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PAPER- II

LIFE SCIENCES

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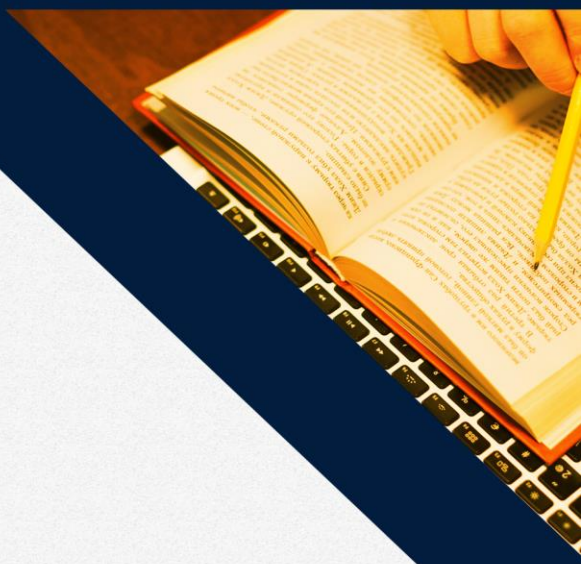


Council of Scientific and Industrial Research

LIFE SCIENCES CODE: 3

UNIT-2:CELLULER ORGANIZATION

2.1CELL MEMBRANE:

Cell is the structural and functional unit of life. Cell membrane protects the cell i.e all internal organelle and the intracellular molecules from the external environment. Cell membrane also participates in communications and transportation of biomolecules between intracellular and extracellular environment.

Cell membrane shows a typical structure having a lipid bi-layer (thick) and embedded protein layer according to the 'Fluid Mosaic Model' proposed by Singer and Nicolson in 1972.

2.1.1. CHEMICAL COMPONENETS OF CELL MEMBRANE:

Lipid- forms the lipid bilayer of the cell membrane.

Protein- forms the membrane proteins which are of two types integral protein and peripheral protein.

Carbohydrate – carbohydrates are present in the cell membrane in the form of glycolipid (bound to lipids) and glycoprotein (bound to protein)

LIPID BILAYER:

Lipid molecules constitute the bulk and thick components of cell membrane, lipid bilayer. There are about 10^9 lipid molecules in the cell membrane of a single animal cell. All the lipid molecules in the plasma membrane are **amphiphilic** in nature that is they have a **hydrophilic** (water loving) or polar end and a **hydrophobic** (water fearing) or non polar end.

POLAR MOLECULES- A polar molecule is a chemical species in which the distribution of electrons between the covalently bonded atoms is not even.

Example – water, ammonia.

NON POLAR MOLECULES - A molecules make of electronegatively similar atoms, which distribute electrons equally.

Example- carbon dioxide, methane.

Three classes of lip[id molecules are present in the cell membrane- phospholipids, glycolipids and sterols.

The most abundant lipid molecule of the cell membrane is the **phospholipids**.

2.1.1.1PHOSPHOLIPIDS- Phospholipids are composed of one primary alcohol, phosphate group, one secondary alcohol, two fatty acid chain. Depending upon the primary alcohol phospholipids can be of two types- glycerophospholipids (glycerol), sphingolipids (sphingosine).

The main phospholipids in most animal cell membrane are phosphoglycerides. Phosphoglycerides contain a three carbon glycerol. Two long chain fatty acids are linked through esters bond with two adjuscent carbon atom of glycerol. These structure form the hydrophobic tail part of the phospholipids as it is non-polar so can not dissolve in water. This hydrophobic parts are dissolved in organic solution. The third carbon atom of the glycerol is attached to the phosphate group(with one negative charge) which in turn attached with a another group, the secondary alcohol (serine, choline, ethanolamine) with a positive charge or neutral. These form the head part of phospholipids which is polar, dissolve in water, i.e., hydrophilic in nature.

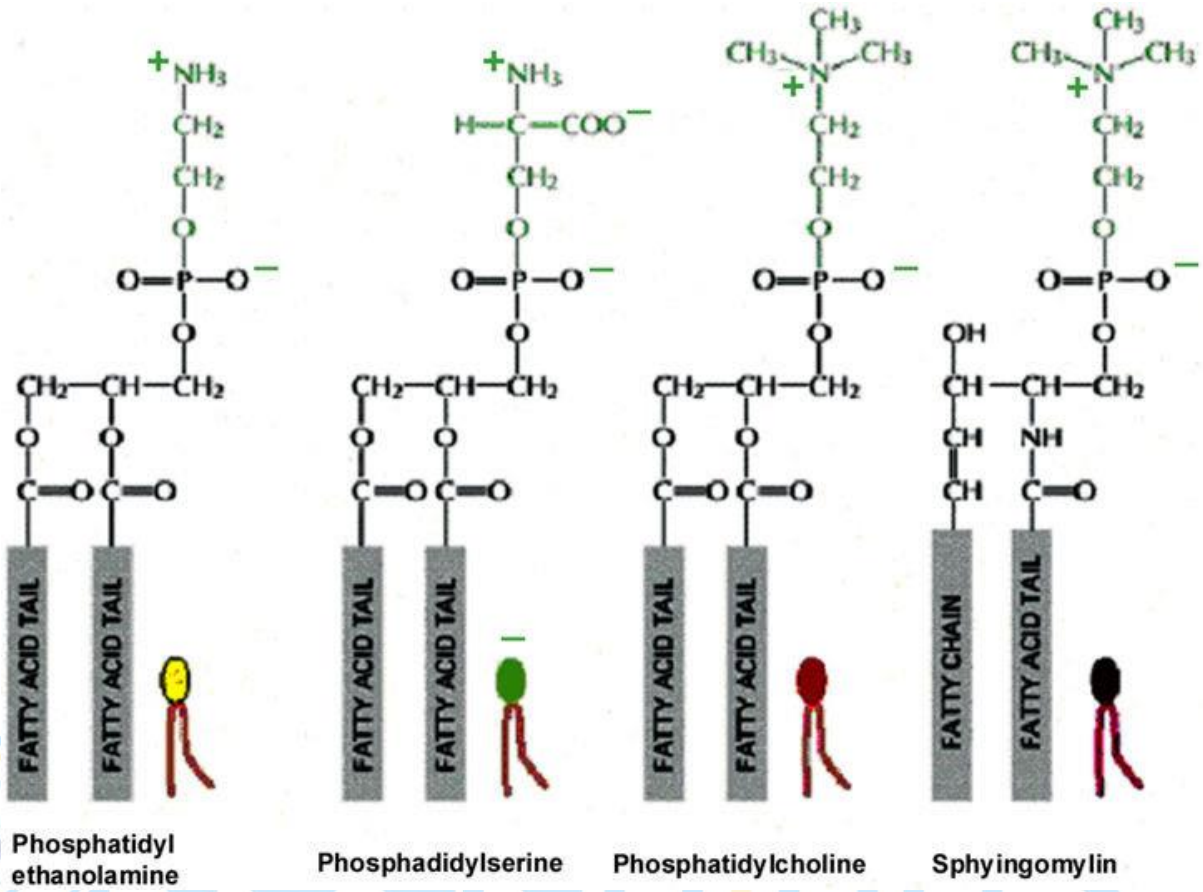


Figure- Phospholipids of cell membrane

The other important class of phospholipid are sphingolipids. It contains sphingosine instead of glycerol. In case of sphingomyeline, the most common sphingolipids, a fatty acid tail is attached to the amino group, and a phosphocholine group is attached to the terminal hydroxyl group.

At neutral pH (pH 7), **phosphatidylecholine** and **phosphatidyleethanolamine** carry no net electric charge, whereas **phosphatidyleionositol** and **phosphatidyleserine** carry a net negative electric charge.

2.1.2 MEMBRANE ASYMMETRY OF LIPIDS:

The lipid composition is different in the cytosolic leaflet and non-cytosolic or exoplasmic leaflet of the phospholipid bilayer. In plasma membrane of human erythrocyte cells sphingomyeline and phosphatidylecholine found more in number in the exoplasmic side of phospholipid bilayer, whereas phosphatidyleserine, phosphatidyleethanolamine and phosphatidyleionositol are preferentially located in the cytosolic side of the phospholipid bilayer. Although these phospholipids molecules are generated in the ER they oriented in preferential manner in the phospholipid bilayer with the involvement of three enzymes **flippase, floppase and scramblase.** After synthesis phospholipid molecules are equally distributed in two phospholipid monolayers, and this is mediated by **scramblase,** a phospholipid translocator protein. Thereafter **flippase,** a P-type active transporter protein transport phospholipids (phosphatidyleserine, phosphatidyleethanolamine, phosphatidyleionositol) from **outside to inside.** On the other hand another **floppase,** another active transporter protein transport phospholipid molecules (phosphatidylecholine, sphingolipids) from **inside to outside. Scramblase** is a ATP independent transporter protein.

FLIPPASE	FLOPPASE	SCRAMBLASE
P-type	ABC type	
ATP dependent	ATP dependent	ATP independent
Outside to inside	Inside to outside	Both direction and lateral direction

However there is a significant differences is available in lipid asymmetry between apoptotic or cancer cell and normal cell. In apoptotic cell or cancer cell phosphatidylecholine found more in cytosolic end and phosphatidyleserine found more in exoplasmic cell. This is a very useful difference between cancer cell and normal cell.

2.1.3 MEMBRANE FLUIDITY: Cell membrane has a quasi-fluid structure. Cell membrane maintains a critical fluidity so as to maintain its semi-permeability. Fluidity is essential to transport gasses, nutrients, signalling molecules, and also for movement of phospholipids molecule along the membrane plane. For example human live in different climatic region. But there a lot of differences between cell membrane compositions among people live in colder region and temperate region. The cell membrane fluidity is controlled by various factors,

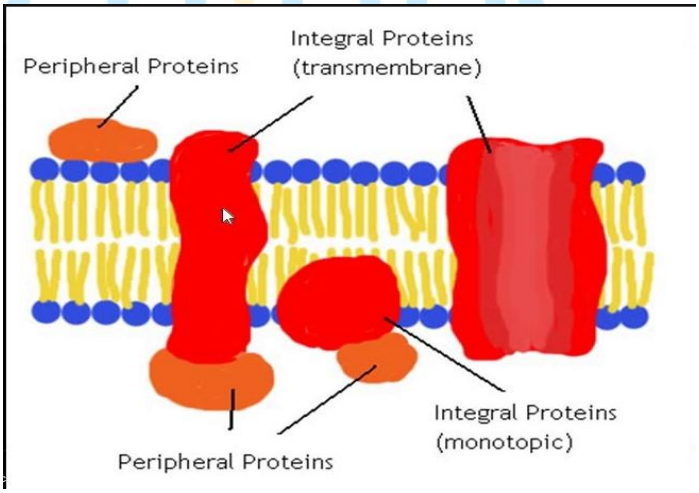
1) **Temperature:** As the temperature increases the fluidity of the membrane increases. Increasing in temperature increase the kinetic energy of phospholipids molecules and also decrease the interaction between lipid molecules thus increase the fluidity. Decrease in temperature in turn decrease the membrane fluidity and make the cell membrane more rigid in nature. The temperature at which membrane behaves like 50% fluid and 50% gel like structure is known as transition melting point **(T_m).**
2) **Fatty Acid Chain Length:** Long saturated fatty acid chains have more tendency to aggregate and packed tightly by van der walls interaction and hydrophobic interactions, thus make the membrane more gel like state or increase the rigidity and decrease the membrane fluidity. Meanwhile presence of short fatty acid chains increase the membrane fluidity as there is less surface area available for van der walls interaction and hydrophobic interaction.
3) **Degree of Saturation:** Phospholipids with unsaturated fatty acid chains that is fatty acid chains having double bond or triple bond structure, increase the membrane fluidity. Unsaturation that is presence of double bond structure in the fatty acid chain form kink structure and increase the distance between adjacent fatty acid chains of neighbouring phospholipid molecules. This in turn reduces the tendency to interact with other phospholipid molecules. So more the unsaturation more will be the membrane fluidity.

Sterol: There is another molecule sterol, is also responsible for maintaining the cell membrane fluidity. Sterols are lipid soluble molecules and these are amphipathic in nature. These are mainly responsible for maintaining of membrane rigidity. In case of animal cell the sterol present is known as **cholesterol,** in case of plant the sterol present

is known as **phytosterol.** Most common phytosterol are **brassicasterol, campesterol, sitosterol, stigmasterol, avenasterol.** In case of fungi the sterol present is known as **ergosterol.** Bacteria contain **hopanoids** instead of sterol. In animal cells Cholesterols mainly interact with phospholipids present in the cell membrane. Cholesterol present in between lipid molecules and interacts with phospholipids by the means of hydrophilic interaction and hydrophobic interaction as cholesterol shows amphipathic character. Basically in cold temperature cholesterol concentration increases so as to prevent possibilities to be more rigid as it prevent phospholipid molecules to came more closer. Whereas in high temperature cholesterol also prevent lipid molecules from being separated out from each other by means of hydrophobic interaction with fatty acid chains. So the main role of cholesterol in the plasma membrane to maintain membrane fluidity.

2.1.4 MEMBRANE PROTEINS:

As we know cell membrane is composed mainly of lipids and proteins. Protein molecules are embedded in the phospholipid bilayer. Membrane proteins play a major role in maintaining the functions of a specific cell. Membrane proteins are amphipathic in nature. Membrane proteins can be classified as



1) Integral protein

2) Peripheral protein

3) Lipid anchor protein

1) Integral Protein: Integral proteins also called trans membrane protein. These type of protein shows three part **cytosolic domain, exoplasmic domain and transmembrane domain.** The cytosolic and exoplasmic domain have hydrophilic

surface which interact with the aqueous of the cytosolic and exoplasmic environment. The transmembrane domain or the membrane spanning segment of the protein is hydrophobic in nature and it interacts with the fatty acid chain of the phospholipid molecules. In all transmembrane proteins examined till date, the membrane spanning domains consists of one or more α-helix or of multiple β-strands. Glycophorin, BAND-3, GPCR, Aquaporin, Ion channels, ATP Binding Cassette, Human Leukocyte Antigen (HLA), are some example of transmembrane protein.

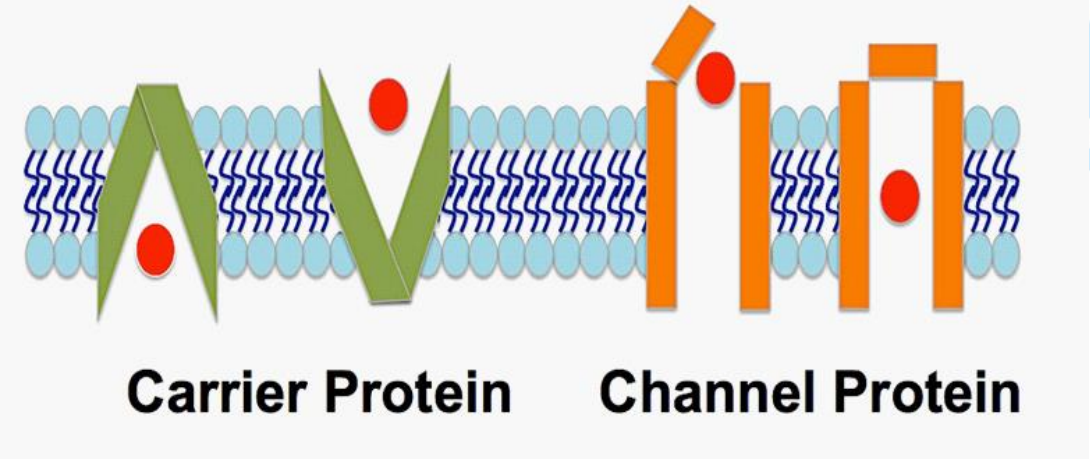
Depending upon the number of time a transmembrane protein pass the lipid bilayer TM protein may be single pass (monotopic) or multi-pass (polytopic). Glycophorin, the major protein the human erythrocyte cell membrane, is a representative single pass transmembrane protein, which contains only one single membrane spanning α-helix. It is composed of 131 amino acid residues. BAND-3 is a multi-pass TM protein which contains 929 amino acid residues and 14 transmembrane segment. The aquaporins are a large family of highly conserved which transport water, glycerol and other hydrophilic molecule across the cell membrane. Aquaporins have 6 membrane spanning α-helix. Porin a class of transmembrane protein have β-barrel strand in transmembrane segment. Porins are present in outer membrane of gram-negative bacteria like E.coli.

Peripheral Protein: Peripheral proteins do not directly contact the hydrophobic core of the phospholipid bilayer. Instead they are bound to the membrane either indirectly by interactions with integral or lipid anchored membrane proteins can be bound to either the cytosolic or the exoplasmic face of the plasma membrane. Peripheral proteins covalently attached to the cell membrane. Spectrin, anchyrin, BAND-4.1 are well known example of peripheral proteins.

Lipid Anchor Protein: Lipid anchor proteins are covalently bounded to one or more lipid molecules. The hydrophobic segment of the attached lipid is embedded in one leaflet of the membrane and anchor the proteins to the membrane. The polypeptide chain itself does not enter the phospholipid bilayer. GPI- anchor protein is a type of lipid anchor protein.

2.1.5 MEMBRANE TRANSPORT:

Transport proteins are of two general classes: carriers or channels. A carrier protein is a fixed topology transmembrane protein, with the ability to cycle between conformational changes. In this way, a solute binding site may be accessible on either side of the membrane, depending on the current conformation of the membrane protein. An intermediate conformation may also exist, in which the bound solute is inaccessible to both, however there is never an open channel through the carrier protein all the way through the membrane. A carrier protein transports either a single or a defined number of solute molecules per conformational cycle. Channels cycle between either a closed or an open conformation, and while open, provide a continuous pathway straight through the membrane bilayer. This allows flux of thousands of solute ions. Control of channel gating, between either the open or closed conformation, may be regulated by one of the following forms: voltage, binding of a ligand regulatory molecule, or by mechanical means such as membrane stretch



2.1.5.1 UNIPRT:

Carrier proteins that mediate the transport of a single solute are classified as a uniporter. These types of transport proteins facilitate the mode of diffusion by providing carrier-mediated solute transport down the concentration gradient, accelerating a reaction that is already thermodynamically favored. The uniport carrier protein allows transport of the non-diffusible solute across the membrane barrier at a rate that is much higher than passive diffusion in which

the solute molecule is never in contact with the hydrophobic core of the membrane. An example is the GLUT1 glucose carrier, found within the plasma membrane of various cells. GLUT1 is a large, 12 alpha helix membrane protein permitting the facilitated diffusion of a single glucose molecule per cycle into the cytosol of the cell to be metabolized and used as energy in cellular processes

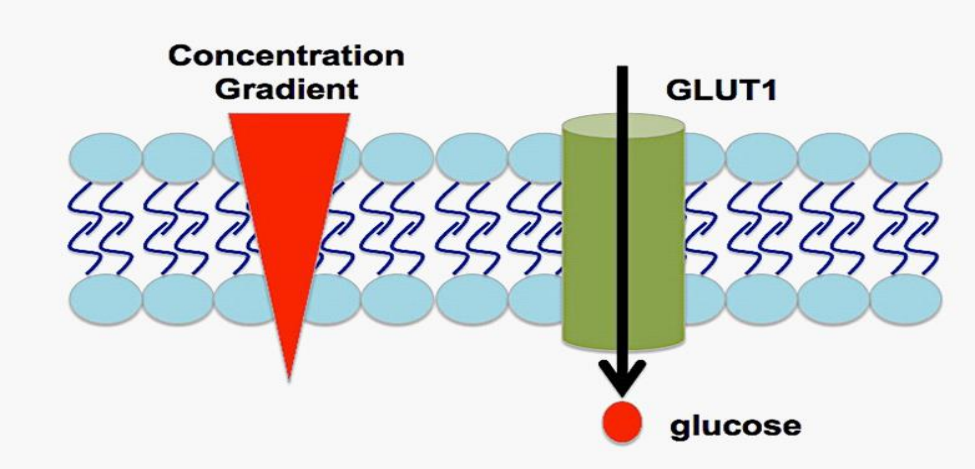


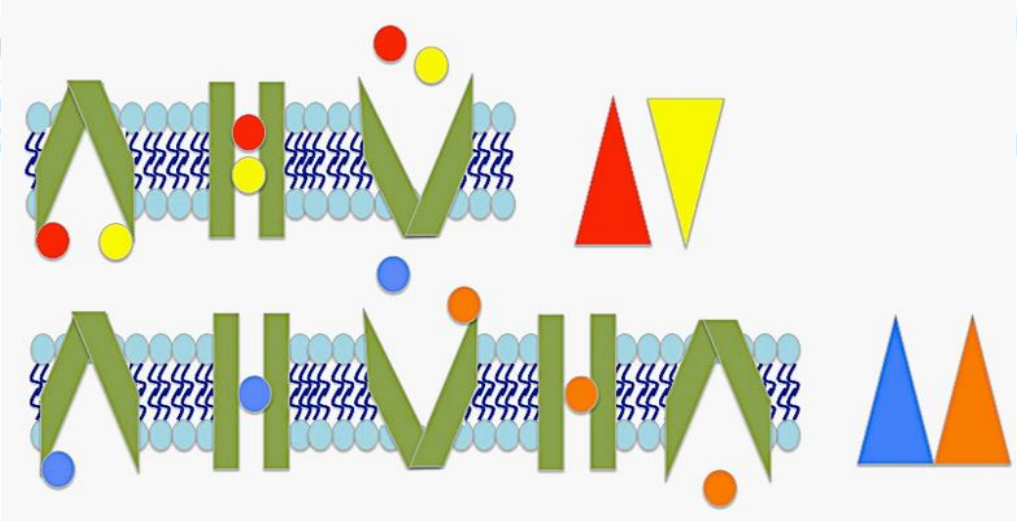
FIG: *Mode of transport for the uniport transport protein, GLUT1. Glucose diffusion across the membrane bilayer is facilitated by the GLUT1 carrier protein*

2.1.5.2 Symport and Antiport

Carrier proteins also hold the ability to couple the movement of one type of ion or molecule down its concentration gradient, with the movement of a second type of molecule against its concentration gradient. The ability to transport two different solutes simultaneously is termed cotransport. In this way, the transport protein mediates transport in which an energetically unfavorable reaction is coupled to an energetically favorable reaction that does not require ATP.

A carrier protein, which binds two dissimilar solute molecules to transport them across the membrane to the same side, is classified as a symport cotransport protein. Transport of the two solutes is coupled obligatorily. An example of a symport carrier protein is lactose permease, while also happen to be the first carrier protein for which an atomic resolution structure has been determined. Lactose permease catalyzes uptake of the disaccharide lactose into E. coli bacterial cells, along with hydrogen ions, driven by the protein electrochemical gradient.

A transport protein in which an exchange exists across the membrane, one solute for another, is classified as an antiport protein. A substrate binds and is transported across the membrane, then another substrate binds and is transported in the other direction. In this way it is an exchange, because the antiport carrier protein cannot undergo the conformational transition in the absence of bound substrate. An example of such is the adenine nucleotide translocase (also referred to as the ADP/ATP exchanger), which catalyzes a 1:1 exchange of ADP for ATP across the inner mitochondrial membrane



About figure: Mechanism of transport for symport (top) and antiport (bottom) carrier proteins. Concentration gradients for solute molecules are shown by corresponding colored triangles in which an energetically favorable reaction catalyzes that of an unfavourable condition.

Dissimilar solutes are transported in both cases, with final transport location on same side of the membrane for symport and opposite sides of the membrane for antiport.

Note: the cartoon with the protein of parallel lines is a hypothetical transition state as carrier proteins are never open to both sides of the membrane at the same time.

Learning outcomes

- A carrier protein cycles through a variety of conformational states.
- Solute binding sites become alternatingly accessible to either side of the membrane, but a complete opening through the membrane never exists with a carrier protein.
- A channel exists between either a closed or an open conformation, providing an open

channel straight through the membrane permitting flux of thousands of solute molecules.

- In uniport, the single solute transport is down the concentration gradient. In symport, dissimilar solutes are transported to the same side of the membrane, with one solute with and the other against the respective concentration gradient.
- In antiport, dissimilar solutes are transported to opposite sides of the membrane again with one solute following and the other opposing respective concentration gradients.

2.1.5.3 Passive transport

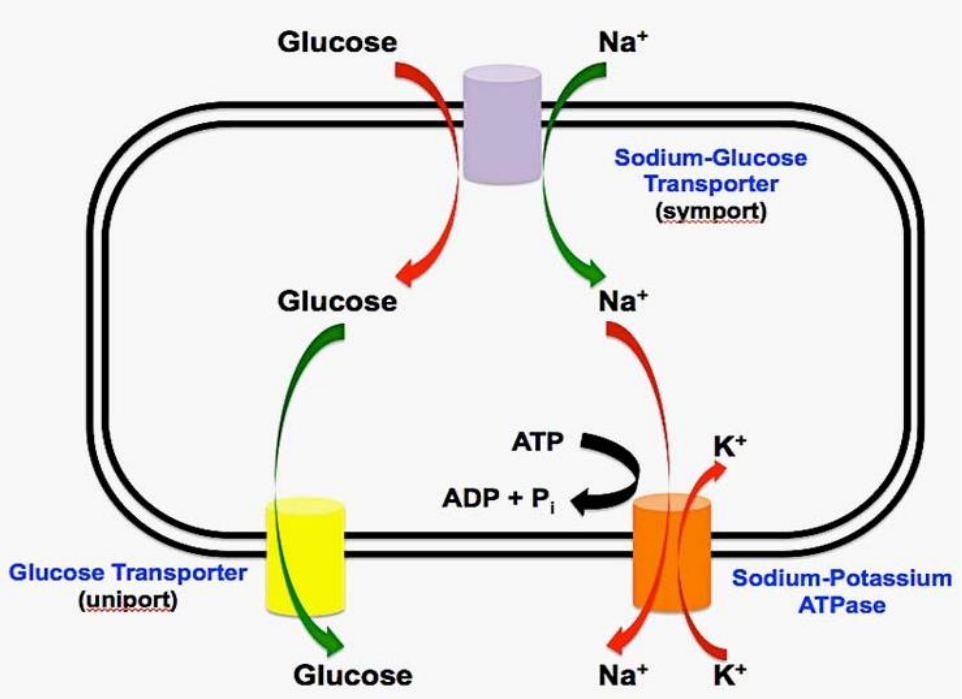
When a solute moves down its respective concentration gradient, a thermodynamically favorable (-ΔG) event, it is classified as passive transport. The solute is carried from an area of higher concentration to lower concentration, along the gradient, and therefore faces no resistance. Due to this lack of resistance, passive transport does not require any input of energy. Both carrier and channel proteins permit passive transport by facilitating the selective diffusion of solute molecules with their concentration gradient, termed facilitated diffusion.

Porins are beta barrel channels located in the outer membranes of bacteria, mitochondria and chloroplasts. Most currently known porins are trimers, in which each subunit is composed of 12 to 18 membrane spanning beta strands that are amphipathic. The alternating hydrophobic- hydrophilic amino acids lead to a water filled core that provides a hydrophilic passageway for the movement of ions or molecules across the membrane, without coming

into contact with hydrophobic membrane. An example of a porin channel protein is E. coli OmpF that forms a barrel structure. In OmpF monomer is composed of 16-strands, with a single loop in each monomer folding down and into the ß-barrel, preventing movement of substances greater than 600 D as well as providing a switch between open and closed conformations. When open, the OmpF protein permits passage of ions or small molecules with their concentration gradient, not requiring the usage of additional energy.

2.1.5.4 Active transport

During active transport, a solute is carried from low to high concentration regions, against its gradient. The solute molecule therefore faces resistance in this transport, and requires an input of energy. The conversion of energy stored in chemical bonds into energy stored in a concentration gradient defines active transport. In primary active transport, the binding and hydrolysis of ATP to ADP+P_i to the membrane transport protein drives the energetically unfavorable transport reaction. Binding of ATP to the protein triggers a conformational change, with the hydrolysis and release of ADP+P_i triggering another change in the conformation to generate a transport cycle, ensuring the mechanism of active transport operate only in a single direction. In secondary active transport, the uphill transmembrane movement of a substance is not directly coupled to the conversion of ATP to ADP+P_i. In this case, the transport protein utilizes a pre-established concentration gradient from an ATPase. The first transport protein, the ATPase, uses ATP in primary active transport to establish a concentration gradient of ions (H+ or Na+), with the secondary transport protein utilizing the stored energy in the established ion concentration gradient to transport solute molecules. Therefore, the secondary active transport of a solute is driven directly by the ATP-dependent formation of a gradient of a second molecule



About figure: Glucose transport into intestinal cells. A concentration gradient of sodium ions is established via the sodium-potassium ATPase in which generates a high extracellular concentration of Na+.

The sodium-glucose symport transport protein couples the energetically favorable movement of sodium ions into the intestinal cell, coupled with the unfavorable movement of glucose molecules also into the intestinal cell. Glucose thereby becomes concentrated inside of the cell, and exits the cell using its downhill concentration gradient via a passive glucose uniport transport protein subsequently moving glucose to the blood stream.

Green arrows depict energetically favorable events with red arrows depicting energetically unfavorable events.

Learning outcomes

- **Passive transport utilized the stored energy in concentration gradient. The transport of the**

solute faces no resistance and thus requires no input energy.

- **Active transport utilized the energy transport solutes against the resistance of their concentration gradient.**

- **Porins create a hydrophilic passageway through the hydrophobic membrane by their beta barrel formation.**

- **Primary active transport utilizes ATP by the direct binding and hydrolysis signalling the cycle through conformational changes to transport the solute molecule uphill against its concentration gradient. Movement of the solute is directly coupled to the reaction of ATP to ADP + Pi**

- **Secondary active transport is not directly coupled to ATP hydrolysis, instead the transport of a substance is driven directly by the ATP-dependent formation of a gradient of a second molecule. The transport protein utilizes the pre-established concentration gradient from an ATPase.**

2.2 ENDOPLASMIC RETICULUM

All eukaryotic cell have the largest single membrane bound intracellular compartment called **endoplasmic reticulum(ER).** ER is organized into a interconnected net like labyrinth of branching tubules and flattened sacs like structure and their membrane is continuous with the outer nuclear membrane. This enclosed compartment is called **ER lumen or ER cisternal.**

2.2.1 Structure of ER

ER is essential to every cell for its diverse structure and function. Sometimes the ribosomes (which synthesize protein) directly attach to the ER membrane. This region of ER is termed as **rough endoplasmic reticulum (RER).** The region of the ER that lack bound ribosomes are called **smooth endoplasmic reticulum (SER).** Centrifugation or homogenization of cells cause the breakdown of ER into fragments and reseals into small vesicles called **microsome**(~100-200nm in diameter**)**. Depending on the presence of the ribosomes on the membrane of microsome, microsome can be divided into rough microsome and smooth microsome. Area of the SER from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to golgi apparatus are called **transitional ER.**

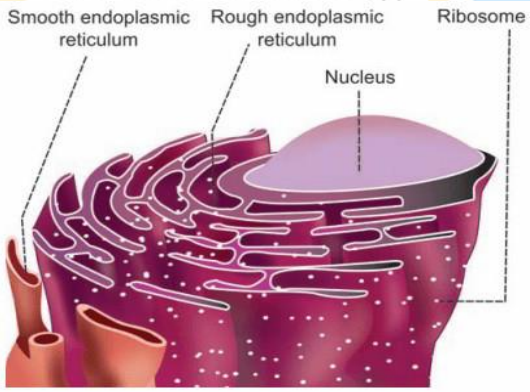


Fig: An endoplasmic reticulum with its division roughER and smoothER

2.2.2 Function of ER

Function of SER

- Synthesis of lipid
- Detoxification of organic compounds in liver cells
- Metabolism of heparin
- The SER serves as a strong place for calcium.

Function of RER

- Structural Protein synthesis by ribosomes
- Modification and the processing of newly synthesized protein : glycosylation in RER; folding of protein; formation of disulfide bonds within polypeptide.
- Transport of the proteins.

2.2.3 Transport of protein across the ER membrane

Rough ER secrete large amount of secretary proteins. Polypeptides are synthesized two distinct locales with in the cell.

1. Approximately one third of the proteins encoded by the mammalian genome are synthesized on the ribosome attached to the cytosolic surface of the RER membrane and reside within compartments of the endomembrane system including ER, glogi complex, lysosome, endosome, vesicles and plant vacuoles. These are secreted protein; integral membrane proteins; soluble proteins.
2. Other polypeptides are synthesized on free ribosomes that is ribosomes which are not attached with RER. These are protein destined to remain in cytosol (i.e. enzyme of glycolysis, protein of cytoskeleton); peripheral protein of cytosolic surface of membrane (i.e sprectrin, ankyrin), proteins are transported to nucleus and the protein to be incorporated into peroxisomes chloroplast and mitochondria. Proteins in the later two groups imported posttranslationally(polypeptides totally synthesize in cytosol then imported into organelle) in to the appropriate organelle.

In 1971 G Blobel, D Sabastini, B Dobberstein demonstrated that

1. Secretory protein contaoin a signal sequence at their N-terminus that directs the emerging polypeptide and ribosome to the ER membrane.
2. It was proposed that the polypeptide moves through the membrane as it is being synthesized, that is cotranslationally.

This is known as **signal hypothesis.**

- *The steps that take place during the transfer and synthesis of protein specially secretory lysosomal, plant vacuolat protein are:*

  Synthesis of polypeptide begins after a mRNA binds to the free ribosome. A signal sequence at N terminal of the nascent peptide, typically contain 6-15 hydrophobic amino acid leads to the compartmentalization of the polypeptide within the ER lumen. As it emerge from the ribosome this signal is recognized by a **signal recognition particle (SRP)** which consis six distnict polypeptide and a small RNA molecule called the 7SLRNA. SRP stops further translation until the SRP-ribosome-nascent chain can make contact with the ER membrane. Binding with ER occurs through two interaction 1. Interaction between SRP and SRP receptor 2. Ribosome and the translocon. **SRP receptor** is a G protein couplrd receptor. In acive state GTP is attached with it and the hydrolysis of the bound GTP turns it off. In eukaryotic cell **translocon** is made up of Sec61 complex (Sec61 contain three subunits sec61α, Sec61β, Sec61γ). In inside view Translocon has an hourglass shape with a plug formrd out of a short α helix at its inactive state. The SRP-ribosome-nascent chain complex binds to ER membrane SRP is released from its receptor and ribosome become attached to the cytosolic end of the translocon. Then signal sequence enter through the translocon where it serves as a **start transfer signal** that open the pore by displacing the plug from the channel and allowing the reminder of the polypeptide to translocate through the membrane cotranslationally. After the nascent peptide pass into the luman of the ER, the signal peptide is cleaved by membrane protein. This protein undergoes the folding.

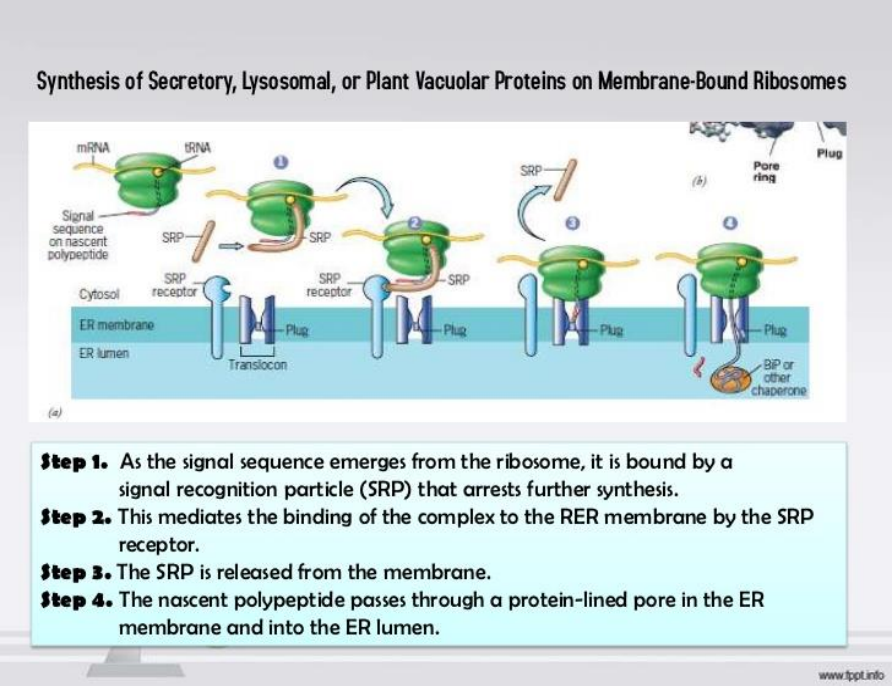


Fig: synthesis of secretory lysosomal or plant vacuolar protein on the membrane bound ribosome.

2.2.4 Steps of translocation of integral membrane protein:

Integral proteinS are also synthesized on membrane bound ribosome of the ER using the same mechanism describe above. Unlike soluble secretary and lysosomal protein which pass entairely through the membrane of ER , integral protein have one or more hydrophobic transmembrane region that are shunted directly from the channel of the translocon to the lipid bilayer. Translocator(translocon) are therefore gated into two direction: 1. It open to form a pore across the membrane to let the hydrophilic protion of protein cross the lipid bilayer, 2. It open laterally within the membrane to let hydrophobic portion of the protein partition into the lipid bilayer. **Lateral gatting** is important during the integration of transmembrane protein. There are four process for the translocation of transmembrane protein:

For single pass transmembrane protein

1. In simplest case a start signal sequence initiate the translocation but an additional hydrophobic sequence known as stop transfer sequence which serves stop transfer signal which terminate the transfer process before the entair polypeptide chain is translocated. Now the stop transfer sequence anchors the protein after the start signal sequence have been cleaved off and release from the translocator. By lateral gattin this stop signal sequence is transferred into lipid bilayer where it remains as a single α helical structure with N terminus in luminal face and c terminus at cytosolic face.
2. If the start signal sequence is internal rather than the N terminal there are two possible type of trans location depending on the position of the signal sequence on the peotide chain. In one case resulting protein have N terminal at luminal side while in other case resulting protein has C terminal at luminal side. There is no stop transfer sequence.

For multipass transmembrane protein

3. In multipass transmembrane protein 1st internl signal sequence serves as start transfer signal which continues the translocation until the stop transfer sequence is encounter by translocon. After that the 2^{nd} internal start signal sequence reinitiate the translocation until the 2^{nd} stop transfer signal comes on and this continues until the last stop signal codon is encountered by the translocon.

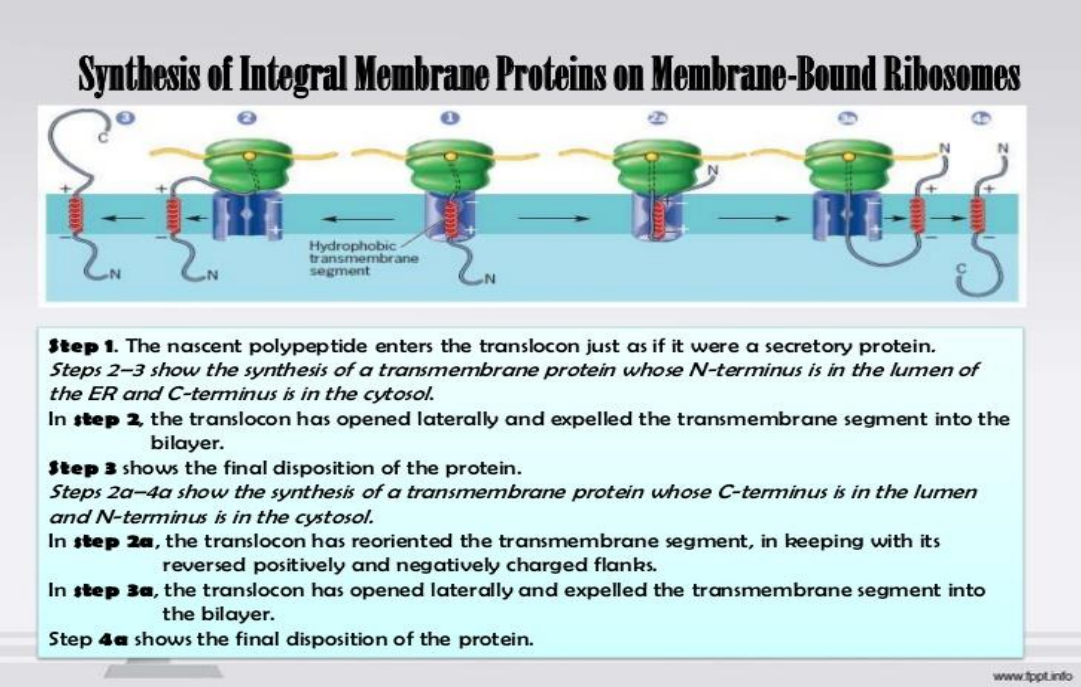


Fig: Ttranslocation of integral protein into the ER membrane

2.2.5 N LINKED GLYCOSYLATION:

As the nascent protein enter RER cistern, signal peptide at N terminal is removed by signal peptidase and thr carbohydrates are added to this protein is commonly known as glycosylation. Most common process of glycosylation is to transferred a preformed precursor oligosaccharide(Glc3Man9GlcNAc2) to proteins that are processed into ER—mostly the protein transported to glogi apparatus, lysosome, plasma membrane or extracellular space. They commonly known as glycoproteins. The addition of sugars in a oligosaccharide chain is catalyzed by a group of membrane bound enzyme complex oligosaccharide transferases. This oligosaccharide chain initially bound with a dolichol phosphate, a lipid carrier embedded in the ER membrane. The process begins with the transfer of N acetylglucosamine 1 phosphate followed by the transfer of another N acetylglucosamine. Then five residues of mannose are added to the last N acetylglucoamine. The dolicol with seven sugars flipped across the membrane and the

remaining sugar i.e. additional four residues of mannose and three glucose molecule are attached on the luminal face of the ER. Now the oligosaccharides are completely assembled and transferred enzymatially to the aspergine residue within in a special tri peptide sequence Asn-X-Ser/Thr (X is any amino acid except proline). An antibiotics— Tunicamycin— blocks the first step of this pathway thus inhibit the synthesis of oligosaccharides. Congenital Diseases of Glycosylation (CDGs) are identified through blood tests that detect the abnormal glycosylation of serum proteins.

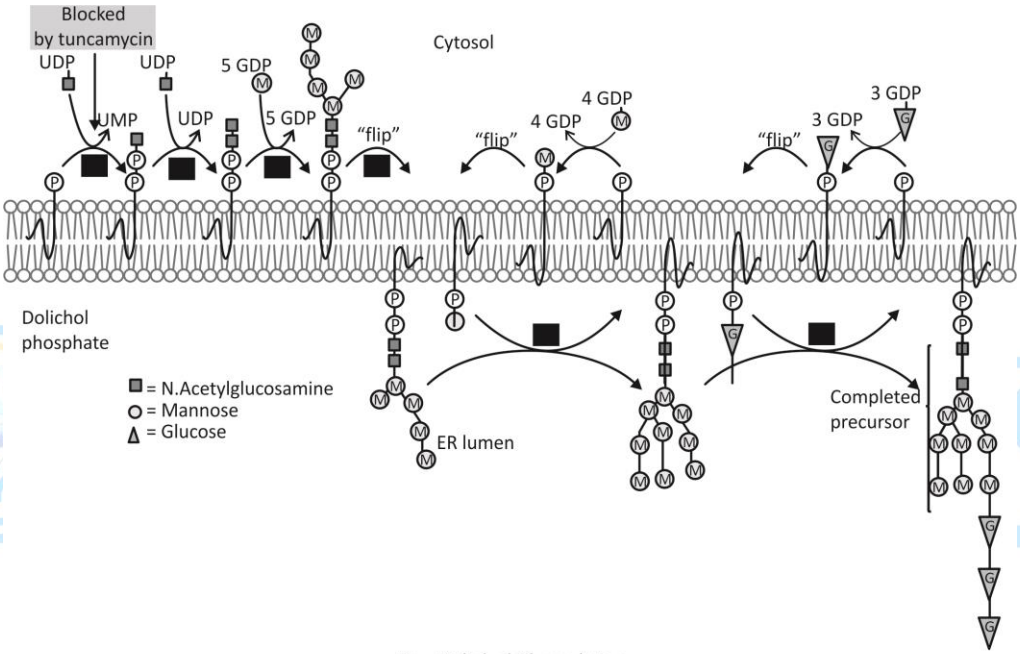


Fig. : N–linked Glycosylation

Types of bonds present in $Glc_3Man_9GlcNAc_2$ on dicol carrier:

Presence of bond	Type of bonds
Bonds between 1st N acetylglucosamin and dolicol	Pyrophosphate bridge
Bonda between 1st and 2nd N acetyiglucosamin	β1,4 glycosidic bond
Bonds between 1st mannose and 2nd N acetylglucosamine	β1,4 glycosidic bond
Bonds between 9th mannose ans 1st glucose	α1,3 glycosidic bond

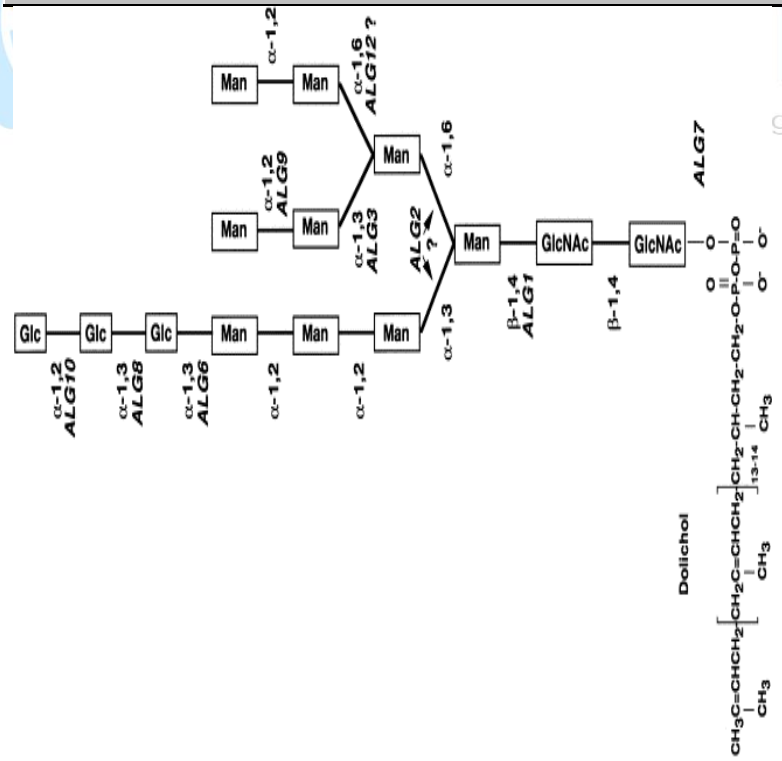


Fig:Bond between N linked oligosaccarides

Function of N linked glycosylation:

- Immune system N linked glycans help to detect the migration pattern of cell
- Provide structural component of cell wall
- Modify protein such as stability and solubility.
- Direct trafficking of glycoproteins.
- Cell cell interaction.

2.2.6 Modification of newly synthesized protein

This modification process is held by enzymetically removal of two glucose residues. In this stage nascent protein are screened by a system called **quality control** which determines whether the protein is move on to the next compartment or not. In this stage the protein contain one glucose binds with the ER chaperon i.e. **calnexin and calreticulin**. If the fold is proper this chaperon is removed by removal of glucose residues by **glucoidase II** enzyme. A conformation sensing enzyme called **UGGT** recognizes incompletely folded or misfolded of protein. UGGT add the single glucose residue back to the mannose residues and give the protein an another chance to fold properly. When the accumulation of misfolded protein **(unfolded protein response; UPR)** is increased in ER another chaperons in ER named **Bif** helps to recover from this situation. Bif leads to the removal of protein which degraded by proteasome complex. This process is called *ER associated degradation* **(ERAD).**

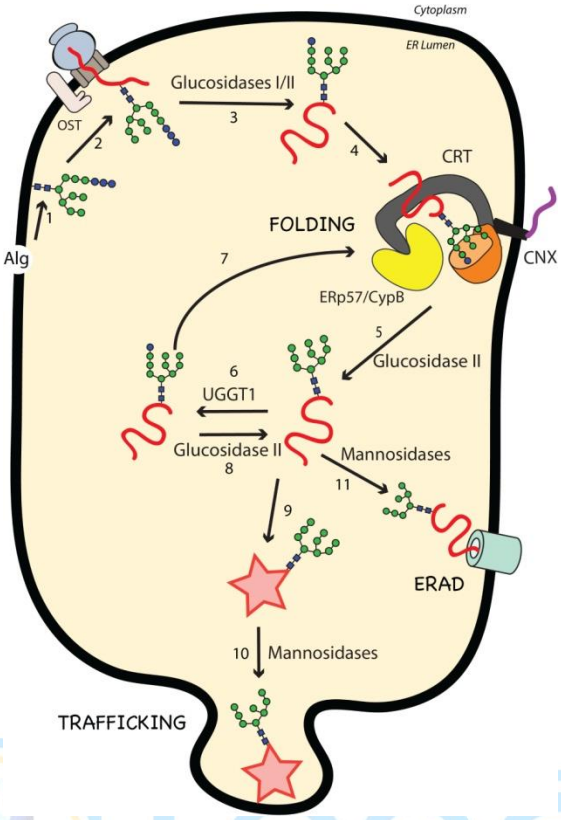


Fig: folding of protein in endoplasmic reticulum

ER membrane also contain a number of protein processing enzyme, such as **protein disulfide isomerase (PDI)** which helps in formation of disulfide bond between the cysteine residues of the newly entered protein. Disulfide bond gives stability to the protein which are present at the *extracellular surface of the plasma membrane or secreted in extracellular space.*

2.2.7 Synthesis of membrane lipid:

Most membrane lipids are synthesized in the smooth ER. There are some exception. That are: 1.*sphingomyelin and glycolipids* are synthesized early in ER and completed in the Glogi apparatus. 2. Some *unique lipid in mitochondria and chloroplast* membrane synthesized by the enzyme present in their membrane.

The major classes of phospholipids and cholesterols are formed in smooth ER. The major phospholipid made is phosphotidylcholin at the outer leaf let of ER by the enzyme present in the membrane of ER.

2.2.8 Detoxification of xenobiotics compound:

Detoxification of xenobiotics compounds such as ethanol and barbiturates are held in liver can leads to proliferation of SER in liver call. Detoxification is carried out by a collection og oxygen transferring enzyme, including the cytochrome P450 family. The results are not allows positive. For an example the relatively harmless compound benzo[a] pyren formed when meat is charred on a grill is converted into a potent carcinogen by the detoxifying enzyme of SER.