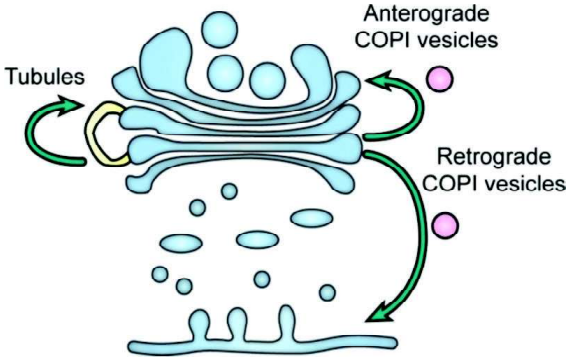

UNIT 6

VESICULAR TRANSPORT AND MEMBRANE FUSION

Structure

6.1 INTRODUCTION

In the previous units you learnt about various types of membrane transport and transporters. You also studied their role in maintaining the life processes at cellular level. Membrane transport proteins and various carrier molecules present in the membrane allow the movement of ions or molecules through semipermeable or selectively permeable biological membrane even at the cost of energy. In this unit you will learn about vesicular transport that is essential for the transportation of soluble and membrane proteins to their destinations. We will also focus on the various types of vesicular transport vesicles and molecular mechanism of vesicular transport. We will also deal with receptor mediated endocytosis in section 6.5 about which you have studied briefly in Unit 4.

Expected Learning Outcomes

After studying this unit, you should be able to:

- write the significance of vesicular transport;
- differentiate between clathrin and COP coated vesicles;
- describe the molecular mechanism of vesicular transport;
- explain membrane fusion; and
- describe receptor mediated endocytosis.

6.2 VESICULAR TRANSPORT

You may revisit about protein from Cell biology (BBCCTT-103) course that you studied in first semester (unit-9). The transport across plasma membrane and vesicular transport are two different phenomena. During membrane transport the transfer of ions or molecules takes place across the membrane while vesicular transport deals with the soluble and membrane proteins i.e. proteins destined along the secretory route. The synthesis and their translocation between different organelles of cell are facilitated by small membrane bound structures known as transport vesicles.

In eukaryotes, vesicular transport is crucial for growth, development and survival of organism because it maintains the correct balance and distribution of various moieties in specific cellular compartments. The selectivity of such transport is important for maintaining the functional organization of the cell. Thus, this transport process must be precise and operate in optimal manner. This is possible due to the presence of specific receptor proteins and signaling molecules present on both the vesicles and target sites.

Recall the co-translational translocation of proteins you have studied in Cell Biology (BBCCT-103 Block III: Protein Trafficking). Proteins destined along the secretory route are transported from endoplasmic reticulum (ER) to Golgi complex for post translational modification as well as sorting. The proteins are transported to lysosomes, secretory granules or plasma membrane according to the signal it bears which is encoded by their sequence. Vesicular transport mechanism helps in sorting and transport of protein to their correct address. A simple question comes to mind. How do these proteins maintain their integrity? To answer this question let us go through vesicular transport models.

The Golgi has been classified into:

1. *cis* compartment closest to ER
2. *medial* compartment
3. *trans* compartment, which exports proteins to different destinations.

If you remember the co-translational modification of proteins, the core glycosylation takes place in ER. In each compartment of Golgi, the carbohydrate units are specifically added or modified. In ***cis* Golgi** three mannoses are removed from the oligosaccharide chain of proteins to be secreted or to be inserted in the plasma membrane. In **medial Golgi**, two

more mannoses are removed and two N-acetylglucosamines and a fucose is added. Finally, in **trans Golgi**, another N-acetylglucosamine is added followed by addition of galactose and sialic acid. Fig. 6.1shows the path how Golgi apparatus modifies and sorts proteins for transport throughout the cell as well as helps in maintaining membrane integrity.

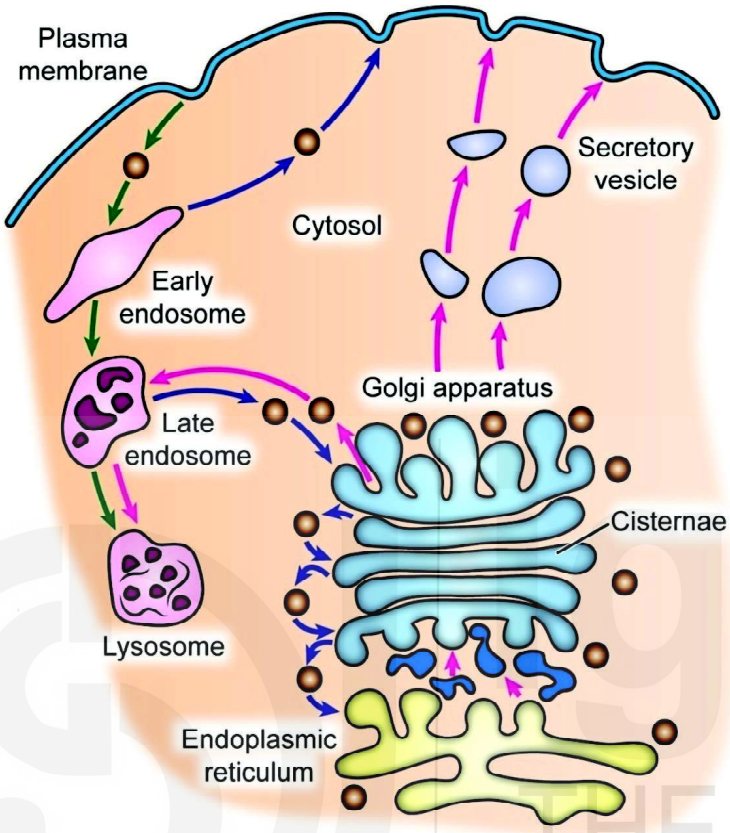

Fig. 6.1: Protein modification and sorting in Golgi apparatus for transport throughout the cell.

What happens when there are defects in Golgi function?

Defects in various aspects of Golgi function can result in congenital glycosylation disorders, some forms of muscular dystrophy, and may contribute to diabetes, cancer, and cystic fibrosis.

In vesicular transport, membrane symmetry is preserved i.e. the cytosolic face of a transport vesicle corresponds to the cytosolic face of donor compartment. After fusion, the cytosolic face of the transport vesicles becomes continuous with the cytosolic face of the target compartment. Accordingly, when a vesicle fuses with the plasma membrane, its luminal surface becomes part of the external side of the plasma membrane. The carbohydrate groups of glycoproteins in plasma membrane are always on the extracellular surface.

There are two opposing models to explain the movement of proteins through Golgi: **Cisternal maturation model** and **Vesicular transport model**.

6.2.1 Cisternal Maturation Model

How do cargo proteins move between the Golgi cisternae? As it was mentioned earlier, Golgi-mediated modifications act as signals to direct the proteins to their final destinations within cells, including the lysosome and the plasma membrane. Initially scientists believed that each Golgi cisterna was transient and that the cisternae themselves moved from the *cis* to the *trans* face of the Golgi, changing over time. So, proteins were travelling as passengers (cargo) within cisternae. Cisternal maturation model proposes

that the enzymes present in each individual cisterna change over time, while the cargo proteins remain inside the cisterna. However, another school of researches believe in the vesicular transport model.

6.2.2 Vesicular Transport Model

It is believed by a group of scientists that instead of entire cisternae, vesicles formed in the Golgi move cargo proteins. This model was given by **George Palade** and **Marilyn Farquhar** in 1998. The vesicular transport model proposes that the Golgi cisternae are a stable compartment which carries protein modification enzymes (such as enzymes to add or remove sugars, add sulfate groups, and perform other modifications). Vesicles arrive at each cisternae carrying cargo proteins; and there it undergoes modification by the resident enzyme. New vesicles carrying the modified protein buds off from the cisternae, and travel to the next stable cisternae till the modification is complete.

To summarize, one model says that cisternae are transient structures and each cisternae physically moves from *cis* to *trans* face while the modification goes on. Another model is of the view that cisternae are stable structures having specific enzymes. And vesicles carrying cargo proteins move from one cisternae to another for modification to take place. It is yet debatable which model is best. Now, the question arises, Do all proteins follow the same path?

SAQ 1

Tick [✓] mark the correct option:

a) *Cis* Golgi compartment is closest to ER. [True/False]

b) Post translational modification occurs in lysosomes. [True/False]

c) During glycoprotein synthesis, fucose unit is attached in ER. [True/False]

d) Specific receptor proteins are present only on the receptors and not on the vesicles. [True/False]

e) The carbohydrate group of glycoproteins in plasma membrane is always present on the extracellular side. [True/False]

f) Vesicular transport model proposes that the enzymes present in each individual cisterna change over time, while the cargo proteins remain inside the cisterna. [True/False]

SAQ 2

Answer in 1-2 sentences:

a) Define vesicular transport.

.....

.....

b) How transport across membrane is different from vesicular transport?

.....

.....

c) Name the models of vesicular transport mechanism.

.....

.....

6.3 TRANSPORT VESICLES

As you already know function of coated vesicles is related to endocytosis and the intracellular transport of membranes and soluble proteins. These coated vesicles vary in size between 50 to 250 nm and are characterised by the presence of a coat made of tiny, regularly spaced bristles that cover the cytoplasmic side of the vesicle.

A vesicle is formed by budding off from a donor membrane that in turn fuses with an acceptor membrane. Sometimes donor membrane undergoes distortion to initiate budding. The first step in vesicular transport is the formation of a coated vesicle by budding from the donor membrane surface. Coated vesicles are involved in the vesicular transport throughout the endomembrane system, as well as in exocytosis and endocytosis. The nature of coat depends on the cargo protein to be transported and the targeted destination/ acceptor membrane. Here, you must note that the protein coat of the coated vesicle is formed from soluble proteins on the cytosolic face of the donor membrane guided by G-proteins. The involvement of GTPases like **dynamin** has been shown in the formation of vesicles. It forms a ring around the neck of the bud and GTP hydrolysis constricts the ring, pinching off the neck to release the vesicle (Fig. 6.2). Transport of vesicles occurs with the help of cytoskeletal proteins such as actin filaments to the targeted site. Let us focus on some of the best characterised coated vesicles.

1. Clathrin-Coated Vesicles
2. COP-Coated Vesicles (COP I and COP II)

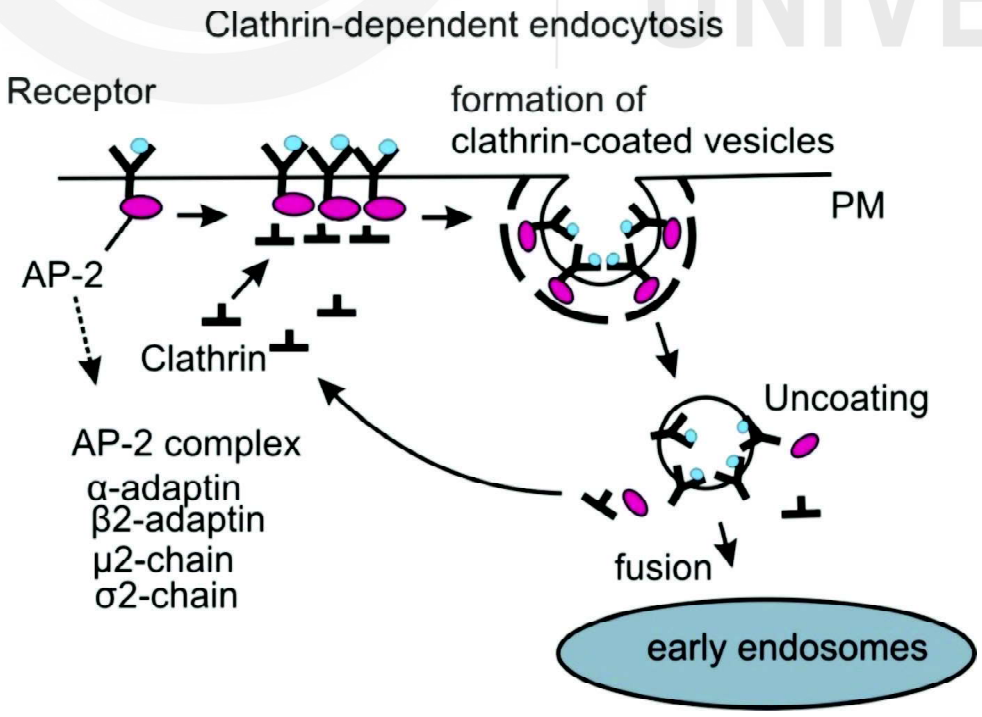

Fig. 6.2: Main steps of the clathrin coated vesicle formation.

6.3.1 Clathrin-Coated Vesicles

Clathrin-coated vesicles are one of the most extensively characterized transport vesicles. They mediate endocytosis of transmembrane receptors and transport of newly synthesized lysosomal *hydrolases* from the trans-Golgi network to the lysosome. Accumulation of clathrin on the plasma membrane stimulates the formation of clathrin coated vesicles. The cell surface receptors (adaptor protein complexes), interact with each other, with membranes, and with the sorting signals found on cargo molecules. Coat constituents not only serve to shape the budding vesicle, but also play a direct role in the packaging of cargo, suggesting that protein sorting and vesicle budding are functionally integrated.

One clathrin molecule is comprised of three molecules of heavy-chain and three molecules of light chain and is called a **triskelion**. Each heavy chain of the structure is of 180 kDa whereas each light chain has the molecular weight of 35 kDa. Clathrin molecules successively assemble into a polyhedral, cage-like coat on the surface of the coated pit. **Triskelion**, a three-pronged protein complex made up of clathrin coat attaches to the membrane via an **adaptor protein (AP) complex**. Adaptor proteins bind both to clathrin and to integral membrane proteins of the vesicle and stimulate its assembly. Much more importantly, by binding to the molecules in the membrane of the vesicle, adaptor proteins appear to be responsible for recognising the appropriate cargo molecules.

Main steps of the clathrin coated vesicle formation are shown in (Fig. 6.2):

1. Recruitment of the G-protein, adaptor proteins and clathrin to defined sites on the plasma membrane.
2. Clathrin concentrates in specific areas of the plasma membrane, forming clathrin-coated membrane invaginations, called **clathrin-coated pits**.
3. Budding and detachment of the nascent clathrin-coated vesicles through a series of highly regulated steps.

6.3.2 COP-Coated Vesicles (COP I and COP II)

COP-coated vesicles (COP I and COP II) transport all type of molecules from the Golgi to the ER and back. Various steps of vesicular transport is assisted by specific type of coat structures. For transport of molecules from *cis* Golgi to ER, COP I coated vesicles are involved, whereas in reverse transport i.e. from ER to Golgi, COP II coated vesicles are used. This differential selectivity is helpful in creation of one way transport system of specific molecules to corresponding organelles in the cell. By this route, the newly synthesized proteins are targeted to specific locations in the cell or secreted out of the cell. The ER-resident proteins have ER retention signals at the c-terminal. **The KDEL(Lys-Asp-Glu-Leu) for lumen protein and KKxx(Lys-Lys-?-?) for membrane protein.** In Table 6.1 details of sorting signals that direct proteins to specific transport vesicles is given.

Table 6.1: 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	COP I
Cytoplasmic Sorting Cells			
Lys-Lys-X-X (KKXX)	ER-resident membrane proteins	COP I α and β subunits	COP I
Di-acidic (e.g., Asp-X-Glu)	Cargo membrane proteins	COP II Sec 24 subunit	COP II
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

The molecules to be transported through COP I-coated vesicles are selected by binding of the molecules to specific membrane receptors. Transport by COP I-coated vesicles pathway is often called the **retrograde transport pathway** (Fig. 6.3) because it is involved in the ER retrieval pathway. COP II coated vesicles are required for transport of molecules from ER to Golgi. This mode of transportation is also known as **anterograde transport pathway.**

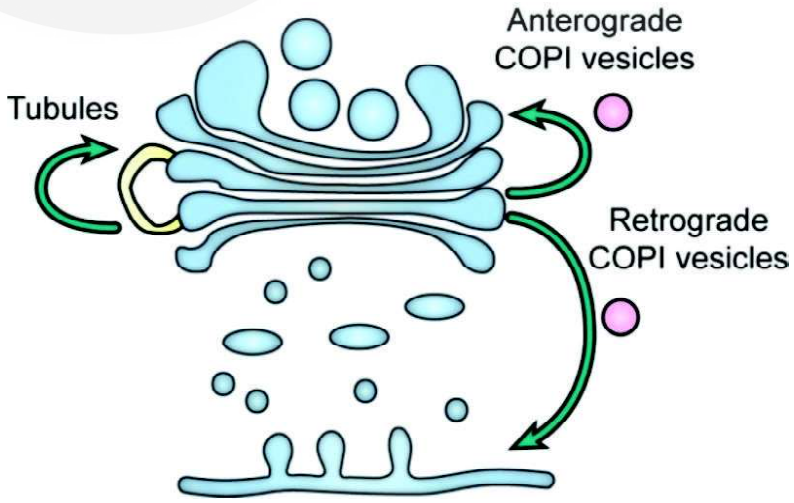

Fig. 6.3: Various types of vesicular transport pathways.

COP I coated vesicles are formed by sequence of events much more similar to that for Clathrin coated vesicles. In case of COP II coated vesicles, the final structure of coat is formed by sequential assembly of specific protein

components. Fig. 6.4 gives an overview of vesicle formation of both the types.

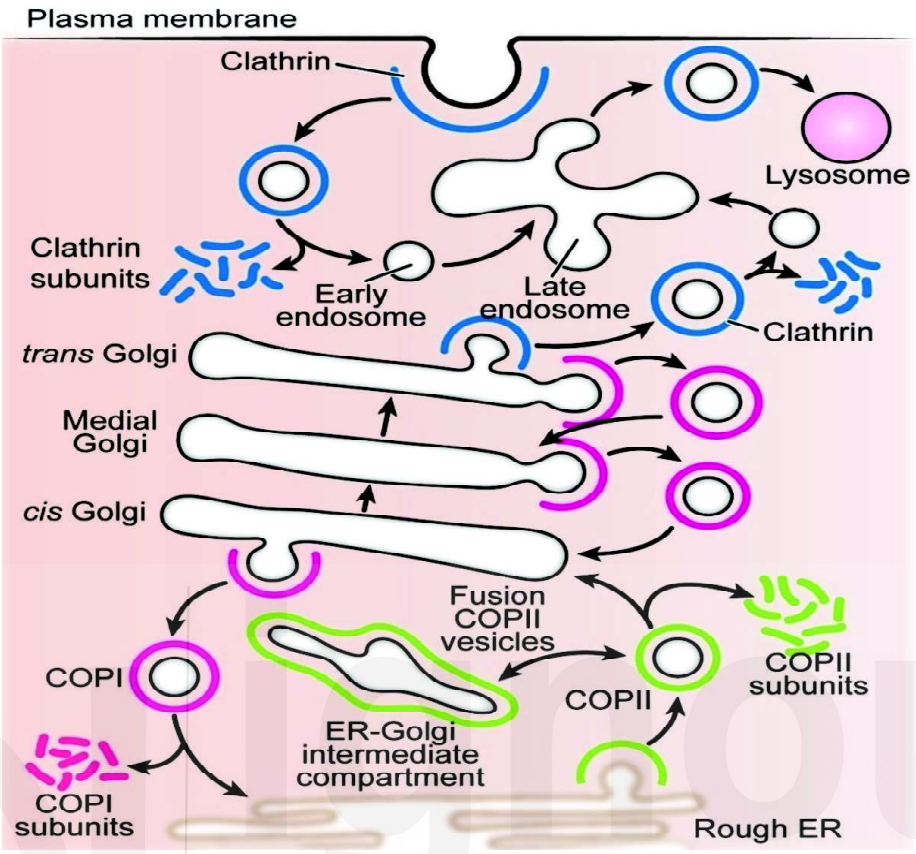

Fig. 6.4: Formation of Clathrin-coated vesicles involved in the endosomal pathways; COPI-coated vesicles involved in retrograde transport from the *trans* Golgi back through the Golgi cisternae to the ER and COPII-coated vesicles for transport from the ER to the *cis* Golgi.

G protein Sar1 is responsible for initiation of budding of COP II coated vesicles. Additionally, two other proteins Sec 23 and Sec 24 are part of the pre-budding complex. The mechanism of action and formation of pre-budding complex is quite different for clathrin and COP II coated vesicles.

SAQ 3

Fill in the blanks with appropriate words:

a) The coat on vesicle for transport of cargo from ER to Golgi is made up of …… (G-protein/ COP II).

b) Budding of COP II vesicles is initiated by G protein …… (Sar 1/ SNARE).

c) Clathrin-coated vesicles mediate …… (exocytosis/ endocytosis) of transmembrane receptors and transport of newly synthesized lysosomal *hydrolases* from the trans-Golgi.

d) ……. (ATPases/ GTPases) are involved in the formation of vesicles.

6.4 VESICULAR TRANSPORT MECHANISM

In the previous section we discussed about different types of vesicle formation. In this section we will learn how these vesicles are transported from their site of formation to their designated targets resulting in membrane fusion.

The vesicular transport is initiated after the formation of the transport vesicles. Vesicles are formed by detachment of a small portion of lipid bilayer. This process is also called **budding**. The initiation of membrane curvature required for vesicle formation requires energy. This process is mediated by proteins such as **epsins**. The newly formed vesicles contain the proteins that were present in the portion of membrane as well as soluble molecules. Fusion of the vesicle with a target membrane is generally a reversal of the process of its formation. The proteins that mediate targeting of the vesicle to the specific cellular location also mediate fusion, and in some systems regulate the precise time at which fusion occurs.

The molecular components of vesicular transport have been discovered using mutant strains of yeast. The formation of transport vesicles and selective incorporation of cargo into them is mediated by their **protein coats**. The coats are supramolecular assemblies of protein on nascent vesicles. The coat formation initiates the shaping of curvature on the flat membranes. The coat proteins are also involved in the recognition of sorting signals in the cargo.

Hsc70 is a chaperone protein with diverse cellulose functions also known as HSPA8. ATPase activity of Hsc70 is important for endocytosis during the uncoating of clathrin coated resides.

Most of the cargo proteins are found in COP II coated buds and vesicles while being exported from ER. These proteins may be attached directly to COP II or are indirectly bound to COP II through some transmembrane export receptors. The sorting signals recognized by COP II are present in the cytosolic domains of transmembrane cargo proteins. Structurally clathrin coats are more complex than COP II and COP I. Clathrin vesicle assembly is regulated by kinases, phosphatases and other accessory proteins. Scission of clathrin coated vesicles depends on accessory factors such as, **dynamin**. In addition, the uncoating of clathrin vesicle is mediated by the chaperons Hsc 70 and auxilin.

After uncoating, the vesicles are targeted to corresponding acceptor compartment where it is fused with the acceptor membrane. N-ethylmaleimide sensitive factor (NSF) binds to α- SNAP (soluble NSF attachment protein) to form a complex molecule. This complex binds to membrane associated receptors called SNAREs. Each type of transport vesicle contains a specific v-SNARE that binds to corresponding t-SNARE on the target membrane. Most SNAREs have C- terminal anchored transmembrane proteins while their N-terminal domain faces the cytosol. They contain a heptad (group or set of seven) repeat motif of 60-70 amino acids which participate in coiled coil formation. The SNARE complex formed by the pairing of v-SNARE and t-SNARE is very stable four helix bundle in which v-SNARE contribute one helix and t-SNARE the remaining three α-helices.

NEM-
n-ethylmaleimide

NSF –
n-ethylmaleimide (NEM) Sensitive Factor

SNAP-

Soluble NSF Attachment Proteins

SNAREs (v- and t-)-
Soluble NSF Attachment protein Receptors

SNAREs seem to perform two major functions.

i) Fusion of membrane

ii) Provide specificity of membrane fusion

Fig. 6.5 gives an overview of various steps of vesicle budding and fusion. As you can note from the figure. Specificity is also provided by tethering proteins that assist in membrane fusion prior to SNARE complex formation.

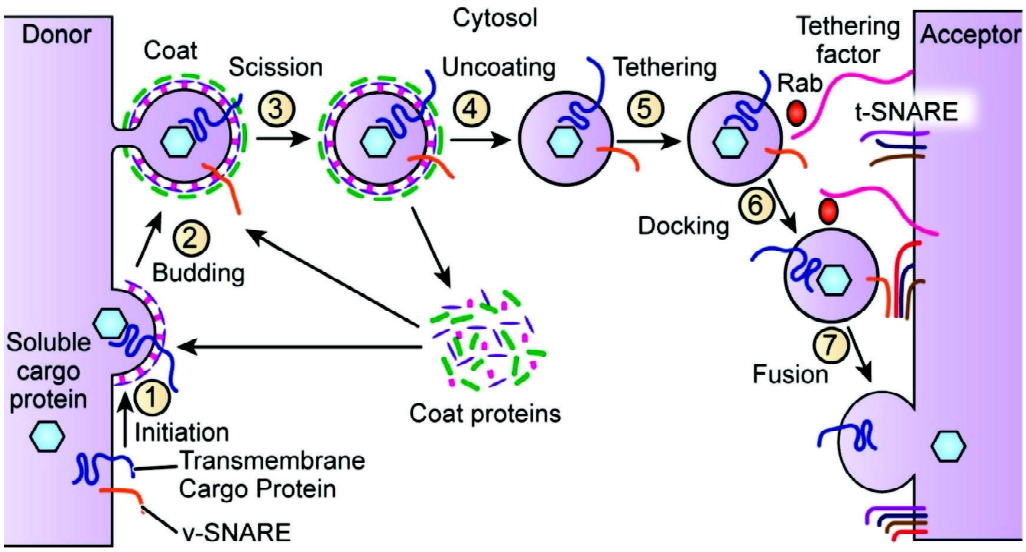

Fig. 6.5: Various steps of vesicle budding and fusion.

6.4.1 Membrane Fusion

Membrane fusion during vesicle transport consists of two reactions. First, the vesicles have to select and recognize the specific target membrane according to its receptor signal. After this, the two membranes fuse and deliver the content of vesicles to the targeted cellular compartment. The specificity is provided by a group of proteins called SNAREs. As you have read in the previous section SNAREs are of two types: v-SNARE and t-SNARE; v-SNAREs are found on vesicles whereas t-SNARES are present on the target membrane. The initial step is **membrane recognition** followed by a loose interaction called **tethering**. In the later stages, the membranes reside much closer to each other, known as **docking** which ultimately results in membrane fusion.

In addition to SNAREs, two other types of proteins are also required for vesicle membrane fusion. The **Rab** proteins are a family of small GTP-binding proteins that are related to the **Ras** proteins. After the formation of complexes between complementary SNAREs and membrane fusion, a complex of two additional proteins (the NSF/ SNAP complex) is needed to complete the process of vesicle transport. These are not required directly for either vesicle/ target pairing or for the fusion of paired membranes. However, the NSF/SNAP proteins act after membrane fusion to disassemble the SNARE complex and thus the components of SNAREs are reutilized for other rounds of vesicle transport. Look at Fig.6.6 to visualize the steps involved in interaction and fusion of the membranes of the vesicle and the target and recycling of SNAREs.

The interaction and fusion of the membranes of the vesicle and the target involves following steps:

1. Specification of the vesicle delivery site,
2. Recruitment of components capable of initiating vesicle ‘capture’,
3. Formation of a bridge between the vesicle and the target membrane,

4. Conformational change that allows the vesicle and target membrane proteins to come close enough to interact,
5. Dissociation of the tethering proteins, to free them for another round of transport.

The tethering process is regulated by a multimeric protein complex, called **TRAPPs** (Transport Protein Particles). It is made of 10 subunits and has the molecular weight of about 1,100 kDa. All the proteins of TRAPP complex are highly conserved and are found embedded in the membrane.

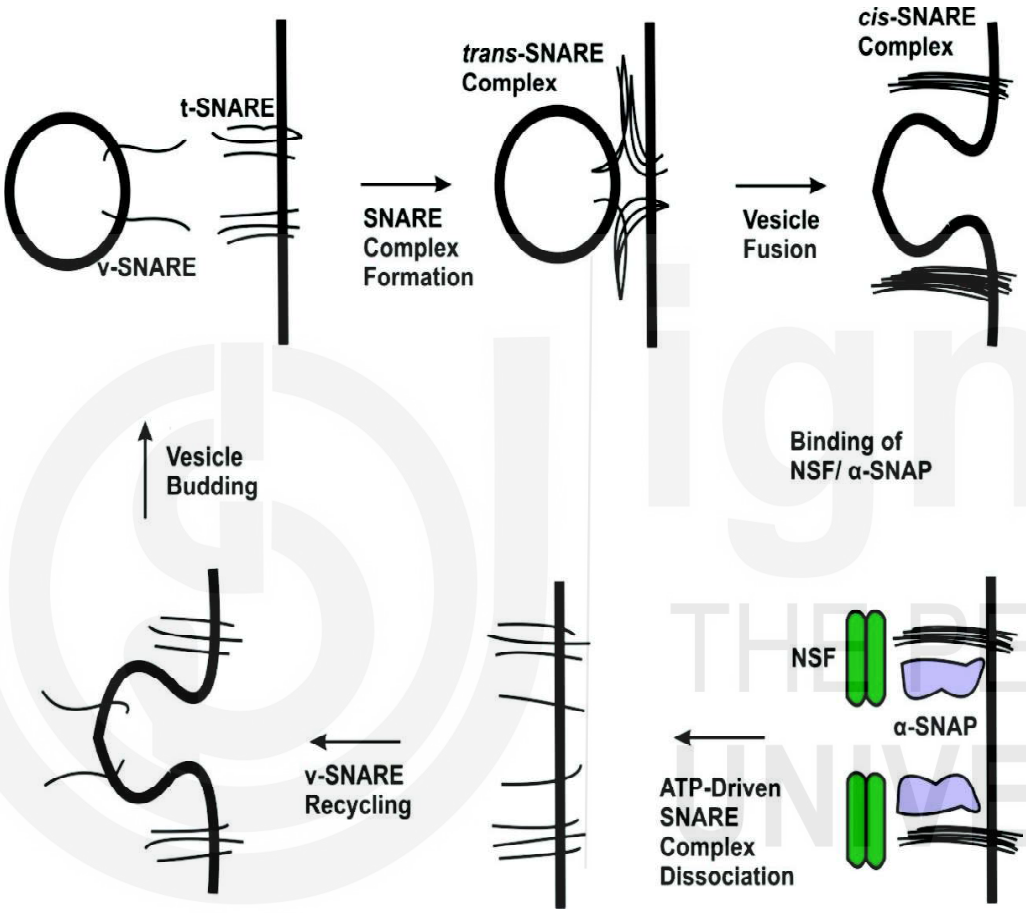

Fig. 6.6: Steps involved in interaction and fusion of the membranes of the vesicle and the target and recycling of SNAREs.

SAQ 4

Fill in the blanks:

a) Uncoating of clathrin vesicle is mediated by ……. (NSF/ chaperons).

b) Vesicle fusion is mediated by interactions between specific pairs of protein called …… (SNAREs/ COP II).

c) Transport protein particle (TRAPP) complex is made of …….. (two/ ten) subunits.

d) v-SNAREs are found on …… (target membrane/ vesicles) whereas t-SNARES are present on the (target membrane/ vesicles).

6.4.2 Receptor Mediated Endocytosis

Some proteins enter the cell from the surrounding medium through the process of receptor-mediated endocytosis. The receptor-mediated endocytosis is a process for import of specific materials in the cell. A specific structure called **endosome** is formed. The receptors on the surface of endosomes are specific and selective. The receptors found on the cell membrane bind to the corresponding ligand/ molecule before its transfer in the cell. The process has following steps:

1. Exogenous ligand binds to specific membrane receptors
2. Formation of clathrin coated vesicles
3. Membrane invagination
4. Vesicle uncoating
5. Compartment of uncoupling receptors and ligand (CURL) and endosome formation
6. Ligand is further modified by the cell, then
7. Receptors are recycled towards the surface or digestive vacuoles, and
8. Receptors merges with cell membrane.

In eukaryotes, the clathrin-mediated endocytic pathway is involved in the selective intake of proteins at the plasma membrane. The clathrin deposition on the membrane initiates the vesicle formation and process of endocytocis of molecules or ligands from outside of the cell to its interior. After the clathrin molecules are assembled, the membrane is deformed to form the vesicle by making an inward curvature. Several ligands can be imported in the cell by a single coated pit. After this process is complete, the clathrin coat is lost and its components are recycled. The transport of cholesterol through low density lipoprotein (LDL) is mediated by receptor mediated endocytosis. This is a crucial process through which cholesterol is distributed to all the cells. When LDL receptors become non-functional, the cholesterol is not released into the cell and LDL remains in the blood stream thereby causing problems to the patients.

6.5 SUMMARY

Let us recapitulate what we have learnt so far:

- Proteins are transported from endoplasmic reticulum (ER) to Golgi complex for post translational modification (addition and alteration) as well as sorting.
- Vesicle formation requires deformation of the lipid bilayer, forming a goblet-shaped invagination of the membrane that will eventually be pinched off to form the vesicle, a process called budding.
- COP I-coated vesicles shuttle molecules from exit sites on the *cis* Golgi complex towards the ER, while COP II-coated vesicles shuttle them from the ER towards the Golgi.

- Most of the cargo proteins are found in COP-II coated buds and vesicles while being exported from ER.
- Each type of transport vesicle contains a specific v-SNARE that binds to corresponding t-SNARE on the target membrane.
- Receptor-mediated endocytosis is an extremely selective process of importing materials into the cell. This specificity is provided by receptor proteins located on specific areas of the cell membrane and is known as coated pits.

6.6 TERMINAL QUESTIONS

1. Name the different compartments of Golgi and associated functions.
2. Differentiate between anterograde and retrograde transport pathway.
3. Write down the steps of clathrin coated vesicle formation.
4. Explain the role of SNAREs in vesicular transport.
5. Explain membrane fusion.
6. Discuss the events involved in target specificity.
7. Enlist various steps of receptor mediated endocytosis.

6.7 ANSWERS

Self-Assessment Questions

1. a) true b) false c) false d) false e) true f) false
2. a) Vesicular transport is a mechanism which helps in sorting and transport of protein to their correct address such as lysosomes, secretory granules or plasma membrane according to the signal it bears.

   b) Transport across plasma membrane and vesicular transport are two different phenomena. During membrane transport the transfer of ions/ molecules takes place across the membrane while vesicular transport deals with the soluble and membrane proteins i.e. proteins destined along the secretory route.

   c) Cisternal maturation model and vesicular transport model.
3. a) COP-II b) Sar 1 c) endocytosis d) GTPases
4. a) chaperons b) SNAREs c) ten d) target membrane

Terminal Questions

1. The different compartments of Golgi are:

   i) *cis* compartment closest to ER

   ii) *medial* compartment

   iii) *trans* compartment, which exports proteins to different destinations. For details refer section 6.2.

2. Transport of secretory molecules from ER to Golgi is known as anterograde transport pathway and COP II coated vesicles are involved in this transportation whereas transport by COP I-coated vesicles pathway is often called the retrograde transport pathway. It is also involved in the ER retrieval pathway. Refer section 6.3.2.

3. Vesicle formation requires deformation of the lipid bilayer, forming a goblet-shaped invagination of the membrane that will eventually be pinched off to form the vesicle, a process called budding. Three vesicle formation are

   i) Recruitment of the G-protein, adaptor proteins and clathrin at the defined sites on the plasma membrane,

   ii) Clathrin concentrates in specific areas of the plasma membrane, forming clathrin-coated membrane invaginations, called **clathrin-coated pits**.

   iii) Budding and detachment of the nascent clathrin-coated vesicles through a series of highly regulated steps. Refer section 6.3.1.

4. SNAREs are membrane associated receptors and play a very important role in vesicular transport. They provide specificity in transportation. Each type of transport vesicle contains a specific v-SNARE that binds to corresponding t-SNARE on the target membrane. Refer section 6.4.

5. The interaction and fusion of the membranes of the transport vesicle with its target involves two types of events. First, the transport vesicle must specifically recognize the correct target membrane; for example, a vesicle carrying lysosomal enzymes has to deliver its cargo only to lysosomes. Second, the vesicle and target membranes must fuse; thereby delivering the contents of the vesicle to the target organelle. Refer section 6.4.1.

6. SNAREs play an important role in determining target specificity. Vesicle fusion is mediated by interactions between specific pairs of proteins, called SNAREs, on the vesicle and target membranes (v-SNAREs and t-SNAREs, respectively). Membrane fusion as a series of events includes:

   i) Specification of the vesicle delivery site

   ii) Recruitment of components capable of initiating vesicle ‘capture’

   iii) Formation of a bridge between the vesicle and the target membrane

   iv) Conformational change that allows the vesicle and target membrane proteins to come close enough to interact

   v) Dissociation of the tethering proteins, to free them for another round of transport. Refer section 6.4 for more details.

7. Receptor mediated endocytosis consists of following steps:

   i) Exogenous ligand binds to specific membrane receptors.

   ii) Clathrin vesicles are formed.

   iii) Membrane invagination

   iv) Vesicle uncoating

v) Compartment of uncoupling receptors and ligand (CURL) endosome forms.

vi) Ligand is further modified by the cell

vii) Receptors are recycled towards the surface or digestive vacuoles

viii) Receptors merge with cell membrane. Refer Section 6.4.2.

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