Q Protein Synthesis on Free & Membrane-bind ribosomes, Uptake into ER, Glycosylation Sorting (in GB) & Trafficking of proteins.

A cell needs proteins in a very diversified manner. The proteins may be membrane proteins present in the plasma membrane or membranes of different cell Organelle, lumen proteins & enzymes present in the lumen of various cell Organelles, Cytoplasm proteins & enzymes, Secretory proteins etc.

These all proteins are synthesized within the cell, processed and then targeted to their specific target located.

All the proteins synthesized within the cell, then having their own definite fate. Within the cell several factors & mechanisms help to determine whether the newly synthesizing protein has to be retained in cytoplasm or in nucleus or in mitochondria or other cell Organelles or whether it will be embeded as a membrane protein in plasma membrane or membrane of other Cell Organelle or, in the lumen of various cell Organelle like lysosomes, ER, GB, peroxisomes etc. or whether it is a Secretory protein which is to be secreted outside the cell.

The determination of fate of the synthesizing proteins is called as "Protein Sorting" and the pathway taken by different group of proteins to reach to their different specific target destinations is called "Protein trafficking"

Protein Synthesis :-

The synthesis of all types of cellular proteins occurs by translation of mRNA on Ribosomes. The synthesis of protein always starts with the attachment of small sub-unit of ribosome with m.RNA, then large sub-unit get attached to it, to form a complete protein. Synthesis initiation complex. Now, ribosomes moves along the mRNA, reading the nucleotide sequence in the form of codons. Complementary to each codon, there is one anticodon present on specific t-RNAs which carry specific amino acids. The t-RNA along with attached amino acid enter into ribosome through A-site. The codon-anticodon matching occurs in P-site. If matching is done, tRNA detaches the amino acid and comes out of ribosome through E-site. If Codon-anticodon mismatches, tRNA along with it is amino acid, comes out of ribosome through E-site.

Similarly the next codon is matches with anticodon of incoming

t-RNA and the process goes on. The P-site of ribosome contains an enzyme Peptidyl transferase, which forms peptide bonds between the amino acids that are left in the P-site after Codon-anticodon matching, thus a gradually increasing chain of amino acids is formed which finally comes out of ribosome & changes freely in Cytoplasm. This process is called Elongation.

As the ribosome moves towards 3' end of mRNA, the last Codons are Stop Codons, for which there are no anticodons in t-RNA. So as ribosome reaches at Stop Codon the entire translation complex get dissociated releasing the newly synthesized protein. This process called Termination.

* Fate of newly Synthesizing Protein :-

If the protein is destined to go into nucleus, mitochondria, chloroplast or peroxisomes, then it is synthesis occur on free ribosomes.

But, if the protein is destined to go into plasma membrane, Lysosomes or if it is a Secretory protein which is to be excreted outside the cell, then synthesis of such proteins occur on membrane-bound ribosomes (ribosomes bound to Endoplasmic Reticulum membrane).

Protein Synthesis (Translation of mRNA)

- On free ribosomes → Nucleus, Mitochondria, Chloroplast, Peroxisomes, etc.
- On membrane-bound ribosomes (on ER) → Plasma Membrane, Lysosomes, Secretory vesicles.

* Protein Synthesis on membrane-bound ribosomes and uptake into ER:-

Signal hypothesis (uptake of Proteins into ER):-

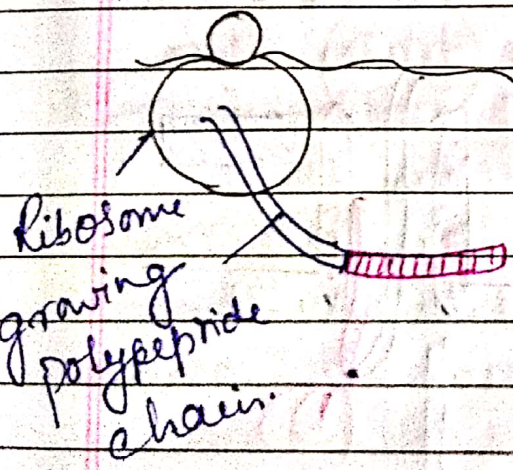
The synthesis of all the cellular proteins start on free ribosomes. If the proteins are destined for plasma membrane, lysosomes or secretory proteins, then the whole protein synthesis machinary (i.e., mRNA, Ribosomes and associated factors) is targeted towards Endoplasmic Reticulum by a special sequence called Signal sequence at the ~~Amino~~ amino terminus of the growing polypeptide chain.

These signal sequences are short stretches of hydrophobic amino acids. The signal sequences can be 20 Amino acids long.

The signal sequence is recognized and bound by SRP (Signal Recognition Particle), which consists of 6 polypeptides and a small cytoplasmic RNA i.e., 7SL RNA. Binding of SRP inhibits further translation and targets the complex (i.e., SRP, Ribosome, mRNA and growing polypeptide chain) to the rough ER.

The rough ER membrane has SRP receptor and ribosome binding site called Sec 61 complex (Translocation Complex), which consists of 3 membrane spanning proteins. The SRP binds to the Sec 61 Complex channel.

Now, the growing polypeptide chain is translocated into the ER lumen through Sec 61 Complex channel. The signal sequence is none



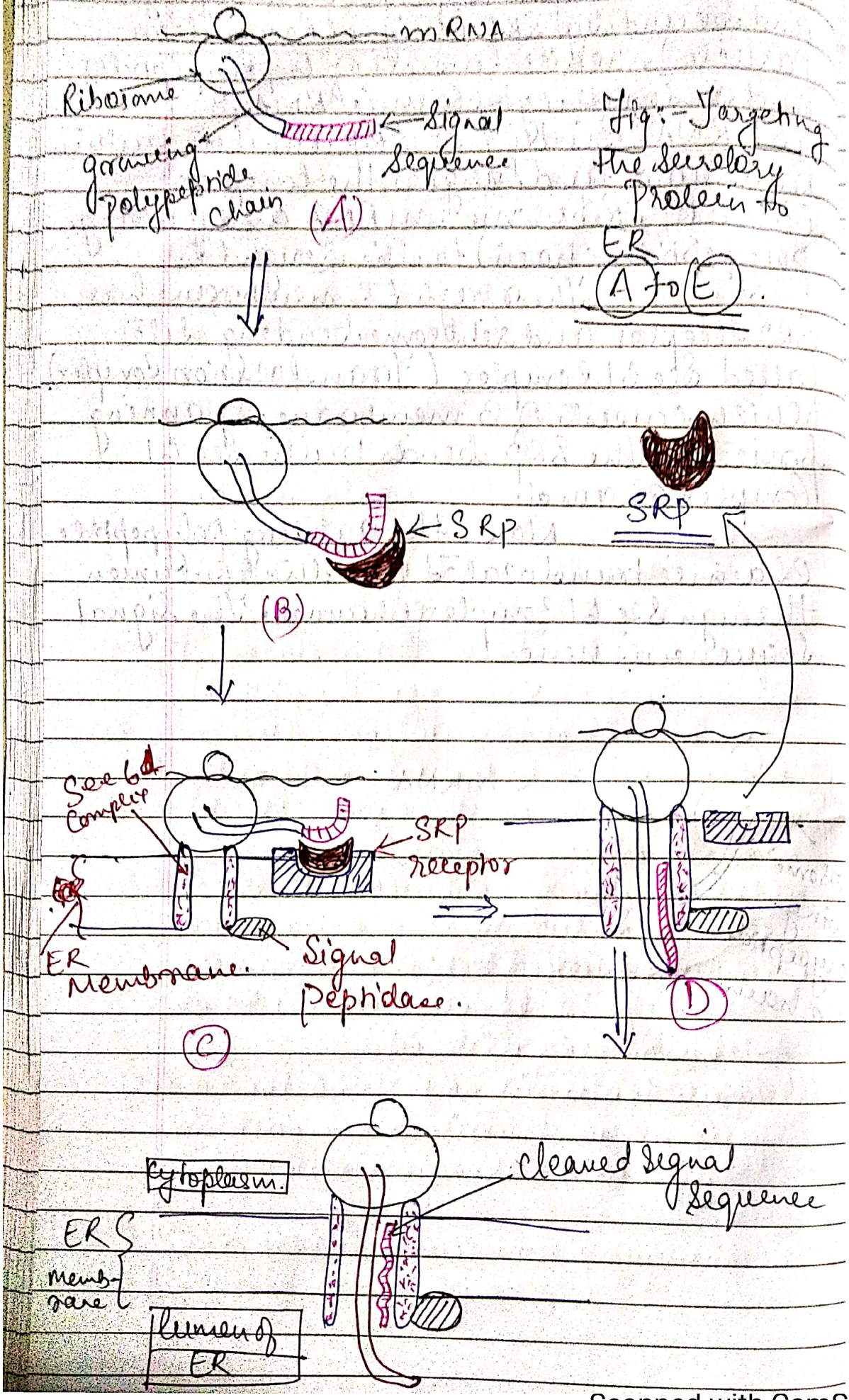


Fig:- Targeting the secretory Protein to ER

A to E

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cleaved by an enzyme called "Signal peptidase" present in association with the Sec 61 Complex.

* Incorporation of Proteins into ER:-

In the proteins destined for secretion or lumen of ER, Golgi body or lysosomes, then the protein is translocated into the lumen of ER. But if the protein is destined as membrane protein. Moreover, the polypeptide (or protein), which are destined as membrane proteins, have internally situated (Stop-transfer sequence), which help the protein to get several membrane spanning regions.

Protein folding and processing (Glycosylation) in ER:-

(Post-translational modifications of ER):-

As the polypeptide grows inside the ER lumen, it's folding disulphide bond formation etc will occurs and the process of glycosylation started.

Protein folding in ER:-

There are special proteins which help in proper folding of the newly synthesized or growing polypeptide chain. One of the protein that helps in protein folding is Bip (Binding protein) present in ER lumen. It is a member of HSP70 (Heat-shock protein) family of Chaperons Bip binds to growing polypeptide chain and mediates its folding or assembly of different protein sub-units. Correctly assembled proteins are then correctly assembled proteins are then transported to Golgi, but incorrectly assembled or folded proteins are retained in ER lumen & degraded.

The disulphide bond (-S-S-) formation is also important in protein folding, which is done by an enzyme "Protein disulphide isomerase".

* Glycosylation of Proteins in ER :-

Glycosylation means addition of Oligosaccharide (Carbohydrate) to the newly synthesizing protein with ER, the Oligosaccharide chain Consisting of 14 sugar residues are added to Asparagine residue (amino acid) of the growing polypeptide Chain. These Oligosaccharide chains are synthesized on a carrier lipid ie. Dolichol found embedded into ER membrane. The Oligosaccharide Chain is then transferred to Asparagine of Asp - X - Ser / Thr. Consensus Sequence of the polypeptide. This transfer of Oligosaccharides from Dolichol to growing polypeptide is done by an enzyme called "Oligosaccharyl transferase", which is a membrane-bound enzyme.

None, 4 sugar residues ~~(3 Mannose~~ (3 Mannose & 1 Glucose) are removed from the Oligosaccharide attached to the polypeptide and further modifications occur in Golgi body. It means, Process of Glycosylation starts in ER but Completes in GB.

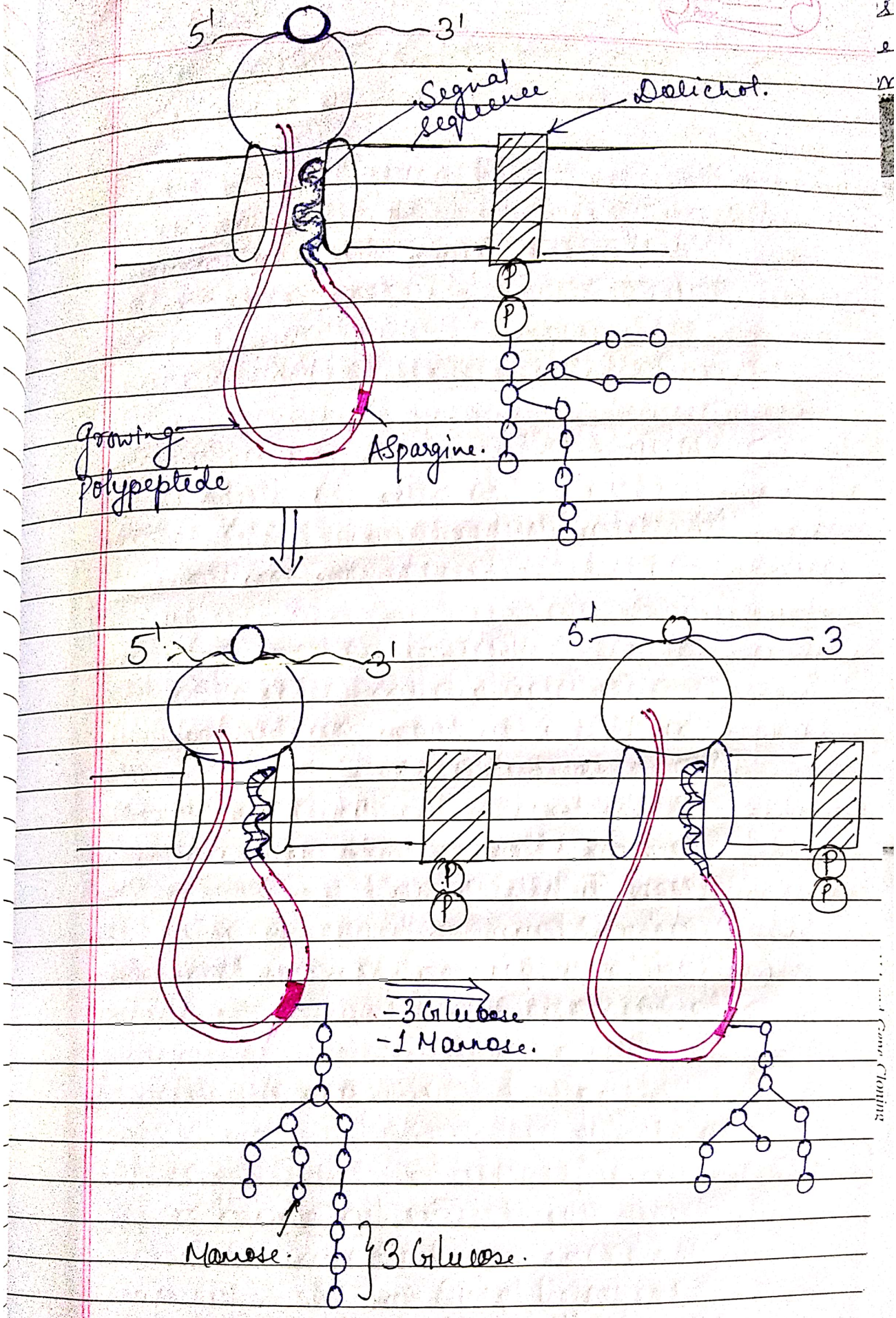


Fig: Process of Glycosylation in ER

* Protein Export from ER:-

The proteins which are synthesized on Membrane-bound ribosomes are either membrane proteins (embeded into ER membrane) or lumen proteins. The proteins are packaged into vesicles are carried to the Golgi body (cis golgi network). However, there is a non-selective bulk flow of proteins from ER to GB. It means, the protein export from ER is non-selective. Therefore, within GB, the sorting of proteins are transported to their target sites. Then, the proteins of ER membrane or ER lumen are exported from ER to GB. But, these proteins have special sequences lumen have a targeting sequence of Lys-Asp-Glu-Leu (KDEL) at their Carboxy-terminus and the proteins of ER membrane having a Lys-Lys-x-x (kkxx) at their C-terminus. Therefore, the proteins having KDEL or KKXX sequences are sent back to ER from GB.

Thus, actually the proteins of ER are also packaged into vesicles and transported to Golgi. These proteins have either ~~KDEL~~ KDEL or KKXX sequences. (targeting sequences). from GB, these ER proteins are recycled back to ER.

* Glycosylation (Post-translational modification) of Proteins in GB:-

The process of glycosylation started in ER, but completes in GB. The glycosylation of proteins in GB involves:-

→ If the proteins are destined for plasma membrane or it is a secretory protein:-

5 Mannose residues are removed, then 1-N-Acetyl Glucosamine (NAG) added, then 2 Mannose again removed, then 1 Fucose added and 2 NAG again added, then 3 Galactose and 3 Sialic acid added.

→ Proteins destined for lysosomes instead removal of Mannose, ~~N-Acetyl~~ N-Acetyl Glucosamine (NAG) phosphate is added to the Mannose residue.

During passage of Proteins from Cis-golgi to trans-golgi network the NAG is removed, thereby Mannose-6-Phosphate residues are left in the protein. The trans golgi having Mannose-6-phosphate receptors which bind to these proteins having Mannose-6-phosphate residues and then these proteins are targeted to Lysosomes.

* Sorting And Trafficking of Proteins in GB:-

The proteins synthesized on membrane bound ribosome on ER, are transported to GB in a non-selective bulk flow, within GB, the proteins are sorted and targeted to their specific target-sites or destinations.

The sorting of proteins according to their specific destinations involves:-

→ If the protein is a protein of ER:-

The lumen proteins of ER has a specific sequence of amino acids Lys-Asp-Glu-Leu (KDEL), and the membrane proteins of ER also having a targeting signal Lys-Lys-XX (KKXX). These proteins are sent back through a recycling pathway from GB to ER.

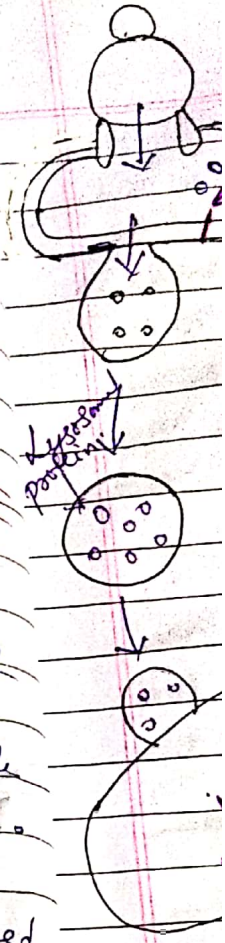
→ If the Protein is a protein of GB:-

The proteins of GB must be retained within GB. The GB membrane proteins have short trans membrane α helices of 15 amino acids which may provide the signal for retention of these proteins within GB. The luminal proteins of GB may have some are retention signals.

→ If the Protein is a Secretory Protein:-

In bulk flow secretory pathway, there is continuous unregulated secretion of proteins i.e. the secretory proteins are packaged into vesicles and exported from trans golgi network to fuse with plasma membrane so as to release the secretory proteins outside the cell. However, in some cells, the secretory proteins are secreted in response to specific signals. Such regulated cell secretion include

hormone secretion, neurotransmitter secretion, secretion of digestive enzymes etc. These proteins are packaged into vesicles, which store them, and as specific signal comes these vesicles fuse with plasma membrane to release their content outside.



⇒ If the protein is a Lysosomal protein:-

If the protein is a protein of Lysosomes, then N-Acetyl Glucosamines phosphate (NAG-P) is added to the Mannose residues of the Oligosaccharide Chain which is attached to the protein. As these proteins move from cis-golgi to trans-golgi network, NAG is removed as result Only Mannose-6-phosphate residues are left in the protein. Now, these proteins are attached to Mannose-6-phosphate receptors in trans-golgi network, from where these proteins are targeted to lysosomes.

→ If the Protein is a membrane protein of Plasma Membrane:-

The membrane protein may have some specific clues which direct the vesicles containing membrane proteins to specific sites on the plasma membrane.
For eg:- Presence of GPI anchors direct the membrane protein to be

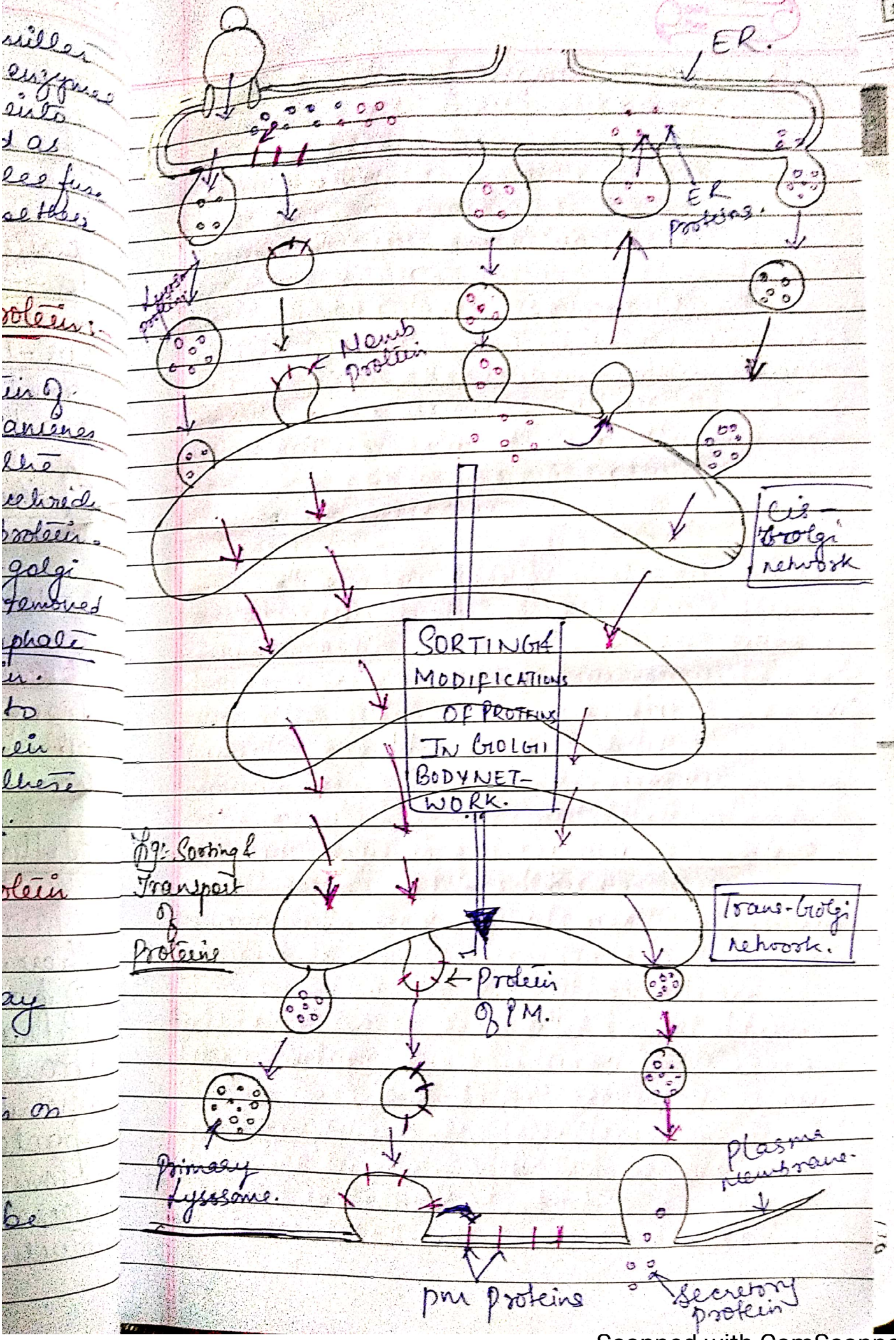


Fig: Sorting & Transport of Proteins

sent to the apical domain of plasma membrane of epithelial cells (mucosal cells) of intestine. However, if there is no specific target site in plasma membrane the membrane proteins are targeted to their target membrane in a non-selective manner.

Differences between RER and SER :-

The Endoplasmic Reticulum occurs in two forms within the cell :- the Rough ER and Smooth ER. The main differences between these two are :-

Rough ER	Smooth ER.
i) Ribosomes are found attached to RER, thereby surface appears granular.	i) No ribosomes attachment with SER membrane, thereby surface is smooth.
ii) Mainly helps in Protein Synthesis & post translational modifications in the newly synthesized proteins.	ii) Mainly involved in Lipid Synthesis including membrane phospholipids, Steroid hormones, Cholesterol Synthesis etc.
iii) Involved in the process of Protein folding and Protein Glycosylation.	iii) No role in such functions.
iv) No role in Bile acid Synthesis and Detoxification	iv) SER plays important role in Bile acid Synthesis and Detoxification.

Rough ER	Smooth ER
v) No role in Synthesis of Triglycerides and steroid pigments	v) Involved in Synthesis of Triglyceride in intestinal cells and visual pigments (Eg Rhodopsin) from vitamin A in cells of Retina of eye.
vi) No role in Glycogen breakdown (Glycogenolysis).	vi) Contains an Enzyme Glucose-6-phosphatase as a membrane protein of SER, which plays an important role in Glycogenolysis.