteins (BBCCT-105). The protein folding pathway is schematically depicted in Fig. 8.10.

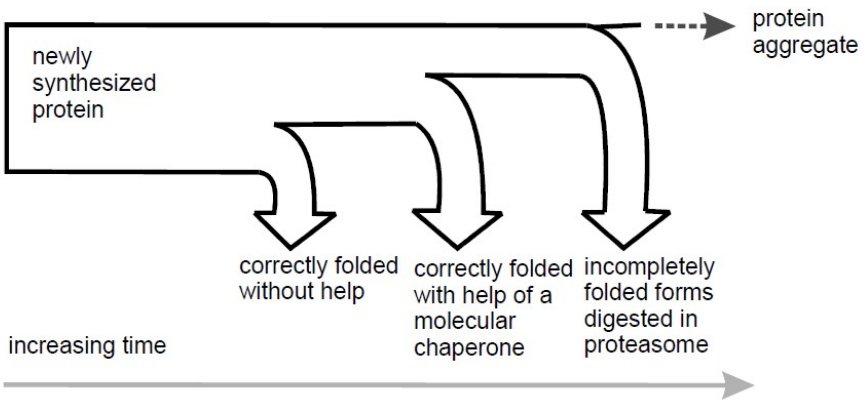


Fig. 8.10: Pathway for protein folding.

The ER lumen is enriched in molecular chaperones that prevent aggregation of unfolded proteins and facilitate their correct folding by providing appropriate microenvironment. The chaperones are grouped in several subfamilies. Many of them such as Hsp40, Hsp60, Hsp70, etc. exist in preassembled multi-chaperone complexes that are required for coordinated catalysis of protein folding. The folding of proteins is an energy dependent process. The chaperones bind and hydrolyse ATP to support protein folding. It is also known that chaperones never work in isolation rather they act in combination to assist in folding. It is however still unclear what specifically do these chaperones recognize and how they know that a protein is properly folded and ready to exit the ER?

Apart from chaperones two other enzymes with known function are PDI and peptidyl prolyl isomerase. The enzyme PDI reshuffles disulphide bonds as folding progresses while the isomerase changes the trans-peptide bond linking proline residues to cis configuration. Finally, the correctly folded protein is transported in vesicles to the cis Golgi complex for further processing and trafficking. It is known that changes in the polypeptide sequences due to mutations may drastically affect the folding efficiency, leading to conformational defects. In such situations the misfolded proteins are retained in the ER and subsequently degraded in the cytosol. Some variants of human diseases including cystic fibrosis, familial hypercholesterolemia and even diabetes have been tracked down to defects in ER functioning.

Quality control in the ER

The proteins that are properly folded and assembled are allowed to exit the ER in vesicles to the Golgi body. Misfolded or incorrectly assembled proteins are bound to chaperones in an attempt to try and fold them to their native conformation or they are targeted for degradation. This is known as the quality control. The proteins that do not fold correctly are retained by addition of glucose residue(s) by an ER **glucosyl transferase (GT).** A glycoprotein with one or more glucose residues is bound to a chaperone that is anchored to the ER membrane. As long as the glycoprotein has glucose residues it will remain in the ER. Indeed, several rounds of glucosylation – deglucosylation occur in the ER. When all attempts to fold a protein do not succeed then it is transported to the cytosol (**retrograde transportation)** for degradation by proteasomes that degrades protein into peptides function at neutral pH.

Another quality control operates by way of returning ER resident proteins back from the Golgi. The C-terminus of these proteins has a tetra peptide sequence, KDEL or related sequence that serves as a **retrieval tag.** It does not block the departure of the protein from the ER. Once they reach the cis Golgi the C-terminal sequence is recognised by specific membrane receptors and the complex is then returned in vesicles to the ER. The protein is released from the receptors into the ER lumen due to the difference in pH in the two compartments. It is likely that these proteins may be modified by enzymes present only in the Golgi and then retrieved back.

8.7.1 Acquisition of Glycolipid anchor

Eukaryotic membrane proteins are also anchored to the plasma membrane by **glycosyl phosphatidyl inositol (GPI)**. The existence of lipid anchors was speculated with the discovery that bacterial phospholipase C could release alkaline phosphatase from the mammalian cell surface. The lipid anchors are acquired post-translationally in the ER. These proteins have a cleavable hydrophobic sequence at the C-terminus (17-30 residues) which signals the addition of the GPI anchor. Some of the well characterised GPI anchored proteins are decay acceleration factor (DAF); trypanosomal variant surface glycoprotein (VSG), human erythrocyte acetyl choline esterase and Thy-1. The proteins that carry a GPI anchor are said to be **glypiated** and such proteins are targeted to the apical side of polarised cells.

The basic steps in the acquisition of a GPI anchor involve the formation of GPI moiety; processing of the nascent chain and the covalent attachment to the protein. The structure of a representative GPI anchored protein is given in **Fig. 8.11**. It is known that abnormalities in GPI synthesis or attachment are implicated in diseases like paroxysmal nocturnal hemoglobinuria.

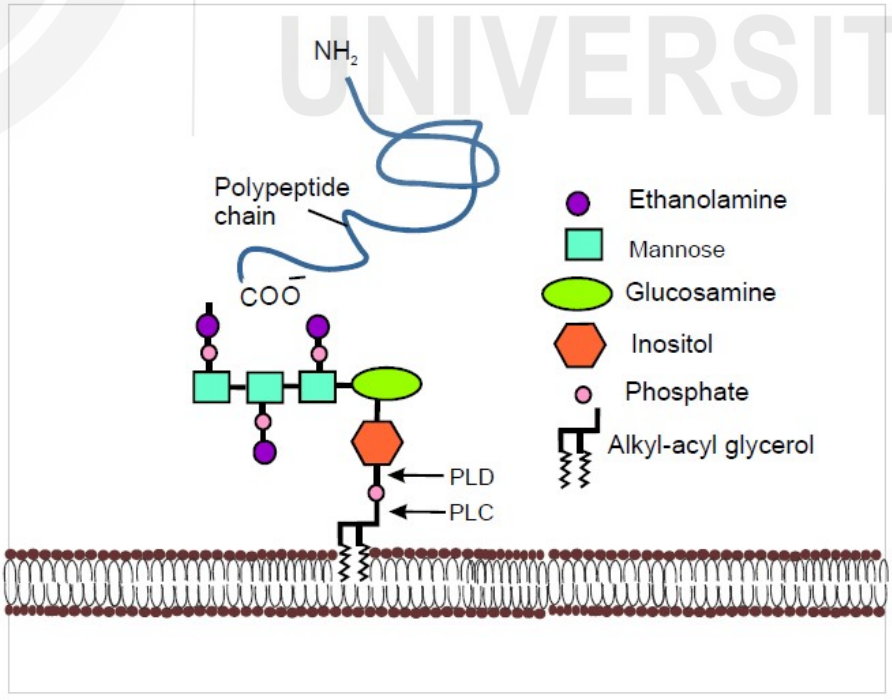


Fig. 8.11: A GPI anchored protein.

SAQ 4

Fill the blanks:

(i) The process of addition of carbohydrates to proteins is called …….

(ii) The N-Glycosylation of proteins occurs in ……..

(iii) The retrieval tag in ER resident proteins is present at the.....

(iv) The preformed oligosaccharide is transferred from dolichol-phosphate to an ---------- residue of the protein.

(v) Protein folding takes place in ……

(vi) Misfolded or aberrant proteins in the ER are exported to the ..... for degradation by .....

(vii) An example of a glycolipid anchor is ..... and it added .....in the ER.

8.8 SUMMARY

After reading this unit, you have learned that:

- The RER is the primary site for production of most secretory and membrane proteins that fall along the secretory route. It is present in almost all eukaryotic cells and works in close collaboration with the Golgi apparatus.
- The synthesis of proteins on RER is initiated by cytosolic ribosomes and then targeted to the ER once the growing polypeptide exposes the signal sequence.
- The impact of the signal hypothesis is beyond the targeting proteins to the ER. It applies to all types of trafficking pathways operative in cells.
- Most proteins are imported to the ER co-translationally through a translocon. Some proteins are first completely synthesised in the cytosol and then translocated post translationally with the assistance of cytosolic molecular chaperone and translocon machinery.
- In eukaryotes, the basic components of co-translational protein translocation across the ER include signal peptide sequence, SRP-SRP receptor and translocon channel. Both SRP and its receptor are G proteins. They bind and hydrolyse GTP and work catalytically to transfer ribosomes with a growing chain to the membrane for further synthesis and translocation. Most proteins have a transient N terminal signal sequence.
- The ER synthesises both soluble and integral membrane proteins. The soluble proteins are completely translocated into the ER lumen. The proteins may either be retained in the ER or may be targeted to other membrane compartments like Golgi or lysosomes. The default fate of a soluble protein is secretion.
- The proteins destined for incorporation into the membranes of ER, Golgi, lysosomes, storage vesicles or cell surface all begin their journey by inserting in the right orientation in the ER membrane. These

proteins carry specific **topogenic sequence** that guides their insertion. There are four types of transmembrane proteins.

- The modifications in the nascent polypeptide chain begin even before the completion of protein synthesis. The co-translational modifications are N-glycosylation and removal of the signal peptide. Both signal **peptidase** and **oligosaccharyl transferase** is integral membrane proteins that are present close to the translocon. The glycosylation of proteins is a key to proteome diversity.
- The other changes like trimming reactions; addition of GPI anchors; protein folding and quality control are post translational events.
- The endoplasmic reticulum is the main the site for protein folding. The main players involved in protein folding are multiple molecular chaperones, protein disulphide isomerase (PDI), peptidyl prolyl cis-trans isomerase and foldase. It also has quality control systems to monitor the status of folded secretory and membrane proteins.
- The misfolded or defective proteins are transferred back to the cytosol where they are degraded by proteasome.

8.9 TERMINAL QUESTIONS

1. How do cells know the final destination of a protein?
2. Who developed the signal Hypothesis? Briefly explain it in your own words.
3. Point out the major differences between the four types of transmembrane proteins.
4. How many sugars residues are present in the core oligosaccharide that is N linked to the growing polypeptide? Name the sugars.
5. Indicate the relevance of protein glycosylation.
6. Explain the significance of protein folding and quality control in the ER.

8.10 ANSWERS

Self assessment Questions

1. (a)

(i) Protein trafficking (ii) Palade (iii) 13-36; N- terminus
(iv) Blobel & Sabatini (v) RNP (vi) Intrinsic signals in proteins

(b)

i. **Signal sequence** is a small peptide sequence generally at the N terminus of the nascent protein that is recognised by a soluble factor as it emerges from the ribosomes.

i. **SRP** is a cytosolic ribonucleoprotein (RNP) that recognises signal sequences and carries the ternary complex of SRP-ribosome -nascent proteins to the ER.

ii. **SRP receptor** (docking protein) recognises the SRP-protein complex during protein targeting of to ER membrane.
iii. **Translocon** is a protein-lined channel embedded in the ER membrane which is essential to transport proteins across the ER membrane.

2. (a)

i. RER
ii. Co translation translocation pathway
iii. Post translational translocation pathway.
iv. GTP
v. SRP
vi. signal peptidase

(b)

(i) False (ii) True (iii) True (iv) True (v) True (vi) False

(c) In co- translational protein translocation the synthesis and translocation across the RER occur simultaneously whereas in post translational protein translocation the protein is first completely synthesised in the cytosol and then imported.

The co-translational pathway usually translocates proteins across ER (**secretory pathway)** while the post translational pathway is generally used for proteins that are imported to the nucleus, mitochondria, chloroplast and peroxisomes.

3 (a) Topogenic sequences are present in integral membrane proteins that are destined to ER, Golgi body, lysosomes and cell surface. They consist of 20–25 hydrophobic amino acid residues near the N-terminus signal sequence of the nascent polypeptide chain .They function as **Stop transfer anchor sequence** that stops the transfer of nascent proteins through the translocon into ER lumen

(b) (i) c (ii) a (iii) d (iv) b

4.(i) Glycosylation (ii) ER (iii) C-terminus

(iv) Asparagine (v) ER (vi) cytosol; proteasome
(vii) GPI; post translationally

Terminal questions

1. The proteins that have to be targeted to different locations have sequences that are recognised by the cellular machinery and carried to their final destination. Some of these include the signal sequences that target the growing chain to the RER membrane ; topogenic signals determine regions of the protein that will be inserted in the membrane and finally some signals help in sorting proteins to organelles other than the ER.

2. The signal hypothesis was originally proposed by **Günter Blobel and David D Sabatini (1971)**. They postulated that all mRNAs for the secretory proteins code for an additional N terminal sequence (**signal sequence**) that is recognised by a soluble factor. This in turn will assist in taking the nascent

chain ribosome complex to the ER membrane. Later Dobberstein and Blobel (1975 included the idea of a protein conducting channel in microsome membranes.

3.There are four types of transmembrane proteins. The type I, II, and III have single membrane spanning alpha-helical segment whereas type IV proteins contains multiple membranes spanning segments.

The single pass TM proteins differ in their orientation and whether the signal sequence is cleaved or not (Fig.8.6). Type I proteins have a cleavable signal sequence whereas in type II and type III proteins it is non cleavable. The orientation of type I and III is identical with their C-terminus facing the cytosol and in type II proteins their N-terminus facing the cytosol. The ER lumen is equivalent to the cell exterior.

4. The N-Linked core oligosaccharide consists of 14 sugars - 9 mannose, 3 glucose and 2 NAG residues.

5. Glycosylation is the most common modification that increases proteome diversity. We are only beginning to understand the importance of extensive glycosylation. Our current understanding of the sugar code is limited. Refer to subsection 8.6.4 for details.

6. All newly synthesized proteins imported to the ER are folded to their native conformation. The ER lumen has a more oxidizing environment as compared to cytosol that helps to maintain disulphide bonds. Protein folding is assisted by molecular chaperones, protein disulphide isomerase, peptidyl prolyl cis-trans isomerase and foldase. It is very efficient and energy dependent.

The proteins that are properly folded and assembled are only allowed to exit the ER. Misfolded or incorrectly assembled proteins are bound to chaperones in an attempt to try and fold them correctly or target them for degradation by cytosolic proteasomes.

8.11 SUGGESTED READINGS

1. Lehninger, Principles of Biochemistry, David L. Nelson and Michael M. Cox
2. Molecular Cell Biology, 6th edition, Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. 2000
3. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.
4. Gerald Karp. Cell and Molecular Biology: Concept and Experiments. 6th Edition. John Wiley & Sons, Inc. 2010.

UNIT 9

PROTEIN TRAFFICKING -II

Structure

9.1 INTRODUCTION

In Unit 8 you have learned about the role of ER in protein synthesis and translocation; processing; protein folding and quality control. The translocation machinery facilitates protein import into the ER lumen or ER membrane by co- or post- translational translocation pathway. This pathway is guided by signal peptide sequences on secretory proteins. In ER, secretory proteins are N-glycosylated by attaching a preformed oligosaccharide to the amide nitrogen of asparagine. Some degree of trimming reactions takes place in the ER itself. The question is how secretory proteins are transported from the ER to the Golgi body or their final destination.

Therefore, in this unit you will learn about protein modifications (terminal N- and O-glycosylation) in the Golgi and the topology of the secretory pathway. The role of Golgi body in protein sorting will be described. The vesicular transport models, types of coated vesicles, vascular fusion process and receptor mediated selective transport mechanism are also discussed here.

Expected Learning Outcomes

After studying this unit, you should be able to:

- define the role of functional compartmentalization of Golgi complex;
- describe the terminal glycosylation in the Golgi;
- explain the process of O-glycosylation of proteins;
- differentiate between N- and O-glycosylation;
- highlight the role of Golgi body in protein sorting;
- indicate the topology of the secretory pathway;
- enumerate the major types of coated vesicles;
- explain vesicular transport models and specificity of the process;
- describe vesicular fusion; and
- explain receptor mediated selective transport.

9.2 MODIFICATIONS IN THE GOLGI

The glycoproteins that reach the cis Golgi by vesicular transport from the ER are further modified step by step by specific enzymes present in the sub compartments of the Golgi body. These changes include terminal N-glycosylation, O-glycosylation, phosphorylation and sulphation. Many glycosphingolipids also undergo glycosylation and sulphation in the lumen of the Golgi apparatus. The addition of complex oligosaccharides can make a significant difference in the mass of the protein. The molecular weight of the glycoprotein is greater than expected. This is important to keep in mind when working with glycoproteins.

9.2.1 Terminal N-glycosylation

In unit 8 you have learnt that following the addition of the core oligosaccharide to the growing polypeptide in the ER there is also removal of some of these sugars (trimming reactions). Generally, all three glucose and one or more mannose residues are removed. The **mannose $_8$intermediate** is the most common N-Glycosylated protein that reaches the *cis* Golgi.

The fate of the oligosaccharide depends on the final destination of the protein. If the protein is a lysosomal enzyme it is modified by the addition of phospho-N-acetyl glucosamine followed by the removal of N-acetyl glucosamine or it is further processed before addition of terminal sugars. In case of **lysosomal enzymes,** mannose -6-phosphate residues function as lysosomal targeting signal. The phosphorylated mannose residue is recognised by mannose-6-P receptor in TGN. The complex is sorted to vesicles that carry them to the lysosomes.

The other proteins are acted upon by α-mannosidase I in the cis Golgi and up to three mannose residues may be removed. The Man_5 oligosaccharide is a substrate for N-acetyl glucosaminyl transferase-I in cis / medial Golgi that transfer N-acetyl glucosamine from the activated donor to the oligosaccharide. This step initiates the conversion of the oligosaccharide into complex carbohydrates by a series of steps involving both addition and removal of sugars.

In order to get the final sugar decorations, the proteins are progressively moving in vesicles to the Golgi compartments as there is **functional compartmentalization** within the Golgi (Fig.9.1). Some of the enzymes that act following the addition of N-acetyl glucosamine are mannosidase II and acetyl glucosaminyl transferase-II in the medial cisternae; galactosyl transferase and fucosyl transferase in trans cisternae and sialyl transferase in TGN. Therefore, the glycoproteins present in different compartments along the secretory route have oligosaccharides that reflect the pathway to that point. The Golgi complex is sometimes dubbed as a **'processing unit'** as proteins entering the Golgi stacks are processed to varying extent en route to their site of action.

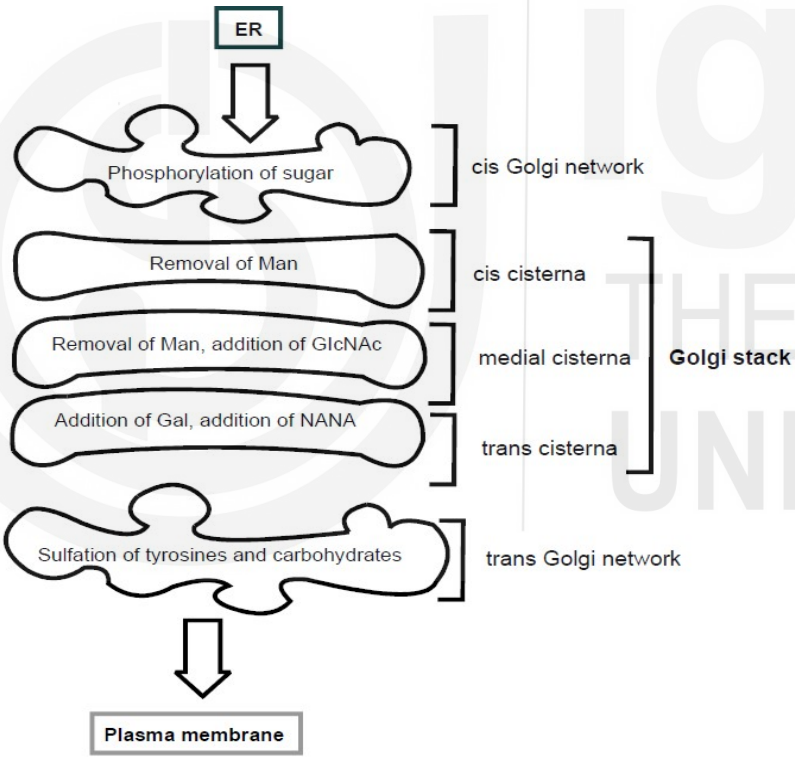


Fig. 9.1: Functional compartmentalization in Golgi complex.

9.2.2 O-glycosylation

The proteins entering the Golgi may be linked to sugars by an O-glycosidic linkage in which the anomeric carbon of a sugar makes a glycosidic bond with the hydroxyl group of either serine or threonine residue. This process is called **O-glycosylation**. It is initiated post translationally and does not depend on a lipid carrier. The sugar that is directly linked to the protein is N-acetyl galactosamine. The second sugar is usually galactose that is added in the *trans* Golgi. The addition of sugars takes in an ordered sequence and many

end with a terminal sialic acid residue. In some proteins the sugars are further modified by sulphation. The activated donors for glycosylation are linked to nucleotides (UDP-N- acetyl glucosamine or galactosamine; GDP-mannose and CMP-sialic acid, etc) and to a large extent they are synthesised in the cytosol except CMP-sialic acid that is made in the nucleus. They are made available in the Golgi lumen (even ER lumen) by specific transporters.

The O-linked oligosaccharides in membrane glycoproteins may be branched although they are not very large. On the contrary the O-linked glycoproteins in body fluids are very large as in mucins. Many proteins have multiple oligosaccharide chains linked along its length in which some are N-linked while others have an O-linkage. Some cytoplasmic and nuclear proteins are also modified by O-glycosylation that occurs in the cytosol.

9.2.3 N- Vs O-glycosylation

Once you have learnt about the two variants of glycosylation in proteins it is always beneficial to compare the two processes. The most obvious differences are given in table 9.1.

Table 9.1: The comparison of O- and N-glycosylation

S.No.	O-glycosylation	N-glycosylation
1	In O-linked oligosaccharides N-acetyl galactosamine is generally linked to the hydroxyl group of serine or threonine.	In N-linked oligosaccharides, N-acetyl glucosamine is linked to the amide nitrogen of asparagine.
2	The oligosaccharides are generally short (1-4 sugars) and may be branched. They lack mannose.	Typical N-linked oligosaccharides contain mannose and N-acetyl glucosamine. They usually have several branches each terminating with a negatively charged sialic acid.
3	The sugars are added one by one by specific glycosyl transferases post translationally.	A 14 sugar preformed oligosaccharide is transferred *en bloc* to the growing polypeptide co translationally. This is followed by removal and addition of sugars in a fixed order.
4	O-glycosylation takes place in Golgi stacks.	N-glycosylation is initiated in the ER and is completed in the Golgi.
5	It is less complex than N-glycosylation.	It is more complex than O-glycosylation.
6	No dolichol linked intermediates are involved.	The oligosaccharide is assembled on a lipid carrier dolichol phosphate in the ER membrane.

The Golgi complex is also the site of synthesis of a variety of complex polysaccharides in both plants and animals. The glycosaminoglycans present in the ECM of animals and the matrix polysaccharides such as hemicellulose and pectins in the cell wall of plants are examples of complex polysaccharides whose structure and role you have learnt in unit 5. The synthesis of the cell wall polysaccharides constitutes major metabolic activity of the Golgi complex in plants.

SAQ 1

Fill in the blanks:

i. The mannose$_8$ intermediate is the most common N-glycosylated protein that reaches the ……. from ……..
ii. The phosphorylated mannose residue is recognized by …… in …….
iii. In O-linked glycosylation, the N-acetyl glucosamine is linked to a……residue in a protein.
iv. The N-linked glycosylation of proteins is initiated in the …….
v. The activated donor of sialic acid is ……

9.3 PROTEIN SORTING AND EXPORT FROM GOLGI APPARATUS

Protein targeting is the biological mechanism that includes protein sorting and delivery of proteins to appropriate destinations within the cell or secreted outside. This is analogous to the sorting of thousands of letters at the post office followed by their delivery to the recipients in different locations. The proteins that are synthesized on RER can either reside in the ER or Golgi compartments or they are targeted to different locations along the secretory route. Recall the structure and functions of Golgi complex from the Unit 5 (Block 2) where you learnt that the **Golgi body** is the 'processing unit 'of the cell. The details of vesicular transport and sorting will be dealt in this unit.

9.3.1 Role of Golgi apparatus in protein transport

The proteins that are synthesized on RER are not released at any point into the cytosol. They are modified, sorted and transported to their final destination. This is achieved by carrying them only in membrane enclosed **coated vesicles** until they are off loaded to their final location. The partially processed and folded proteins leave the ER and enter at the cis face of the Golgi which is oriented towards the nucleus of the cell. They are then transported through the Golgi stacks and TGN before they exit from its concave *trans* face.

In the preceding section you have already been introduced to the functional compartmentalization within the Golgi stacks.

There are three major destinations for the secretory proteins:

- **Plasma membrane**
- **Lysosomes**
- **Extracellular secretion / stored in secretary vesicles**

In addition, there are two distinct mechanisms that operate so that a protein is ultimately reaches the right Golgi compartment. The first one is by not allowing a protein to exit a Golgi compartment and the other is retrieving the protein back so that they can catalyse specific biochemical reactions or help to maintain its structure. Such proteins are known as **resident proteins**. Some of the resident proteins are Glycosyl transferases and glucan synthetases.

The proteins that are targeted to the plasma membrane are always bound to the cisternae membrane and are not discharged into the lumen. They remain in this way even after glycosylation. They form the wall of vesicles that later fuses with the plasma membrane and helps to renew and proliferate the membrane.

We have already discussed the sorting of lysosomal proteins in subsection 9.2.1. These proteins are packed into vesicles in TGN that move to late endosome and then to the lysosomes. Finally the proteins that are secreted outside are released either constitutively or in response to signals. The vesicles carrying them fuse with the plasma membrane to release their contents.

9.3.2 Topology of the secretory pathway

The secretory pathway is essentially a system of functionally interconnected organelles. Approximately one third of all newly translated proteins in a typical eukaryotic cell are translocated into the endoplasmic reticulum (ER), for processing and delivery along with secretory pathway. The transport of a membrane protein from the rough ER to the plasma membrane is depicted schematically in Fig. 9.2.

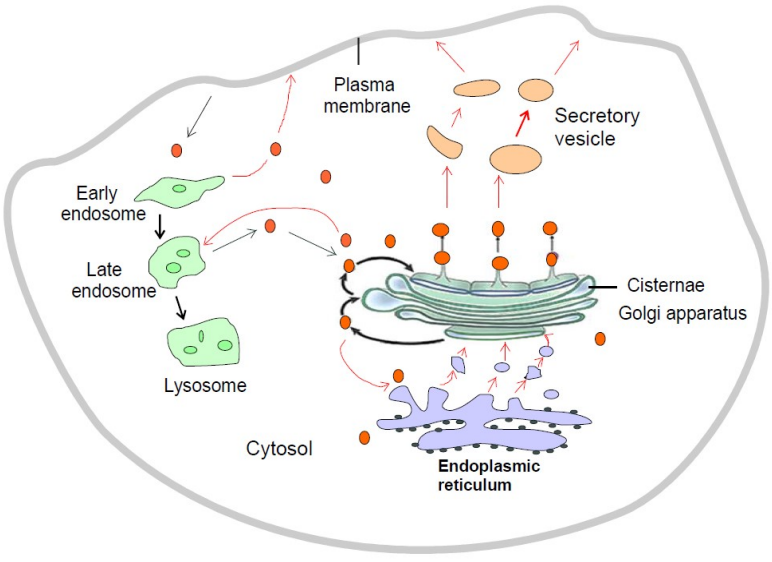


Fig. 9.2: Vesicular transport of a protein from ER to the cell surface.

It must be recognised that the lumen of endoplasmic reticulum and Golgi are topologically equivalent to the cell exterior. The anterograde movement of proteins intended to be targeted through the secretory pathway is:

Rough ER → ER-to-Golgi transport vesicles → Golgi body (cisternae) → trans Golgi network (secretory or transport vesicles) → cell surface → exocytosis

SAQ 2

(a). Choose the correct pathway for targeting a secretory protein:

(i) Rough ER → ER-to-Golgi transport vesicles → Golgi body (cisternae) → TGN (secretory or transport vesicles) → cell surface → exocytosis

(ii) Rough ER → cell surface (exocytosis) → Golgi body (cisternae) → TGN (secretory or transport vesicles) → ER-to-Golgi transport vesicles

(iii) Rough ER → ER-to-Golgi transport vesicles → Golgi body (cisternae) → cell surface → exocytosis

(iv) TGN (secretory or transport vesicles) → rough ER → ER-to-Golgi transport vesicles → Golgi body (cisternae) → cell surface → exocytosis.

(b) Fill in the blanks:

(i) The newly synthesised secretory proteins from the ER enter from the …….. face of the Golgi and finally exit from it's ……..

(ii) The sorting of proteins occurs in the ……

(iii) (The proteins that remain in Golgi body are called ……

(iv) The export and import of secreted proteins occurs via ……

(c) What is the fate(s) of proteins that are delivered from the ER to the Golgi complex?

Let us understand how proteins are exported from the ER to the Golgi body followed by sorting and delivery to their site of action.

9.4 VESICULAR TRANSPORT

Recall the co-translational translocation pathway discussed in the preceding unit. The synthesis and translocation of the protein is the first step in the life of the protein that is destined along the secretory route. Most of the proteins function in other sub cellular compartments or only after they are secreted. This imposes the need to sort and transfer them to the correct destination. This is achieved by vesicular transport mechanisms about which you will study briefly now and later in the 3rd Semester in Membrane Biology and Bioenergetics.

The cells depend on coated vesicles both for import and export of proteins. A vesicle is formed by budding off from a donor membrane that in turn fuses with an acceptor membrane (Fig.9.3). A typical eukaryotic cell uses two pathways for export and only one for import of proteins. A protein may be constitutively secreted / incorporated into the plasma membrane or its release may be regulated. The former route of export is used for bulk flow of proteins and lipids whereas the latter route is regulated release of contents from preformed secretory vesicles in response to signals such as hormones; neurotransmitters or digestive enzymes. The import of proteins is by endocytosis.

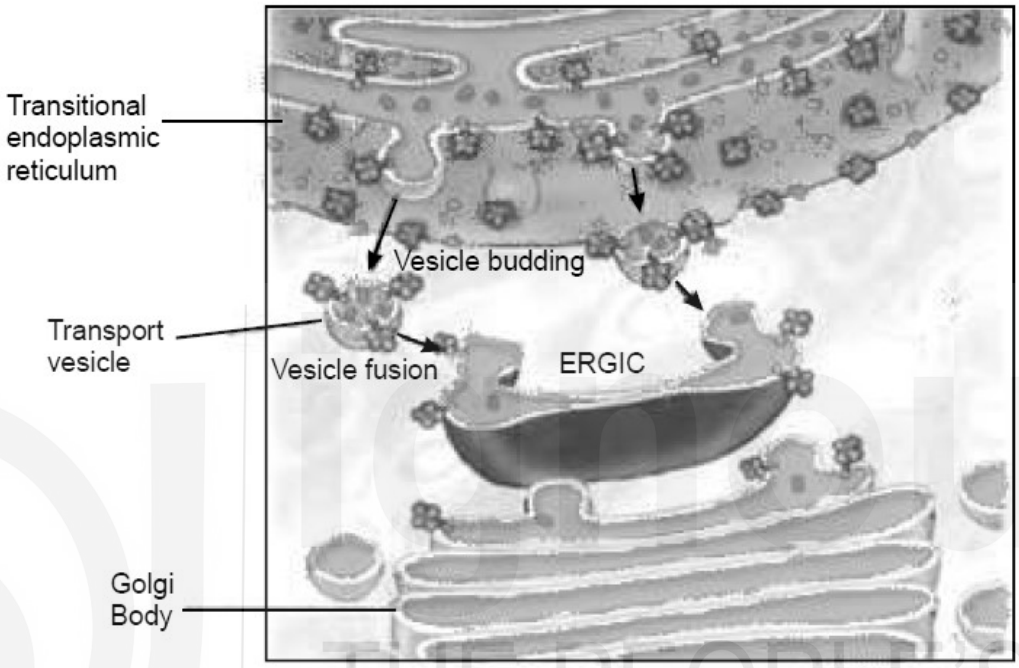


Fig. 9.3: Vesicle formation and fusion during vesicular transport. (Courtesy: Nelson & Cox, Lehninger Principles of Biochemistry)

Randy Schekman

James Edward Rothman

The transport via vesicles is responsible both for traffic between membrane bound compartments and selectivity of transported substances. Thus there is a need to exercise selectivity during packaging in the donor compartment and fusion with the appropriate acceptor membrane so as to maintain the desired functional compartmentalization.

Our current understanding of vesicular transport has been gained by a combination of experimental approaches. They include classical genetic tools of isolating and characterising mutants in yeast that are defective in either transport or sorting; reconstitution of a cell free system of vesicular transport and studies on synaptic vesicles that store neurotransmitters and release their contents in response to signals. It was **Randy Schekman** and his group who pioneered the isolation of yeast mutants defective in vesicular transport and **James Rothman** and his colleagues developed the first cell free system for analyzing protein transport between Golgi compartments (vesicular transport), including the process of membrane fusion. They showed that vesicle formation requires cytosol and a source of energy in addition to the membrane. Their contributions received the final honors when they were named along Thomas Südhof for the 2013 Nobel Prize in Physiology or medicine.

Now let us attempt to answer a very obvious yet important question, how do cargo proteins move between the Golgi cisternae and yet maintain their integrity?

9.4.1 Movement of cargo proteins in Golgi apparatus

Two opposing models emerged over time to account for the movement of materials between Golgi compartments. One of them is the **cisternal maturation model** and the other is **vesicular transport model** (Fig. 9.4). The former model is based on the idea that Golgi cisternae are transient structures and each cisterna physically moves from the *cis* to the *trans* end of the stack as it matures. The process of maturation is dependent on the fusion of membrane bound carriers that move in a retrograde direction to carry medial and *trans* enzymes. This model was prevalent up to the mid-1980s. The alternate model considers that Golgi stacks are stable compartments and functional compartmentalization within the stacks is accomplished by an anterograde vesicular transport involving fusion of vesicles derived from a donor membrane with that of the acceptor membrane. This model gained support from the in vitro demonstration by Rothman's group of vesicles budding from one Golgi cisterna and fusing with the other.

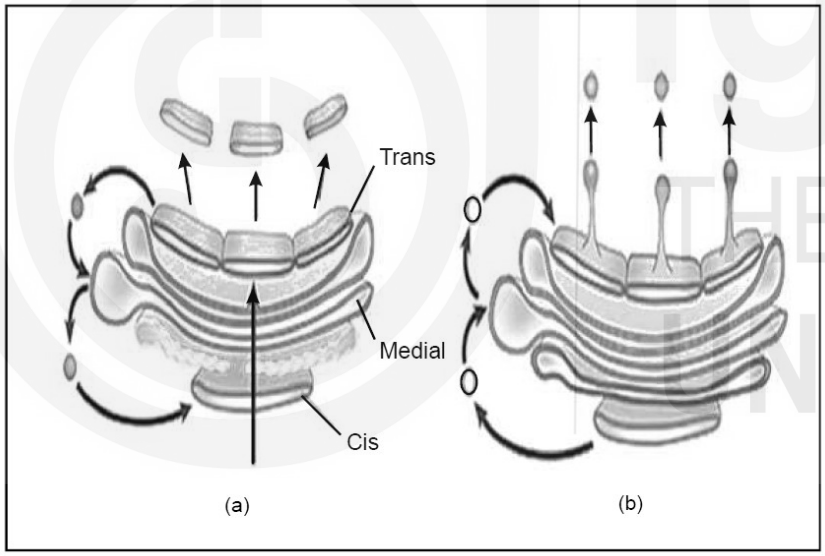


Fig. 9.4: (a) The cisternae maturation model (b) The vesicular transport model.

However, as often happens in science (and in fashion), old ideas sometimes come back in new ways. It is now known that vesicular transport takes place in both directions and features of both models may provide the best answer.

9.4.2 Vesicular Transport mechanism: Vesicle formation

Vesicular transport is the key mechanism for exchange of proteins and lipids between membrane bound subcellular organelles in eukaryotic cells. The ER-to-Golgi trafficking is the first step in the vesicular trafficking network of the endomembrane system. **The first step** in vesicular transport is the formation of a coated vesicle by budding from donor membrane surface. In the process

of vesicle formation, they concentrate the cargo and acquire a coat of proteins whose composition is dependent on the type of coat. The protein coat in general performs two functions: (i) They select the contents of the vesicle that includes the cargo and the machinery for targeting and docking to an acceptor membrane and (ii) They can distort the selected membrane surface to initiate budding. Coated vesicles are involved in vesicular traffic throughout the endomembrane system, as well as in exocytosis and endocytosis. It is worth noting that a protein coat is formed from soluble proteins on the cytosolic face of the donor membrane guided by G-proteins.

All kinds of vesicles are pinched off from the donor membrane following vesicle closure. The involvement of GTPases like dynamin has been implicated in this event. It forms a ring around the neck of the bud and GTP hydrolysis constricts the ring pinching off the neck to release the vesicle. Finally, the vesicles are transported with the help of cytoskeletal proteins such as actin filaments to the docking site. Many distinct classes of vesicles have been identified but in this course you will study only three best characterised coated vesicles, namely:

- **Coat protein (COPI) coated vesicles**
- **Coat protein (COP II) coated vesicles**
- **Clathrin-coated vesicles**

(i) COPI coated vesicles

COPI coated vesicles is a coatomer, a protein complex (Fig.9.5) that coats vesicles meant to transport proteins from the *cis* end of the Golgi complex back to the RER. This type of transport is termed **retrograde transport**. The name "COPI" refers to the specific coat protein complex that initiates the budding process on the *cis*-Golgi membrane. The coat consists of large protein sub complexes that are made of seven different protein subunits, namely α, β, β', γ, δ, ε and ζ. The seven proteins are assembled as a complex before attaching to the membrane. The assembly of vesicle coat also requires a GTP binding protein called ARF (ADP-ribosylation factor). The budding of COPI vesicles can be inhibited by brefeldin A as it inhibits the ARF-GTP exchange activity associated with the Golgi membranes.

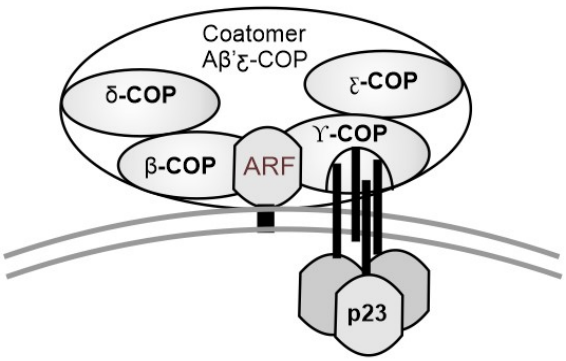


Fig. 9.5: Structure of COP I protein complex.

The components of the COPI coat have been shown to interact with the cytosolic tail of an integral membrane **KDEL receptor** that in turn is bound to soluble ER resident proteins having a C-terminal KDEL retrieval tag. The KDEL receptors have high affinity for KDEL in tubular clusters and Golgi (low pH) whereas they have low affinity for KDEL in endoplasmic reticulum (neutral pH). Even integral membrane proteins of the ER have a C-terminal retrieval tag that binds to COPI coat and it can be retrieved back from the cis Golgi.

(ii) COP II coated vesicles

COPII is a coatomer, a type of vesicle coat protein that transports proteins from rough endoplasmic reticulum to the Golgi sub compartments. This type of transport is termed **anterograde transport**. The coat consists of large protein sub complexes that are made of four different protein subunits (Fig.9.6). The Sec 23 / Sec24 heterodimer forms an inner coat and capture the proteins to be carried whereas the Sec13 / sec31 heterodimer forms the outer coat. The GTP binding protein in this case is Sar1that regulates the assembly and disassembly of the protein coat. The Sec23 subunit is a sar1 specific GTPase activating protein (GAP). In addition, an integral membrane glycoprotein Sec12 that remains in the ER guides the budding machinery; it is a GTP exchange protein.

The G-protein, Sar1 is recruited to the ER membrane in the GDP bound form and assembles the COPII proteins in a particular order to form a vesicle. This event is coupled to a GDP-GTP exchange that helps in stabilizing the coat. The coat proteins assist in selecting integral membrane proteins by recognizing the signal sequences on their cytosolic tails.

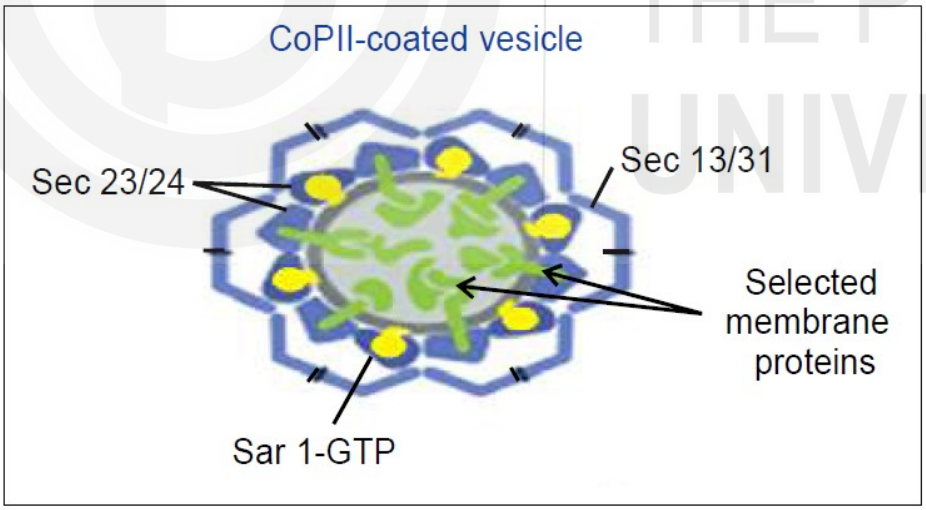


Fig.9.6: Structure of COP II protein complexes.

(iii) Clathrin coated vesicles

Clathrin coat was the first type of coating that was discovered on transport vesicles by **Barbara M.F. Pearse** in 1976.These vesicles are assembled from two types of protein complexes, an outer complex made up of clathrin and an inner shell of adaptor proteins (AP) that assemble on the cytosolic side of the membrane. The former has a structural role in the formation of coated vesicles by distorting the membrane while the latter links two different components.


Barbara M.F. Pearse

The outer surface of the adaptor binds clathrin to the membrane and the inner surface interacts with the cytosolic tails of membrane proteins that bear the sorting signals. The G protein, [ARF –GTP] is also required to assist in the choice of bud site and facilitates the binding of AP complex to the membrane.

The clathrin coat has a thick cage like layer composed primarily of the fibrous protein, clathrin. It assumes a triskelion shape, made up of three heavy chains (180Kd) and three light chains (35Kd). When the triskelia interact; they form a polyhedral lattice surrounding the vesicle (Fig.9.7). This is how the coat gets its name; the Latin word *clathratus* meaning like a lattice.

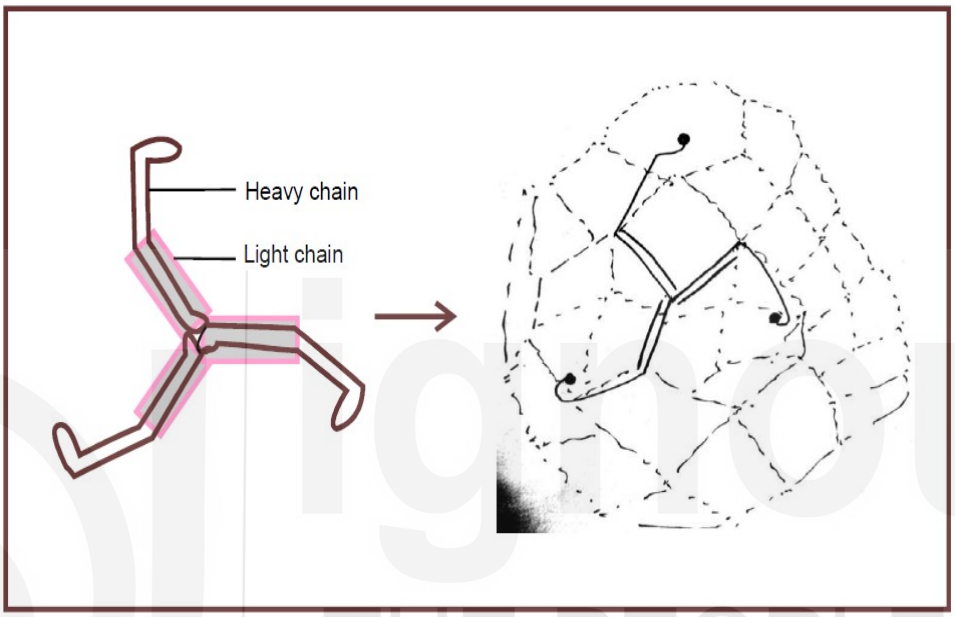


Fig. 9.7: Structure of Clathrin protein along with the resultant lattice structure.

The clathrin coated vesicles are responsible for the regulated uptake of extracellular molecules by endocytosis and transport from the TGN to the lysosomes or vacuoles. In some situations like in case lysosomal soluble proteins, the integral membrane protein (mannose-6-phosphate receptor) in TGN binds to soluble lysosomal enzymes by recognizing mannose -6-phosphate residues on one hand and to the coat proteins via the adaptor on the other hand. This results in the concentration of proteins into clathrin coated vesicles.

There are different adaptor protein complexes known for assembly at plasma membrane and lysosomes. This is logical as they have a role in selecting their cargo. The adaptor protein (AP) complex is numbered as AP-1, AP-2 and so on. The individual proteins of the AP complex are called adaptins. It is the β- or β'-subunits of AP complex that binds clathrin.

By now you must have understood that a cell handles the mammoth task of transporting proteins to their correct destination by recognizing sorting signals that help in directing proteins to specific transport Vesicles. The Fig. 9.8 gives the steps in the formation of a clathrin coated vesicles and it's uncoating prior to fusion. All kinds of vesicles follow a similar theme.

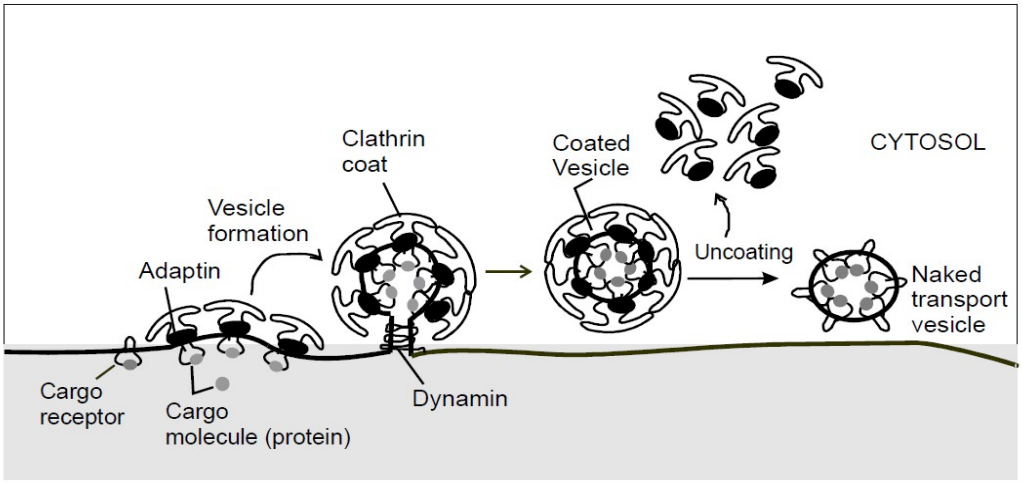


Fig. 9.8: The formation of a coated vesicle & its uncoating before fusion.
(*Source*: Essential Cell Biology, Garland Science)

Before proceeding to the next stage, the information on the three types of coated vesicles, their specific role and the sorting signals that guide vesicles in concentrating the cargo is summarised in tables 9.2 and 9.3.

Table 9.2: Types of coated vesicles in protein trafficking

Vesicle type	Transport step Mediated	Coat Proteins	Associated GTPase
COPII	ER to *cis*-Golgi	Sec23/Sec24 & Sec 13/Sec31 complexes	Sar1
COPI	*cis*-Golgi to ER	Coatomer containing seven different COP subunits	ARF
Clathrin and adapter proteins	*trans*-Golgi to endosome	Clathrin + AP1 complexes	ARF
	Plasma membrane to endosome	Clathrin + AP2 complexes	ARF
	Golgi to lysosome, melanosome, or platelet vesicles	AP3 complexes	ARF

Table 9.3 Sorting Signals that Direct Proteins to Specific Transport Vesicles

Signal Sequence*	Proteins with signal	Signal Receptor	Type of transport vesicles
Luminal sorting Signals			
Lys-Asp-Glu-Leu (KDEL)	ER-resident soluble proteins	KDEL receptor	COPI
Cytoplasmic Sorting Cells			
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins	COPI α and β subunits	COPI
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins	COPII Sec 24 subunit	COPII
Asn-Pro-X-Tyr	LDL receptor	AP2 complex	Clathrin/AP2
Tyr-X-X-ϕ(YXX ϕ)	Membrane proteins	AP1 and AP2	Clathrin/AP1 and AP2
Leu-Leu (LL)	Plasma membrane proteins	AP2 complexes	Clathrin/AP2

9.4.3 Vesicular fusion

This section discusses how a vesicle fuses with an acceptor membrane. Prior to fusion the coat must be shed because it impedes fusion. **Fusion is a property of membrane surfaces**. The disassembly is simply a reversal of the coat assembly mechanism. GTP hydrolysis is essential to initiate the release of coat proteins. The uncoated vesicle has proteins that direct it to the target membrane and bring in proteins that initiate fusion. You would wonder why in the first place the vesicles were coated. According to the vesicle budding-fusion couple hypothesis of **Elazer *etal*** (1994) the fusogenic proteins in the vesicle must be concealed by the coat proteins to prevent premature fusion among Golgi cisternae. This is an additional role of coat proteins to those mentioned in the preceding section.

The proteins involved in vesicular fusion were initially identified by J.E. Rothman's lab. His group succeeded in purifying N-ethyl maleimide (NEM) sensitive fusion (NSF) protein that led to the purification of soluble NSF attachment protein (SNAP) and receptors for SNAPs (SNARE) on membranes. These receptors are present on both vesicle and target membranes. In addition, the Rab family of G proteins has been recognised as important players in targeting and fusion of vesicles. The analysis of vesicular transport in reconstituted systems led Rothman to propose the SNARE hypothesis that explains how do coated vesicles go to the right place and fuse with the target membrane. All types of transport vesicles contain surface molecular markers that assist in identifying the vesicle according to its origin and cargo. They must also be recognized by complementary receptors on the target membrane. This recognition is thought to involve a family of proteins – SNARES and Ras associated binding (Rab) proteins. Each organelle is believed to carry a unique SNARE. These SNAREs seem to perform two major functions; one is to promote fusion itself and the other is to help ensure the specificity of membrane fusion.

Rab proteins have emerged as a critical component of membrane trafficking. They are members of the Ras superfamily of monomeric G proteins and have a prenyl tail attached at their C terminus through which they can insert in the membrane. Approximately 70 different Rab proteins have been reported so far in humans. These proteins have about 200 amino acids and a similar overall structure. Rab protein plays a major role in the specificity of the vesicular transport. The vesicles are directed by them to their specific targets after which SNARE proteins helps in the fusion of the target membrane. The initial contact between the Rab protein on vesicle and the target membrane is mediated by tethering fibrous proteins.

Fig. 9.9 depicts schematically the process of vesicle docking and membrane fusion. **The v-SNAREs** (**vesicle-SNAP receptors**) are found on vesicles and the complementary t**-SNAREs** (**target-SNAP receptors**) are found on target membranes that facilitate specific recognition between vesicles and their targets. The docking occurs when v- and t-SNAREs bind. However, docking does not ensure fusion. Fusion requires a much closer approach, probably catalyzed by specialized proteins. Following membrane fusion NSF/ SNAP complex is required to complete the process. They are not directly involved in

targeting or fusion but have a role in disassembling the SNARE complex that can be reutilized.

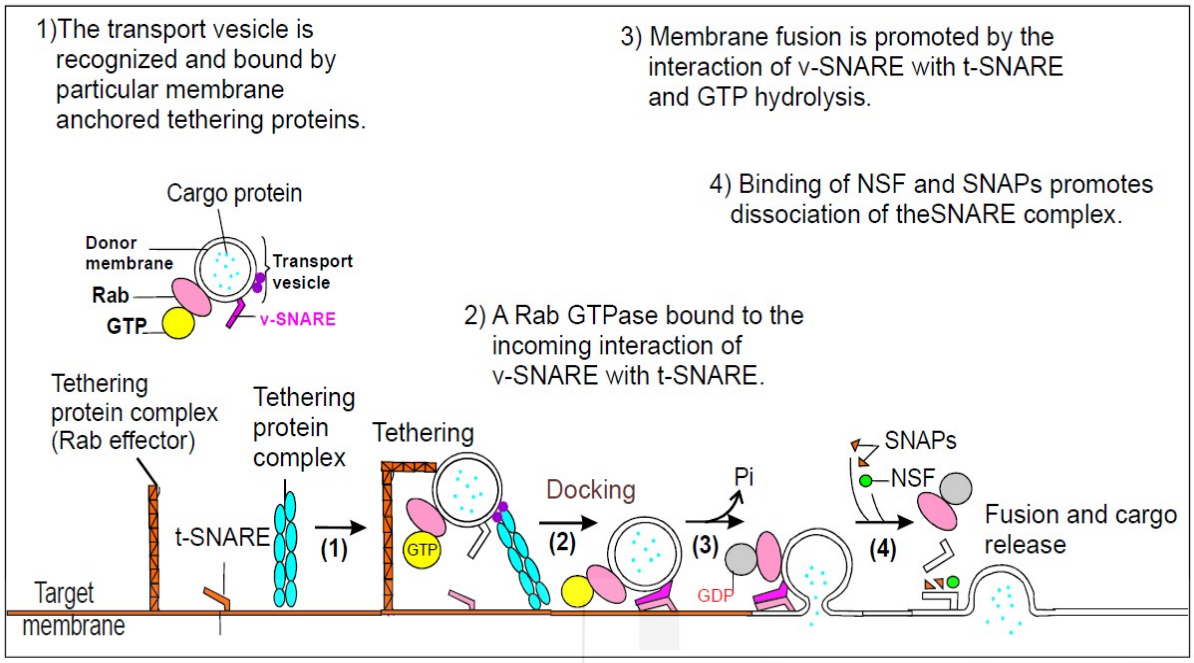


Fig. 9.9: Vesicle docking and membrane fusion mechanism.

SAQ 3

a). Explain how transport vesicles are formed? Why are they coated?

b). Match the items in Column I with that in Column II

Column I	Column II
1. Coatomer I	a. Participate both in endocytosis & transport from TGN.
2. Coatomer II	b. Retrieval tag for ER resident proteins
3. Clathrin	c. Vesicle for Retrograde transport of proteins.
4. C terminal signal sequence Lys-Lys-X-X	d. transports proteins from the RER to the Golgi apparatus

c). How do transport vesicles find the right acceptor membrane?

9.5 RECEPTOR MEDIATED SELECTIVE TRANSPORT

There are some proteins that enter the cell from the surrounding medium by receptor mediated endocytosis. A classical example of this in eukaryotes is the uptake of low-density lipoprotein (LDL). The cell surface has coated pits that contain the endocytic receptors such as LDL receptors in preference to other

cell-surface proteins. These pits are coated with clathrin on the cytosolic side where they form a lattice like structure.

Let us discuss how Cholesterol esters enter the cell by receptor mediated endocytosis (Fig.9.10). This mechanism was proposed by **Michael Brown and Joseph Goldstein** for which they were awarded the 1985 Nobel Prize in Physiology or Medicine. Their work provided the molecular basis of familial hypercholesterolemia.

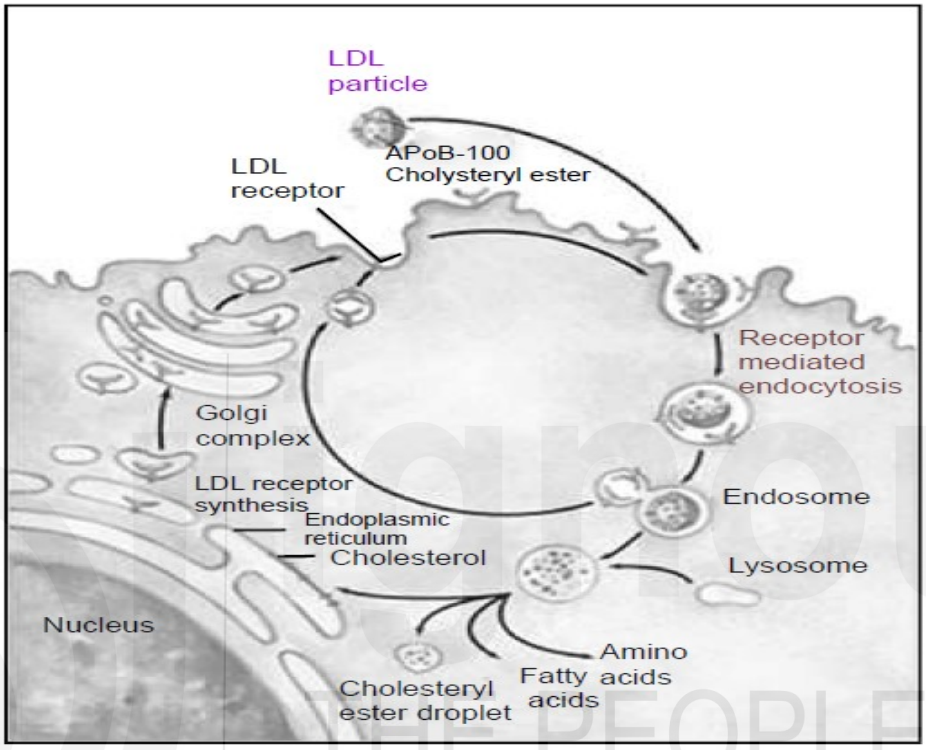


Fig.9.10: Uptake of cholesterol by receptor-mediated endocytosis (Credit: Nelson & Cox, Principles of Biochemistry).

A LDL particle is a carrier of cholesterol esters in the blood and it has a protein apoB-100 on its surface that serves as a ligand for **LDL receptors**. The binding of LDL receptors to LDL initiates endocytosis and the complex is internalized in a clathrin coated pit that pinches off as a coated endocytic vesicle. The coat is then shed to form an early endosome. The endosome fuses with a sorting vesicle called CURL or late endosome where low pH leads to dissociation of the receptor ligand complex and from now on both will have independent fates. The receptor is packaged into separate vesicles and recycled back to the plasma membrane. The vesicle carrying the LDL particle fuses with the lysosomes where cholesterol esters are hydrolysed to fatty acids and cholesterol; Apo B-100 of LDL is also broken down into amino acids that are released into the cytosol. The released cholesterol may be incorporated into the membrane or used to synthesise hormones or even stored.

Many viruses and toxins exploit the host system of receptor mediated endocytosis to gain entry. One of the well understood viral system is the influenza virus whose envelope harbors a protein hemagglutinin (HA) that binds to cell surface sialic acid residues of the target cell leading to virus internalization.

SAQ 4

(a) Why receptor mediated endocytosis is also known as Clathrin-mediated endocytosis? Give an example of receptor mediated endocytosis.

(b) Give another example where receptor mediated endocytosis is exploited by viruses and some toxins.

9.6 SUMMARY

In this unit, you have learnt that:

- The proteins synthesized on RER are not released at any point into the cytosol. They have to be modified, sorted and transported to their final destination. This is achieved by carrying them only in membrane enclosed **coated vesicles** until they are off loaded to their site of action.
- The glycoproteins that reach the cis Golgi by vesicular transport from the ER are further modified step by step by specific enzymes. Broadly these changes can be grouped into terminal glycosylation of the N-linked oligosaccharide and de novo modifications like O-glycosylation, phosphorylation, sulphation.
- There are three major destinations for the secretory proteins, namely plasma membrane, lysosomes and extracellular secretion / secretary vesicles.
- The anterograde movement of proteins intended to be targeted through the secretory pathway is: **Rough ER → ER-to-Golgi transport vesicles → Golgi body (cisternae) → TGN (secretory or transport vesicles) → cell surface → exocytosis**
- The cells depend on coated vesicles both for import and export of proteins. A vesicle is formed by budding and pinching off from a donor membrane that in turn fuses with an acceptor membrane. A typical eukaryotic cell uses two pathways for export and only one for import of proteins. A protein may be constitutively secreted / incorporated into the plasma membrane or its release may be regulated.
- The most studied coated vesicles are COPI, COPII and clathrin. The type of coat proteins found on a vesicle surface determines the destination of the vesicle. The COPI coated vesicle is involved in retrograde transport (cis Golgi to ER); COPII transports in the forward (anterograde) direction and the clathrin coated vesicles participate in the regulated uptake of extracellular molecules by endocytosis and transport from the TGN to the lysosomes or vacuoles. Each kind of vesicle requires G proteins for vesicle assembly and closure.
- In vesicular transport pathway, each vesicle contains additional proteins to ensure protein delivery to their correct destination. These

proteins are SNARES and Rab. The SNAREs seem to perform two major functions; one to promote fusion itself and the other is to ensure the specificity of membrane fusion. Rab is a subfamily of Ras GTPases with unique members on each organelle and play important roles in tethering, fusion micro domain assembly, and trans-SNARE complex formation.

- According to SNARE hypothesis sorting and targeting of vesicles involves two families of SNARE (SNAP receptor) proteins: v**-SNAREs** (vesicle-SNAP receptors) are found on vesicles and **t-SNAREs** (target-SNAP receptors) are present on target membranes which facilitate specific recognition between vesicles and their target when they dock. In addition to complementary SNAREs, membrane fusion also requires GTP hydrolysis by Rab GTPases.
- For an efficient membrane fusion, vesicle and target membrane have to be in close proximity to release the cargo. As the process is completed, the NSF protein stimulates the disassembly of SNARE complex and Rab.
- The receptor mediated selective transport is required for the uptake of specific proteins into the cell. This mechanism is discussed with reference to the uptake of cholesterol carrying LDL particles.

9.7 TERMINAL QUESTIONS

1. How is a lysosomal protein targeted to lysosome?
2. Why Golgi complex is called a “processing unit” of the cell?
3. What is the role of membrane enclosed coated vesicles?
4. What are three major destinations for the secretory proteins?
5. Explain the two models proposed for the movement of cargo proteins in the Golgi. Which one is more accurate?
6. What are the three major types of coated vesicles?
7. Explain how cholesterol is made available to cells from circulation.
8. How does glycosylation in the Golgi complex differ from that in the RER?
9. Enumerate the role of G proteins in vesicular transport and membrane fusion.

9.8 ANSWERS

Self-Assessment Questions

1. (i) *Cis* Golgi; ER (ii) Mannose-6-phosphate receptor; TGN

(iii) Serine or threonine (iv) ER (v) CMP-sialic acid

2. (a). (i)

(b). (i) cis face; trans face. (ii) TGN

(iii) Resident proteins (iv) Coated vesicles

(c). (i) The proteins are modified by terminal N-glycosylation, O-glycosylation, sulphation and phosphorylation depending on the protein under consideration.

(ii) They may remain as resident proteins or may be retrieved back to the ER.

(iii) They may be sorted in the TGN to vesicles targeted to the lysosomes, cell surface, and storage vesicles or secreted.

3. a) A vesicle is formed by budding and then pinching off from a donor membrane. All well characterised vesicles are surrounded by a protein coat.

The coat performs multiple roles: (i) It helps in the selection of cargo (ii) It distorts the selected membrane surface to initiate budding and (iii) the coat prevents premature fusion of vesicles.

b). 1—c; 2—d; 3---a; 4---b

c). All types of transport vesicles contain surface molecular markers that assist to identify the vesicle according to its origin and cargo. They must also be recognized by complementary receptors on the target membrane. This recognition is thought to involve a family of SNARE proteins and Ras associated binding (Rab) proteins. Each organelle is believed to carry a unique SNARE. These SNAREs seem to perform two major functions; one is to promote fusion itself and the other is to ensure the specificity of membrane fusion.

4. a) The endocytic receptors like the LDL receptors are concentrated in coated pits present on cell surface. These pits are coated with clathrin on the cytosolic side where they form a lattice like structure. Hence it is also called clathrin mediated endocytosis (CME).

(b) Some of the viruses that use CME to gain entry include the enveloped single strand RNA viruses (Semliki forest virus and Vesicular stomatitis virus) and non-enveloped DNA viruses (Adenovirus). // There are many other examples also.

Terminal Questions

1. The process of targeting an enzyme to the lysosome depends on its acquisition of a lysosomal targeting signal. This happens in the cis Golgi where specific mannose residues are phosphorylated to mannose -6-phosphate. These proteins are then sorted in the TGN where the phosphorylated mannose residue is recognised by a membrane receptor, Man-6-P receptor and it facilitates the sorting of such proteins in TGN to vesicles that carry them to the lysosomes.

2. The Golgi complex is considered as a **'processing unit'** because the proteins entering the Golgi stacks are processed to varying extent en route to their site of action. Some of the modifications are in the form of terminal changes in sugars; O-glycosylation, phosphorylation and sulphation. In both plants and animals complex glycosylation also takes in the Golgi.

3. The membrane enclosed coated vesicles carry specific cargoes to the correct destination. The coat prevents premature fusion of the vesicle.

4. The three major destinations for the secretory proteins are: (a) plasma membrane, (b) lysosomes and (c) extracellular secretion.

5. Two opposing models emerged over time to account for the movements of materials through the Golgi. They are the cisternal maturation model and the vesicular transport model. The cisternal maturation model is based on the idea that Golgi cisternae are transient structures and each cisterna physically moves from the cis to the trans end of the stack as it matures. The vesicular transport model considers that Golgi stacks are stable compartments and functional compartmentalization within the stacks is accomplished by an anterograde vesicular transport followed by fusion of vesicles derived from a donor membrane with that of the acceptor membrane. It is now known that vesicular transport takes place in both directions and features of both models may provide the best answer.

6. The three types of well studied coated vesicles are coat protein (COPI) coated vesicles, coat protein (COP II) coated vesicles and clathrin-coated vesicles. Each of them transports specific cargos (Refer subsection 9.4.2. for details).

7. Cholesterol esters are carried in circulation by LDL particles. The cells that express LDL receptors internalize it by receptor mediated endocytosis in clathrin coated pits that pinches off as a coated endocytic vesicle. The coat is then shed to form an early endosome. This is followed by the fusion of the early endosome with the lysosomes where the low pH results in the dissociation of the receptor ligand complex and the hydrolysis of cholesterol esters. The released cholesterol is now available to the cells.

8. In the ER, N-glycosylation is initiated co-translationally / an oligosaccharide made up of 14 sugars is added en bloc and later trimmed to some extent / the oligosaccharide is linked to a lipid carrier (dolichol phosphate) ; in the Golgi, sugars are added one by one from activated donor (NDP or NMP) / terminal sugars are added to the N-linked oligosaccharide/ O-glycosylation is initiated / Some sugars are further modified by phosphorylation and sulphation / All glycosylation occur post translationally.

9. G proteins are required for assembly of vesicle coat /vesicle closure/ vesicle targeting and membrane fusion.

9.9 SUGGESTED READINGS

1. Lehninger, Principles of Biochemistry, David L. Nelson and Michael M. Cox
2. Molecular Cell Biology, 4th edition, Harvey Lodish, Arnold Berk, S Lawrence Zipursky, Paul Matsudaira, David Baltimore, and James Darnell. 2000
3. Molecular Biology of the Cell: The Problems Book, by John Wilson and Tim Hunt, Garlend Science, 2002
4. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

UNIT 10

PROTEIN TRAFFICKING III

Structure

10.1 INTRODUCTION

You have studied in the preceding two units of this block about signal directed co-translational protein translocation and processing in ER. Then you learnt about trimming reactions; protein folding and quality control. This was followed by the role of Golgi in protein modification and sorting. Finally you were introduced to the types of vesicles and their role in vesicular traffic.

Now in this unit, you will study about post translational protein trafficking into the nucleus, mitochondria, chloroplast and peroxisome. The proteins required for these subcellular organelles and their membranes are synthesised completely on free cytosolic ribosomes. Each organelle uses a specific translocation machinery for protein and / RNA import. In some cases, the protein has to traverse more than one membrane while in others the movement is bidirectional. In each case they have the signals and the machinery to do the needful.

For better understanding of this unit, it is advised to revise the Unit 5 Structure and function of subcellular organelles (Block 2).

Expected Learning Outcomes

After studying this unit, you should be able to:

- indicate the targeting signals for nuclear protein import and export;
- explain nuclear import and export cycle;
- highlight the role of Ran in bidirectional movement;
- indicate the characteristics of signals for mitochondrial targeting and to its sub-compartments;
- describe mitochondrial protein import;
- describe protein translocation pathway to the stroma and sub compartments of chloroplast; and
- elaborate the Peroxisomal targeting sequence (PTS) and routes of protein import to peroxisomal matrix and its membrane.

10.2 BIDIRECTIONAL NUCLEAR PROTEIN TRANSPORT

As you know, eukaryotes have a well defined nucleus demarcated by a double membrane that harbors the genetic material (DNA). This compartmentalization offers opportunities to control gene expression. The flow of biomolecules between the nucleus and cytosol is **bidirectiona**l and energy dependent. The nucleocytoplasmic transport is through a large number of nuclear pore complexes (NPC). A variety of proteins, enzymes and ribonucleoprotein (RNP) complexes are continuously moving between the two compartments. These include DNA and RNA polymerases; histones, topoisomerases, different species of RNA and snRNPs. Inherent in the bidirectional movement of substances across nucleus membrane is the need to have a way to provide directionality to the transport process.

In unit 5 you have learnt that the NPC (Fig.10.1) is a macromolecular complex that forms an aqueous channel of approximately 9nm in diameter facilitating

the diffusion of metabolites up to 50kD. The movement of larger macromolecules (up to 26-28nm in diameter) is an active process and is characterised by signal dependence and saturability (as it is carrier mediated).

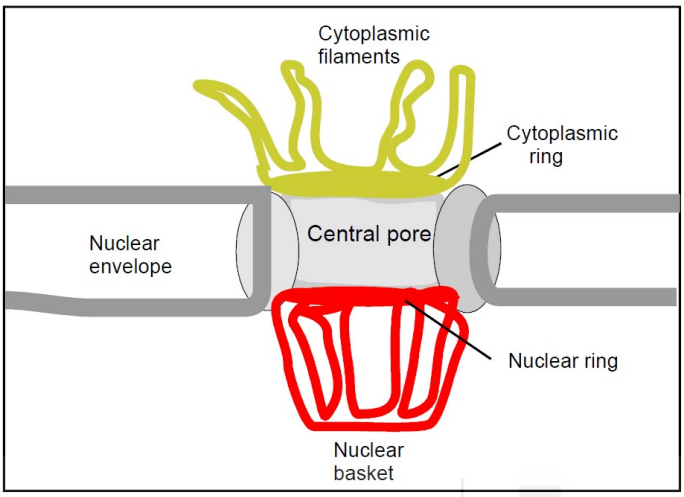


Fig. 10.1: Structure of Nuclear pore complex (NPC).

Before understand how proteins / RNP get into or out of the nucleus, we will first discuss the signals that guide the translocation machinery and the key translocation factors.

10.2.1 Nuclear import and export signals

The first characterised signal shown to be necessary and sufficient to direct nuclear import is the 'classical' nuclear localisation signal (NLS) of simian virus (SV40) large T antigen and the bipartite signal in nucleoplasmin (Fig.10.2) by **Alan Smith** and colleagues in 1984. Since then, many other nuclear signals have been described.

The best characterized NLS sequences consist of a small sequence of basic amino acids generally ranging from four to eight amino acids. There may be one or more such clusters of basic amino acids. Unlike the N-terminal signal sequence in ER, the NLS can be located almost anywhere along the primary sequence of the protein. The signal sequence is retained in the mature protein following import.

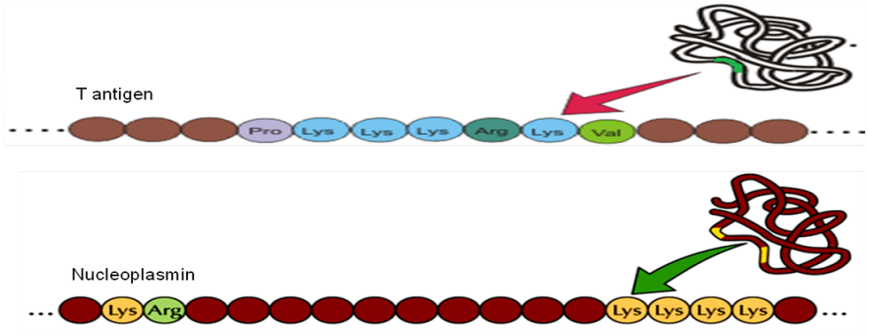


Fig. 10.2: Mono- and bi-partite NLS of SV40 T antigen & nucleoplasmin, respectively. (Source: M. Cooper, The cell: A Molecular Approach)

The nuclear imports are complemented by an equally large number of export processes for proteins and RNA / RNP. The first such signal for an active export was identified in HIV Rev protein and cAMP dependent protein kinase inhibitor (**PKI**). It is characterised by a short stretch of **hydrophobic amino acids** rich in leucine residues:

10.2.2 Translocation factors

Nuclear transport is facilitated by specific soluble receptor proteins that are collectively referred to as **"karyopherins**". Both import and export surprisingly is mediated by members of the β-importin superfamily called β-karyopherins. Depending on the direction of cargo transport they are generally called **importins and exportins.** The individual family members recognise distinct classes of transport proteins and share the ability to bind a subset of nuclear pore proteins thereby providing specificity. According to the National Center for Biotechnology Information (NCBI) database, there are at least 18 importin and 6 exportin genes in humans and almost a similar number in mice. Some of the α-importins are differentially expressed that may account for tissue specific import.

The **Importin** family members recognize the nuclear localization signal (NLS) sequences and transport protein cargo into the nucleus. They are heterodimeric complexes; α- subunit binds NLS sequence with high affinity in the cytoplasm and the β- subunit interacts with components of the nuclear pore and Ran (Fig. 10.3).

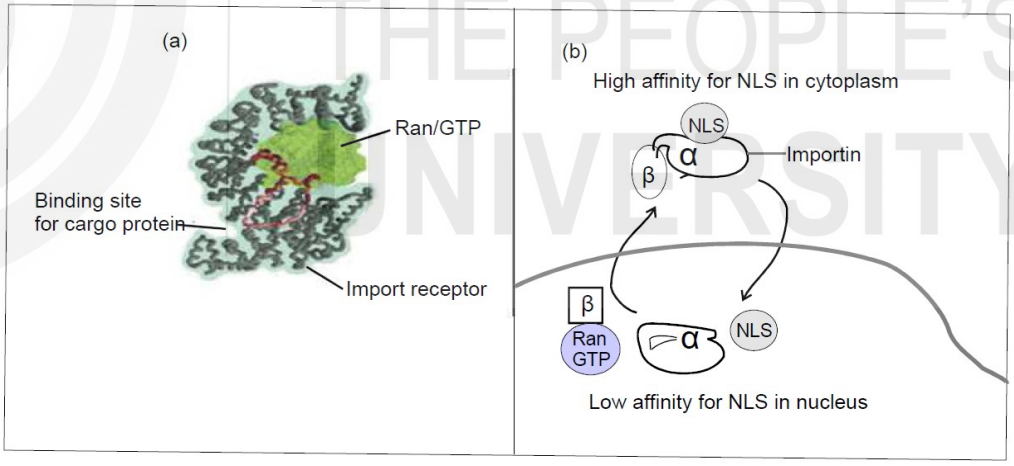


Fig.10.3: (a) Structure of importin receptor (b) A complex of NLS-α-importin and Ran-GTP-β subunit.

(Source: Albert et.al, 2002. Molecular Biology of the Cell)

Many nucleoporins that make up the NPC have a number of short amino acid repeats containing Phe and Gly (FG repeats) that serve as binding site for the import receptor and line the central channel of the pore. It is worth noting that the affinity of the receptor for NLS and Ran-GTP changes between the nucleus and cytoplasm.

The **exportins** work like importins, but mediate transport in the reverse direction. They are also heterodimers of alpha and beta subunits. An exportin recognizes nuclear export signal (NES) on nuclear proteins. They are associated with Ran-GTP during nuclear export.

Nuclear export signal (NES) of PKI -$^{+}H_3N$-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Leu-..

NES of HIV Rev Protein: …..$^{+}H_3N$-Leu-Pro-Pro-Leu-Glu-Arg-Leu-Thr-Leu-….

Ran (ras related nuclear protein) is a monomeric guanine-nucleotide binding and hydrolyzing protein. It is predominantly a nuclear protein (80-90%) and it can switch between GTP- and GDP-bound states. It is a key player in conferring directionality to nuclear transport by controlling the binding and release of cargo. This is accomplished by a compartmentalised distribution of proteins that regulate the two states of the protein. The regulators are Ran guanine-nucleotide exchange factors (GEFs) in the nucleus and GTPase-activating proteins (GAPs) in the cytoplasm. Therefore, nuclear Ran will be predominantly bound to GTP and the cytosolic version will be in the GDP bound form.

The Ran-GDP/GTP cycle is depicted in Fig. 10.4. In the cytoplasm, Ran-GAP initiates hydrolysis of Ran-GTP to Ran-GDP. It then binds to its own transport receptor to get into the nucleus where Ran is stimulated by GEF (Guanine nucleotide exchange factor) to exchange GTP for GDP and reform Ran-GTP. Basically, how the nucleotide state of Ran is used to regulate cargo binding and release will be explained in the next section. Finally, as GTP (guanosine triphosphate) is an energy rich molecule it has an additional role of providing energy during nuclear transport.

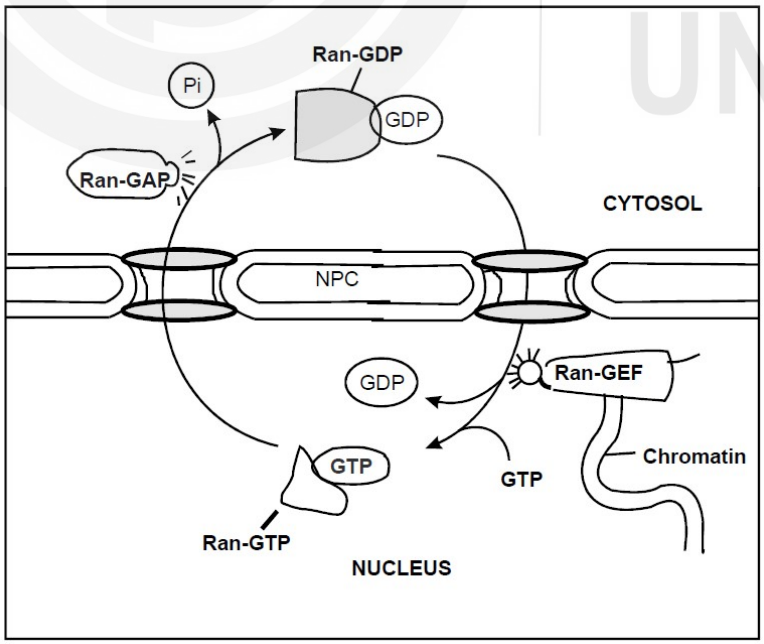


Fig. 10.4 Ran GTP/GDP cycle

The above mentioned components of the translocation machinery work together in a coordinated manner to bring about bidirectional movement across the nuclear pore complex. We will now highlight the steps of nuclear import and export cycle.

10.2.3 Nuclear import and export cycle

The import /export cycle is summarised in Fig.10.5. Consider the import of a hypothetical nuclear protein (cargo) from the cytosol. The protein is translocated into the nucleus in a folded state. The cytoplasmic import receptor, an **importin α/β complex** recognizes and binds the NLS sequence of the cargo through the repeat domain of importin α and form loop or patch on the protein surface. The importin α also has an importin β binding domain (IBB) at its N- terminus. It therefore functions as an adaptor linking the cargo with importin β via its IBB domain.

Next the **NLS- importin α/β trimeric complex** docks on the cytoplasmic side of the NPC via β-importin. In the presence of Ran-GDP and other protein factors the complex is translocated through the NPC channel in an energy dependent manner. Once inside the nucleoplasm, a GDP/ GTP exchange by GEF converts Ran into a GTP bound form. This results in a change in its conformation and interactions with the import receptor. The importin subunits dissociate followed by the release of the cargo bound to importin α by release factors that include a region on IBB, a nucleoporin (Nup50 in vertebrates) and the export receptor for importin α.

Finally, the importin subunits are returned back separately to the cytoplasm for another round of nuclear import. The export receptor for importin α is CAS / Ran-GTP. As the importin-Ran/GTP reaches at cytoplasmic side of NPC, the Ran interact with **GTPase-activating protein** (Ran-GAP) which stimulates the hydrolysis of GTP to GDP and triggers its release into the cytoplasm.

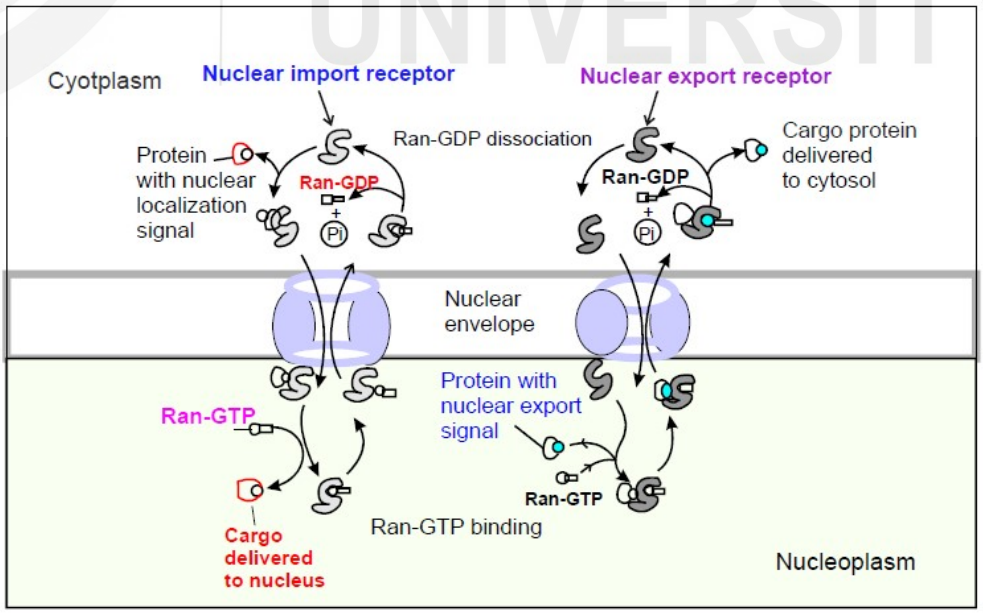


Fig. 10.5: Nuclear protein import/export cycle.

The bidirectional transport across the NPC is mediated by equally effective nuclear export pathway. The basic theme is the same for both import and export of proteins and RNA / RNP. The **exportin receptors** bind **NES sequence** on the cargo. The process is also dependent on Ran with a difference. During export Ran/ GTP promotes the binding of cargo to the exportin in the nucleus, followed by binding and translocation through the NPC. As the complex enters the cytoplasmic side of NPC, cargo protein is dissociated from the exportin receptor as Ran-GTP is converted to Ran-GDP in the cytoplasm. Exportins are then returned back to the nucleus through the nuclear pore complex and recycled.

We end the discussion on nuclear transport by taking representative examples of protein, RNA and RNP transport.

The HIV-1-Rev protein is a mediator of unspliced HIV-1RNA export. It is an adaptor that can bind to unspliced RNA at multiple sites and has an activation domain (a NES sequence), to link it to the export machinery.

Some proteins are known to possess both NLS and NES sequences and so they can easily move in both directions. They are dubbed as 'shuttling proteins'. One well studied example of a shuttling protein is the **hnRNP A1** that has a 38 amino acid long motif, the **M9 domain** responsible for its behaviour. It has a role in mRNA export and the protein shuttles between the nucleus and cytosol to participate in multiple rounds of export. Incidentally most RNA species are exported as RNPs. A rare example of direct nuclear export of t-RNA has been shown to be mediated by exportin-t.

A complex series of movements take place during **ribosome biogenesis** in the nucleolus. The mRNA coding for ribosomal proteins are synthesised and processed in the nucleus. They are exported as rRNP for translation in the cytosol and the proteins are imported to the nucleus for ribosome biogenesis in the nucleolus where they associate with the rRNA. Finally, the ribosomes (RNP) are exported to the cytosol to translate cellular proteins.

SAQ 1

(a) Fill in the blanks:

(i) Nuclear localization signal (NLS) delivers proteins to the…….

(ii) The importin family members recognise…….sequence while the exportins recognise…… sequences on the cargo.

(iii) Ran is predominantly in…… form in the nucleus and in ……. bound form in the cytoplasm due to the …….. distribution of its ……..

(iv) All nucleocytoplasmic transport is through ……

(v) The source of energy for nuclear transport is ……..

(vi) The Rev protein of HIV binds ---------- and has a ……. sequence.

(b) What is the role of Ran protein in nuclear transport?

10.3 PROTEIN TRANSPORT INTO THE MITOCHONDRIA

You have studied in Block 2, Unit 5 that mitochondria are involved in a plethora of biochemical activities in a cell. Apart from the generation of adenosine triphosphate (ATP), mitochondria is a metabolic hub that interconnects various anabolic and catabolic processes such as oxidative phosphorylation, tricarboxylic acid (TCA) cycle, β-oxidation of fatty acids and the synthesis of some amino acids; heme and ketone bodies. To fulfill these multiple roles, mitochondria require about 1000 different type of proteins that are largely encoded by nuclear DNA (~90%) and translated on free cytosolic ribosomes. The completely synthesised proteins are then imported and trafficked to appropriate subcompartments within the mitochondria (Fig.10.6). Now you can appreciate the importance of protein import in mitochondrial biogenesis. The proteins destined to the mitochondria are synthesised as precursors that are about **12 -70 amino acids** longer than the mature proteins. Proteins imported into the mitochondria have to be partially unfolded. Cytosolic molecular chaperones such as Hsp70 or Hsp90 assist to keep the proteins in a partially unfolded state. The Hsp70 also provides the driving force for protein translocation across the mitochondrial membrane. Once the protein reaches the mitochondria it is refolded to its native conformation by another set of chaperones

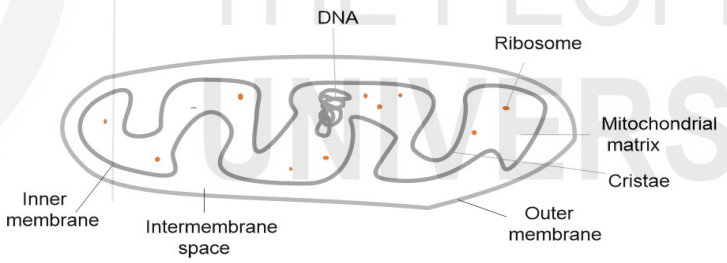


Fig.10.6: A schematic view of a mitochondrion.

10.3.1 N-terminal mitochondrial matrix targeting presequences

Most mitochondrial proteins contain a cleavable signal sequences at their N-terminus. It is also called **N-terminal matrix targeting sequence or presequence.** The signal sequence is necessary for protein import into mitochondrion. The presequences consist of 20-35 amino acids that are usually hydrophilic with uncharged amino acids, interrupted by basic amino acids. They can fold to form an **amphipathic α-helix** with the positively charged residues clustered on one face of the helix and the non polar residues on the opposite side. They lack acidic amino acids (Fig. 10.7). These were first

characterised by the scientist, **Gottfried Schatz.** The default fate of a protein imported into mitochondria is the matrix where the pre sequence is cleaved by a protease. Those that are destined to other compartments have a **bipartite signal** that functions in a hierarchical manner; the first part targets it to the organelle and the second part to a sub compartment other than the matrix. The initial transfer event is the same for all proteins, irrespective of their final location. Once the matrix targeting signal is cleaved, the protein may either be retained there in the absence of other signals or it may travel further depending on the nature of the second signal exposed.

Gottfried Schatz

+H_3N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-

Fig.10.7: A typical mitochondrial N-terminal presequence.

10.3.2 Mitochondrial protein import machinery

The transport of proteins is mediated by membrane spanning protein channel (commonly known as translocon) that are driven by electrochemical gradient across the mitochondrial inner membrane (Fig 10.8). There are three major multiprotein membrane translocons in the mitochondria: TOM, TIM and OXA.

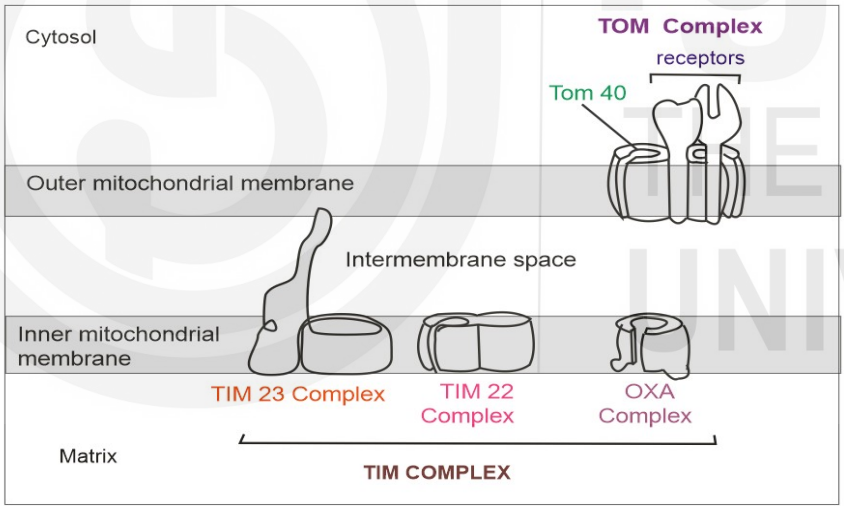


Fig. 10.8: Mitochondrial protein import complexes.

TOM complex is present in the outer mitochondrial membrane (OMM). This complex includes Tom40 (protein lined pore) and receptor proteins (**Tom20, Tom22** and **Tom5)** which recognize and bind presequences on mitochondrial proteins.

TIM complex is an inner mitochondrial membrane (IMM) protein complex that contains two major protein channels, Tim23 and Tim22 complex. Tim23 protein channel has a Tim44 receptor protein which is used to translocate the mitochondrial protein into the matrix while Tim22 protein complex is used to import mitochondrial membrane protein to the IMM.

OXA complex is also located in the inner mitochondrial membrane that mediates the insertion of IMM and intermembrane space (IMS) proteins. These proteins are synthesised either in the mitochondrial matrix or cytosol. Those that are synthesised by cytosolic ribosomes generally have both a presequence and a second sorting signal while others have only one signal. It is primarily involved in the translocation of transmembrane proteins to the inner membrane.

Let us discuss one by one in the following subsections how proteins are imported to the different compartments of the mitochondria beginning with mitochondrial matrix.

10.3.3 Protein import to the mitochondrial matrix

Fig. 10.9 depicts the steps in protein import to the matrix. Even many proteins destined to the outer membrane initially begin by translocating through the Tom40 complex and then their further transfer is stopped and they pass laterally in the membrane.

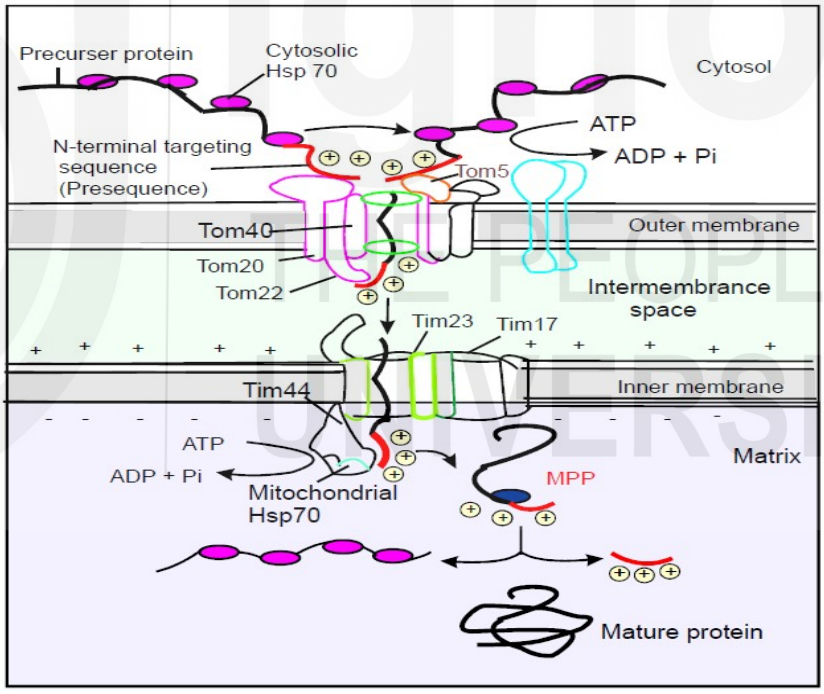


Fig. 10.9: Protein import into the mitochondrial matrix.

The newly synthesized precursor proteins destined to the mitochondria are bound to cytosolic chaperone, Hsp 70 that helps to keep them in an unfolded state and prevent their aggregation. The import of a protein is initiated when the N-terminal matrix targeting sequence (red color) on the precursor protein is recognized by a receptor protein on the outer mitochondrial membrane. These receptors are part of the protein complex required for translocation across the outer membrane **(Tom complex).** The presequence first binds Tom20 and then it is transferred to Tom 5 before it reaches the import channel (Tom 40).

Once the presequence emerges through the Tom40 into the intermembrane space, it binds to a domain on Tom22 before it is transferred to **Tim23 complex** in the inner mitochondrial membrane (IMM) for translocation to the matrix. Both translocons are in close proximity and this arrangement allows unidirectional protein translocation to the mitochondrial matrix. The movement of a protein across the IMM is powered by electrochemical potential maintained by electron transport chain. As the presequence enters the matrix, it interacts with the mitochondrial Hsp70-Tim44protein receptor that assists in protein translocation into the mitochondrial matrix at the expense of ATP hydrolysis. Finally, the proteins acquire its final functional form by cleavage of presequence from most proteins by mitochondrial matrix processing .peptidase (MPP) and molecular chaperones.

10.3.4 Protein import into the inner mitochondrial membrane (IMM)

The mitochondrial proteins destined to the inner membrane, outer membrane and inter membrane space are translocated by multiple ways. Some of them have in addition to matrix targeting presequence an internal signal that is exposed once the matrix peptidase cleaves the presequence. The second signal reroute its export to other compartments depending on the protein under consideration. Others have stop transfer signal sequences that functions both to prevent transfer of the protein into the matrix and anchors it as an integral membrane protein. The second signal for membrane proteins not only ensure their sorting but also the orientation of the protein (Fig. 10.12).

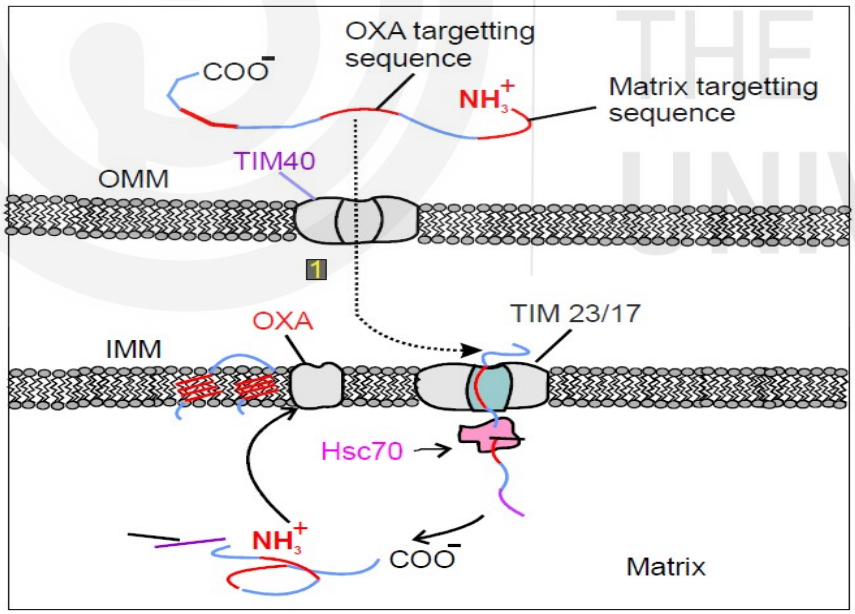


Fig. 10.12: Protein import into the Inner mitochondrial membrane (IMM).
(Source: Credit Lodish: Molecular Cell Biology)

The inner membrane proteins encoded by the nuclear genome have either a cleavable N-terminal MTS or sometimes an internal non-cleavable MTS. A matrix targeting sequence is absent from IMM proteins encoded by the mitochondrial genome and synthesised in the matrix itself. The proteins

synthesised in the cytosol and those synthesised in the mitochondrial matrix are translocated either to the IMM or IMS by a protein translocator called **OXA**. The OXA protein complex is encoded by the mitochondrial genome and synthesized in the mitochondrial matrix. It is speculated that this translocator is also found in the plasma membranes of bacteria and thylakoid of chloroplasts, where it helps to insert membrane proteins by a similar mechanism.

10.3.5 Protein import into the mitochondrial inter membrane space (IMS)

Protein targeting to the intermembrane space requires an IMS-targeting signal sequence (a hydrophobic signal segment) which is strategically placed after the N- terminal MTS. Both signal sequences are cleaved after translocation of protein into IMS. The initial steps are therefore similar to the import of a typical matrix protein and it is translocated to the matrix through Tom and Tim complexes. Once the MTS is cleaved in the matrix, the intermembrane space targeting sequence is exposed. As the protein changes its course the second signal sequence is also cleaved by a protease in the IMM resulting in its release into the IMS (Fig.10.13). The association with the inner membrane is therefore transient.

Some proteins take an alternate route. They do not travel to the matrix. They are translocated through the Tom40 channel and remain in the IMS by not interacting with the Tim23 complex.

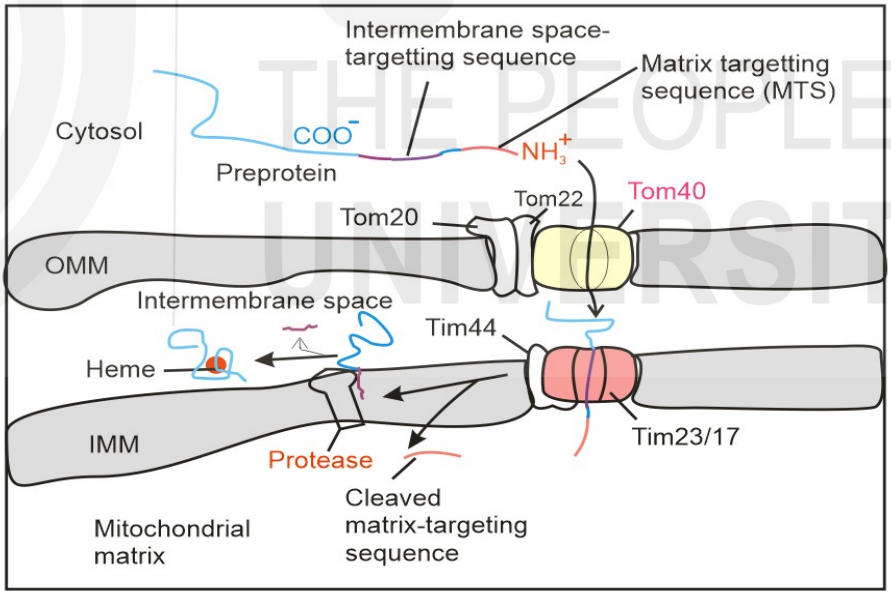


Fig. 10.13: Protein import into intermembrane space.

SAQ 2

State whether the following statements are true or false. If false, point out the error:

(i) Mitochondrial proteins are translocated in a partially unfolded state.

(ii) Hsp70 is a chaperone that helps to maintain proteins in an unfolded form.

(iii) Translocator for mitochondrial matrix proteins is the Tim complex.

(iv) The Tom complex is located in the IMM.

(v) The N-terminal presequence is a signal for mitochondrial protein targeting.

(vi) The presequence of proteins is cleaved during transit through the OMM.

(vii) All IMM proteins are synthesised in the cytosol.

(viii) OXA protein channel is only required for the transport of proteins synthesised in the mitochondrial matrix.

(ix) All matrix targeting sequences are transient sequences.

10.4 PROTEIN TRANSPORT INTO THE CHLOROPLAST

Recall from unit 5 the structure and functions of chloroplast. In this section you will learn how protein trafficking and targeting to specific parts of the chloroplast takes place. About 90% proteins in chloroplast are encoded by nuclear genes .A chloroplast has at least six subcompartments that include the outer and inner membrane, intermembrane space, stroma, thylakoid membrane and lumen into which proteins are specifically targeted post translationally (Fig. 10.14). Due to the complex organisation of the chloroplast, some proteins have to be translocated through three distinct membrane systems.

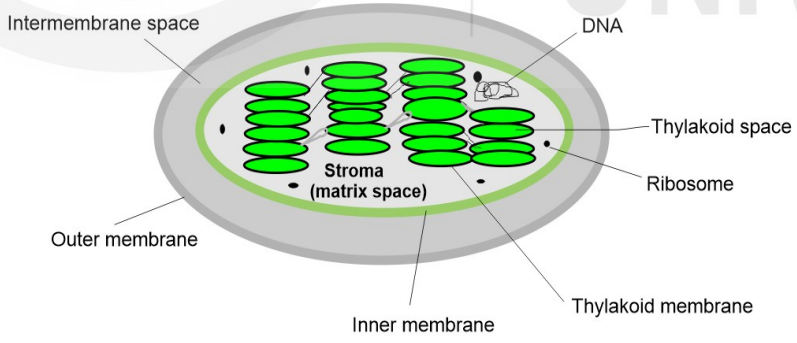


Fig. 10.14: Schematic view of chloroplast.

10.4.1 Chloroplast N-terminal sequence and translocation factors

Most proteins destined to the chloroplast have an **N-terminal cleavable sequence** that functions as a chloroplast targeting peptide (CTP) sequence (Fig.10.15). A number of CTPs have been identified and they are highly

divergent in length, composition and organisation. Inspite of the apparent variation, CTPs do share some common features. They have a high proportion of hydroxylated amino acids, lack acidic amino acids and can fold to form α-helical structure.

$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-

Fig. 10.15: **Structure of a Chloroplast Targeting Peptide (CTP) sequence.**

The chloroplast and mitochondrial protein import pathways exhibit similarly although the translocation machineries of both organelles have evolved independently. The CTP guide precursor proteins to the chloroplast stroma. The import to the stroma is dependent on five translocation factors. They are two protein translocator complexes (TOC and TIC) in the outer and inner membrane; chaperone, Hsp70; stromal processing peptidase (SPP) and a source of energy in the form of ATP and GTP.

10.4.2 Protein import to chloroplast stroma

The proteins destined to the chloroplast are bound by cytosolic chaperone, Hsp70 after the completion of translation. This is a prerequisite step as proteins can be imported only in an unfolded state. The signal sequence is recognised and bound by a soluble guidance complex that in turn interacts with the translocator on outer chloroplast membrane (TOC complex). The energy for crossing the outer membrane is derived from the hydrolysis of ATP and GTP (Fig.10.16).

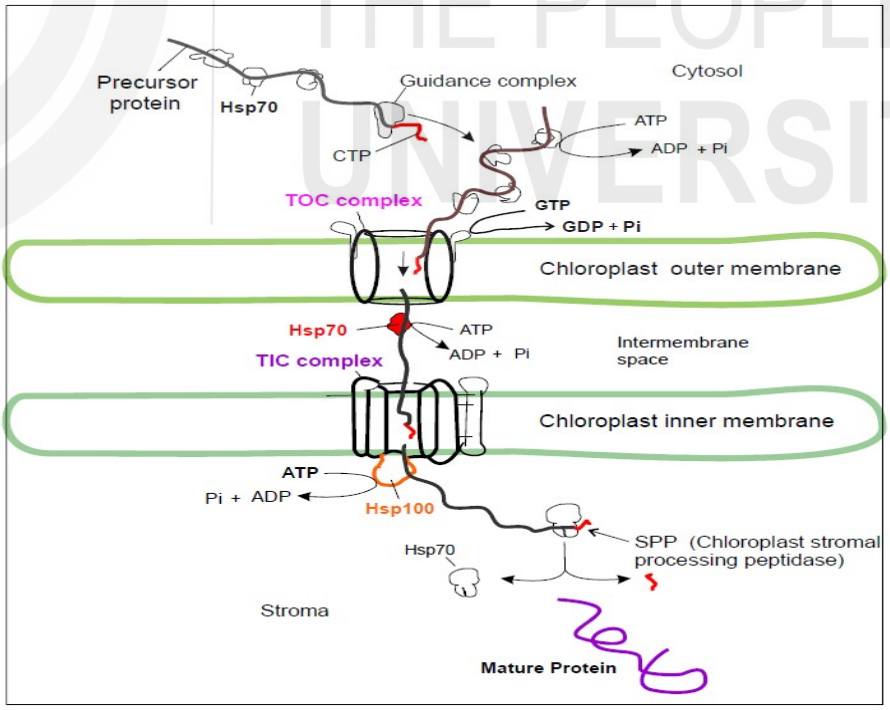


Fig. 10.16: Protein import into chloroplast stroma.

Once the protein reaches the intermembrane space it makes its way through the TIC complex in the inner membrane, a step that requires ATP hydrolysis by Hsp70. As the protein crosses the inner membrane it is bound by a stromal chaperone Hsp100 that hydrolyses ATP to generate the driving force for pulling the protein into the stroma. Finally, the transit peptide is cleaved by chloroplast stroma processing peptidase (SPP) and the protein acquires it native conformation assisted by stromal chaperone, Hsp70.

10.4.3 Protein transport into thylakoid space or membranes of chloroplast

The newly synthesized precursor proteins that are destined either to the thylakoid or inner and outer membranes of chloroplast have additional signal sequences. They have a second hydrophobic internal signal sequence following the N-terminal transit sequence. The pathways show similarity to those used to import mitochondrial proteins into various sub compartments (Fig.10.17).

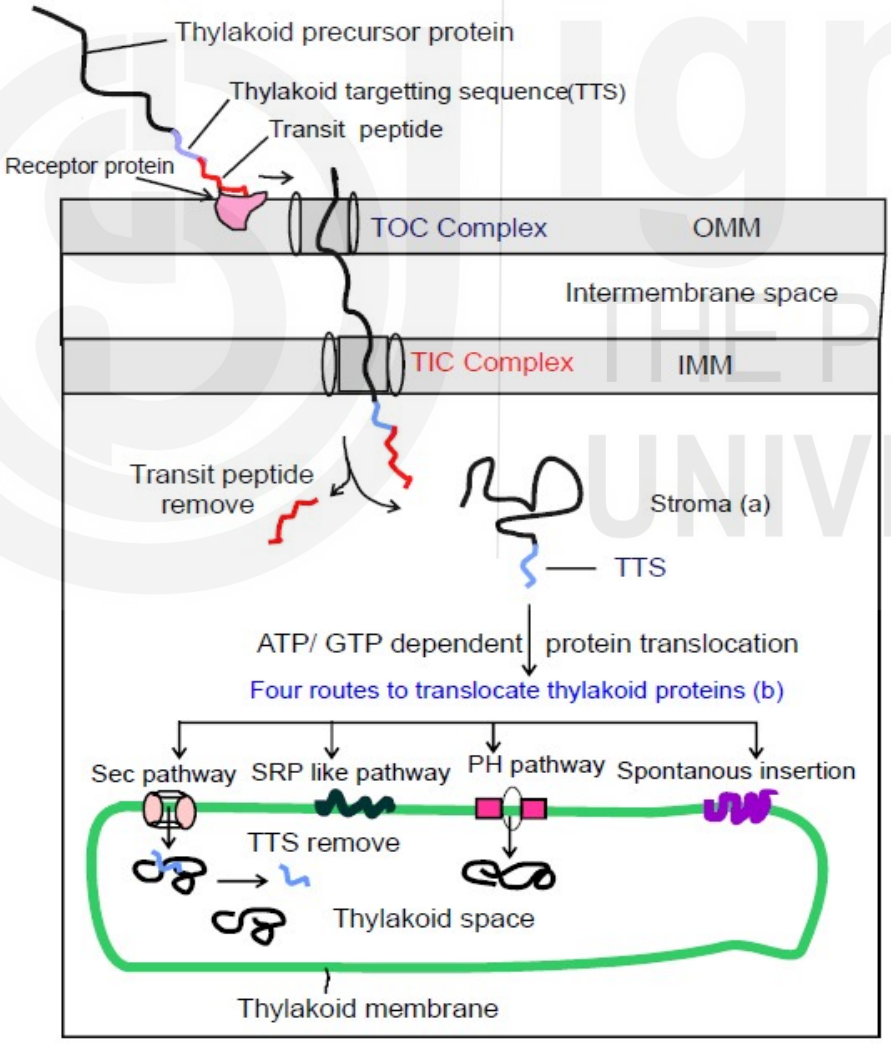


Fig. 10.17: Protein insertion into thylakoid space and membrane.

Protein targeting to thylakoid space is directed by two signals, (i) Transit peptide and (ii) thylakoid targeting signal. Both signal peptides are removed by two different signal processing peptidases once their role is completed. These two peptidases are present in the stroma and thylakoid lumen and function sequentially to remove respective signal sequences. The removal of the transit peptide in the stroma unmasks the thylakoid signal sequence. Both signal sequences guide and direct step by step the translocation of proteins across the chloroplast into the thylakoid space or to thylakoid membrane.

The thylakoid signal sequence initiates transport across the thylakoid membrane. There are at least four routes for proteins to cross or become integrated into the thylakoid membrane (Fig.10.17b). They are:

(i) **Sec-dependent**: This pathway uses proteins that are homologous to the bacterial Sec proteins for translocating proteins across the bacterial plasma membrane. It imports proteins into the thylakoid space (lumen) in an ATP dependent manner.

(ii) **SRP (Signal Recognition Particle) like pathway:** The chloroplast homologue of SRP binds to the protein signal sequence to import proteins into thylakoid membrane. This process requires ATP.

(iii) **ΔpH pathway**: It is dependent on a proton gradient across the thylakoid membrane to transport protein into thylakoid space.

(iv) **Spontaneous insertion**: Some proteins with a signal sequence specific for thylakoid membrane can insert spontaneously. It does not depend on a protein translocator for membrane integration.

SAQ 3

Indicate the role of following:

(i) Stroma targeting sequences……

(ii) TOC……..

(iii) SPP……..

(iv) Hsp70 ……..

(v) Hsp100…….

10.5 PROTEIN TRAFFICKING TO PEROXISOMES

Recall the structure and functions of peroxisomes from Unit 5 Block 2 of this course. Peroxisomes are roughly spherical in shape with a crystalline core and surrounded by a single membrane (Fig.10.18). The core is enriched in a wide variety of enzymes that catalyze oxidative reactions in the cell. They were named ‘peroxisomes’ because of the presence of enzymes that produce and destroy hydrogen peroxide.

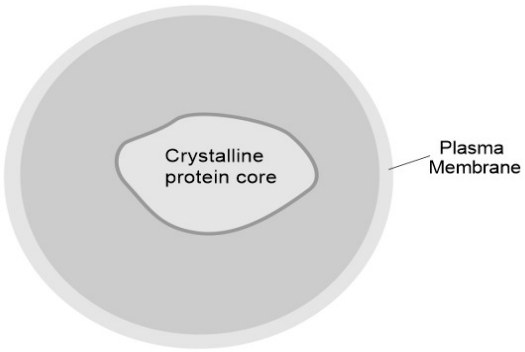


Fig. 10.18: Structure of peroxisome.

Another characteristic of peroxisomes is heterogeneity in the set of enzymes they possess that makes them competent to undertake specialised roles such as critical steps in ether phospholipids synthesis in animals or glyoxylate cycle in germinating seedlings of some plants.

All peroxisomal proteins are encoded by the nuclear genome and synthesized on cytosolic free ribosomes. They are imported post translationally in their native conformation. The proteins not only fold but can also oligomerise and acquire cofactors prior to import. This is in sharp contrast to import to other organelles like mitochondria and chloroplast. As expected the proteins have signals that guide their import either to the matrix or peroxisomal membrane. They also acquire a subset of proteins (& lipids) from the ER.

10.5.1 Peroxisomal Targeting Signals (PTSs)

Peroxisomal targeting sequence was initially identified in the firefly luciferase enzyme and it is both necessary and sufficient to target proteins to the peroxisomes. Most peroxisomal proteins targeted to the matrix have C-terminal peroxisomal targeting signal (PTS) on the protein while few of them such as thiolase have a PTS at their N-terminus. They are generally referred to as PTS1 and PTS2, respectively. PTS1 is a tripeptide sequence of Ser-Lys-Leu (SKL) motif or a related sequence while a nine residue consensus sequence functions as PTS2.The C terminal PTS1is not cleaved following protein import but PTS2 is a cleavable presequence.

The proteins required for peroxisome biogenesis are called **peroxins (Pex).** They have been conserved through evolution**.** A number of peroxins are needed for the import of matrix and membrane proteins. The PTS1 and PTS2 on matrix proteins are recognized in the cytoplasm by specific receptor proteins, Pex5 and Pex7, respectively and the complex is transferred to the translocation complex on peroxisomes. The receptors shuttle between the cytosol and peroxisomes.

10.5.2 Protein import into peroxisomal matrix

Let us consider the import of catalase into peroxisome matrix. It is a heme containing tetrameric enzyme that is imported without unfolding or subunit dissociation (Fig. 10.19). It requires ATP both for protein import and recycling of receptors. The import pathway can be broadly divided into four steps:

- **Receptor binding**: The signal sequence (PTS1) on the protein is recognised by a C-terminal tetratricopeptide repeat domain of Pex5 receptor (step a).
- **Docking**: The binary complex of **Pex5 receptor** - peroxisomal protein docks onto the docking complex composed of **Pex 13, Pex 14 and Pex 17 proteins** (step b).
- **Translocation**: The receptor inserts in the membrane complex and becomes a part of the import pore along with the docking complex proteins. The receptor and Pex 14 have been shown to constitute the minimum translocon. In the matrix the cargo is released from the receptor (Step c). The actual mechanism of cargo release is still unclear.
- **Recycling**: Finally, the Pex5 receptor is returned back to the cytoplasm to participate in another round of import. The recycling of receptor through the peroxisomal membrane requires ubiquitination by Pex4 (step d) and release from the membrane through Pex1 and Pex6 translocon channels (step e). Finally the receptor undergoes deubiquitination before it is ready for reuse (step f).

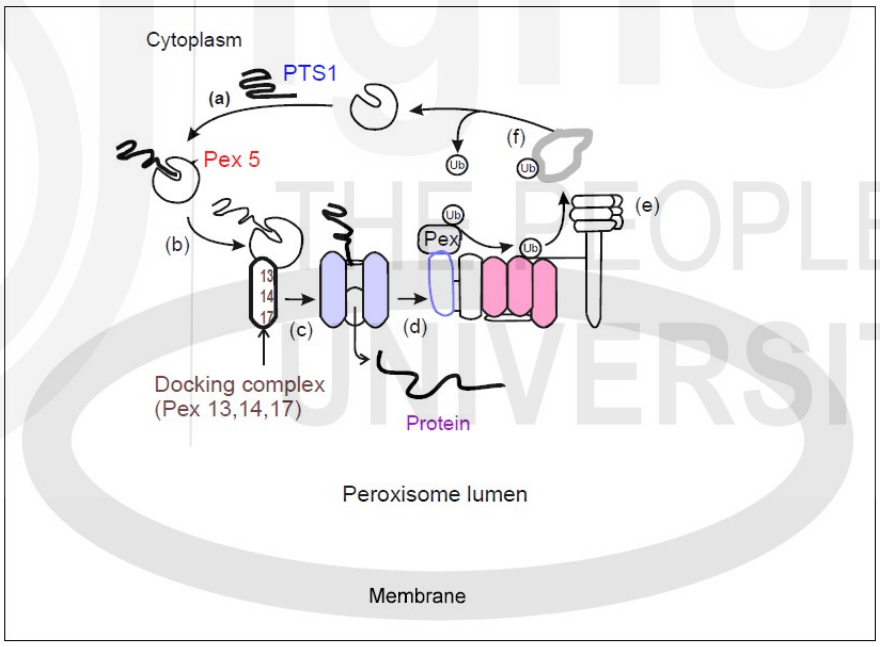


Fig. 10.19: Protein import into peroxisomal matrix in Saccharomyces cerevisiae.

10.5.3 Protein import to peroxisomal membrane

Peroxisomes are unique organelles that can import membrane proteins synthesised in the cytosol. They use a different set of peroxins than the one utilised for matrix proteins. The proteins that are destined to the membrane bear membrane peroxisome targeting signals (mPTS). The mPTS sequences consist of either a cluster of positively charged amino acids or a combination of both positively charged and hydrophobic residues that are flanked by one or

more transmembrane segments. There is evidence in support of at least two routes for membrane import; one is a direct route (class I PMP) and the other is indirect via ER (Class II PMP). Fig. 10.20 gives an overview of the two pathways of peroxisomal membrane protein (PMP) import.

The mechanism of direct import is dependent on two peroxins, namely Pex 19 and Pex3. The Pex19p is a recycling cytosolic receptor / chaperone protein which binds mPTS on PMPs. Some proteins have multiple mPTS sequences and they bind multiple Pex 19 proteins. The complex then docks on a resident peroxisomal membrane protein, Pex3 that is responsible for mediating direct membrane insertion of PMPs and Pex16 may be required for direct insertion of Pex3.

In contrast, class II PMPs is either first transported to or synthesised in the endoplasmic reticulum and then targeted to the peroxisomal membrane. The indirect transport of Pex3 has been investigated in both yeast and humans. There is more than one speculated way by which ER to peroxisome membrane transfer may take place.

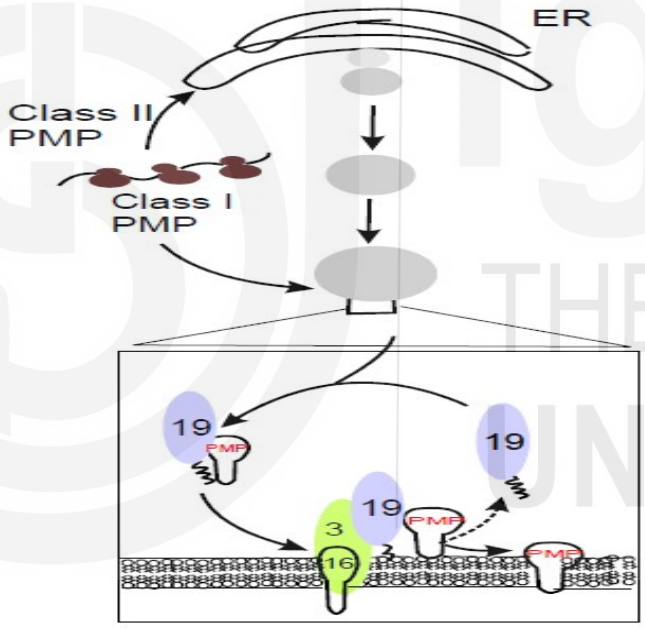


Fig. 10.20: Peroxisome membrane protein import.

SAQ 4

Fill in the blanks:

(i) The C-terminal SKL tripeptide is a ……matrix targeting sequence.

(ii) A protein with a PTS1 sequence is recognised by ……in the cytoplasm.

(iii) The proteins required for peroxisome biogenesis are called ……..

(iv) The proteins are translocated across the peroxisome in their ……state.

(v) The recycling of Pex5 receptor requires ……..by ……...prior to its release from peroxisome into the cytoplasm.

10.6 SUMMARY

In this unit, you have learnt about post translational protein trafficking to the nucleus, mitochondria, chloroplast and peroxisomes.

- Proteins synthesised by cytosolic free ribosomes are either targeted post translationally to different organelles or they continue to remain and function in the cytosol. The proteins may be imported to the nucleus, mitochondria, chloroplast, peroxisomes and few even to the ER. Their final destination is dependent on specific signal sequences present on the protein. Signal sequences are necessary and sufficient for protein sorting and trafficking to their right destination, where they perform specific role(s).
- Signal sequences include NLS, presequence, transit peptide and SKL that targets proteins to the nucleus, mitochondria, chloroplast and peroxisome, respectively.
- Most signal sequences are located at N-terminus of the protein which is cleaved following translocation while most peroxisomal matrix proteins have a non cleavable C-terminus signal sequence. Another set of proteins with a non cleavable sequence have internal signal sequences.Each organelle has dedicated machinery for recognition of signal sequences, translocation and removal of the signal sequence, if required. The process is energy dependent.
- Protein transport to the nucleus is **bidirectional.** The receptors that recognise NLS and NES sequences are called importin and exportin, respectively. All transport is through the nuclear pore complex (NPC). A monomeric G protein, Ran confers directionality to the process due to the asymmetric distribution of its regulators. Ran is predominantly in the GTP form in the nucleus and GDP bound form in the cytosol.
- Most mitochondrial proteins have a cleavable MTS signal sequence at their N-termini. The precursor proteins are imported to the mitochondria in an unfolded form and this state is maintained with the help of cytosolic chaperones. The default location of the imported protein is the matrix. Those that are destined to other compartments have a bipartite signal that functions in a hierarchical manner. The membranes have translocons to allow the entry of the protein.
- The protein import to the sub compartments of chloroplast or its membrane system occurs through TIC and TOC complexes. The transit sequence directs the protein to the chloroplast lumen. The

presence of additional signals dictates the final destination of the protein within the chloroplast such as the thylakoid membrane or space. The energy for protein transport is provided by Hsp70 and Hsp100. There are at least four routes for proteins to cross or become integrated into the thylakoid membrane.

- The peroxisome is unique as it does not follow the rules established for protein import to other organelles. The proteins are imported in their native conformation. Even oligomeric proteins can be trafficked to the organelle. The proteins are targeted either to the membrane or matrix. Most matrix targeted proteins have a C-terminal non cleavable sequence. The peroxisomal membrane proteins also have signals (mPTS) to guide their import and membrane insertion. Some proteins (and lipids) are imported indirectly through the ER.

10.7 TERMINAL QUESTIONS

1. Explain briefly the steps in protein import into the nucleus.
2. Compare the nature of signal sequences for proteins destined to the mitochondria and peroxisomes.
3. In what respect is protein import to the peroxisome different from other post translational import processes?
4. What is the role of heat shock proteins in protein trafficking?
5. Describe the steps in protein targeting from the cytosol to the thylakoid lumen.
6. Explain how proteins are imported to the matrix of peroxisomes?
7. What is the fate of the signal sequence following protein targeting?
8. What are the similarities in protein import between mitochondria and chloroplast?

10.8 ANSWERS

Self assessment questions

1. (a)

(i) nucleus (ii) NLS; NES

(iii) GTP; GDP; asymmetric; regulators (iv) NPC

(v) GTP (vi) unspliced HIV-1 and RNA; NES

(b) Ran is the key player in conferring directionality to nuclear transport by controlling the binding and release of cargo. This is accomplished by compartmentalization of proteins that regulate the two states of the protein. The regulators are Ran guanine-nucleotide exchange factors in the nucleus and GTPase-activating proteins in the cytoplasm (refer to Fig.10.4).

2.

(i) T (ii) T (iii) F

(iv) T (v) T (vi) F

(vii) F (viii) F (ix) F

3.

(i) It guides precursor proteins to the chloroplast stroma.

(ii) It is a translocon through which proteins cross the chloroplast outer membrane.

(iii) SPP cleaves the CTP as it reaches the stroma.

(iv) It is a cytosolic chaperone that maintains proteins in an unfolded state.

(v) It generates the driving force for pulling the protein into chloroplast stroma.

4.

(i) peroxisome (ii) Pex5 receptor (iii) peroxins

(iv) native (v) ubiquitination; Pex4

Terminal questions

1.

The proteins destined to the nucleus have NLS sequence that is recognised in the cytosol by specific soluble receptors (α β) called **importins**. The α-importin is an adaptor that binds both to the cargo and β-importin. Next the NLS-importin α/β trimeric complex docks on the cytoplasmic side of the NPC via β-importin. In the presence of Ran-GDP and other protein factors the complex is translocated through the NPC channel in an energy dependent manner. Once inside the nucleoplasm, a GDP/ GTP exchange by GEF converts Ran into a GTP bound form. This results in a change in its conformation and interactions with the import receptor. The receptor subunits dissociate and the cargo is released. The importin subunits and Ran are returned back.

2.

Most mitochondrial proteins have a 20-35 amino acid long **cleavable** N-terminal MTS sequence that directs a newly synthesized protein to the mitochondria. It consists of an alternating pattern of hydrophobic and positively charged amino acids those folds to form an **amphipathic helix**. Mitochondrial targeting signals may also contain additional signals that subsequently target the protein to regions of other than the matrix.

Most peroxisomal proteins targeted to the matrix have a **non cleavable, C-terminal tripeptide signal sequence** (PTS1) and some have PTS2 (a

nonapeptide) at the N-terminus of the protein. The membrane proteins have mPTS sequence and they may be inserted directly or indirectly via ER.

3.

(a) Proteins are imported to the peroxisomes in their **native conformation**. The proteins not only fold but can also oligomerise and acquire cofactors prior to import.

(b) The proteins are targeted either to the membrane or matrix, so they have only one signal (PTS or mPTS). The PMP can be trafficked either through a direct or an indirect route.

4.

Heat shock proteins (Hsp) are indispensable in protein trafficking. They assist both in protein folding and unfolding. They also provide a microenvironment for assembly and disassembly of protein complexes and prevent their aggregation. In some cases they generate the driving force by ATP hydrolysis for pulling the protein into the organelle. Heat-shock proteins of the hsp60 and hsp70 families are called 'molecular chaperones'. In the case of mitochondria, the imported polypeptides are transferred to the 'foldase' hsp60, which mediates their folding and oligomeric assembly.

5.

A chloroplast has six subcompartments that include the outer and inner membranes, inter membrane space, stroma, thylakoid membrane and lumen into which proteins are targeted post translationally. Protein targeting to thylakoid space is directed by two signals, (i) Transit peptide and (ii) thylakoid targeting signal. Both signal peptides are removed sequentially by two different signal processing peptidases once their role is completed. The removal of the transit peptide in the stroma unmasks the thylakoid signal sequence. Both signal sequences guide and direct step by step the translocation of proteins across the chloroplast and into the thylakoid space or to thylakoid membrane. The thylakoid signal sequence initiates transport across the thylakoid membrane. There are at least four routes for proteins to cross or become integrated into the thylakoid membrane (Fig.10.17).

6.

The import pathway can be broadly divided into four steps: (i) Receptor binding (ii) Docking (iii) Translocation and (iv) Recycling of receptors (Refer to section 10.5.2). It requires energy in the form of ATP both for protein import and recycling of receptors.

7.

The targeting signals are the pieces of information on proteins that enable the cellular transport machinery to accurately target a protein to its site of action. The signal sequences may either be the termini (generally N-terminal) or internal. The N-terminal sequences are most often transient. The N terminal sequence may at times contain more than one signal and the removal of the first signal exposes the second signal that routes the protein to its final

destination. The internal signal sequences are non cleavable and some of them become part of the TM domain of an integral membrane protein.

8.

Protein import into chloroplast and mitochondria share many common features:

(i) Generally it is an energy-requiring process.

(ii) The proteins are imported in a partially folded/ unfolded state.

(iii) The precursor proteins have a cleavable N-terminal MTS / CTS. In some cases, the signals are internal and non cleavable.

(iv) The default destination of a protein in most cases is the matrix/ stroma; those that are destined to other subcompartments have bipartite signals that function in a hierarchical manner.

(v) The outer and inner membrane has translocons through which the proteins enter.

10.9 SUGGESTED READINGS

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