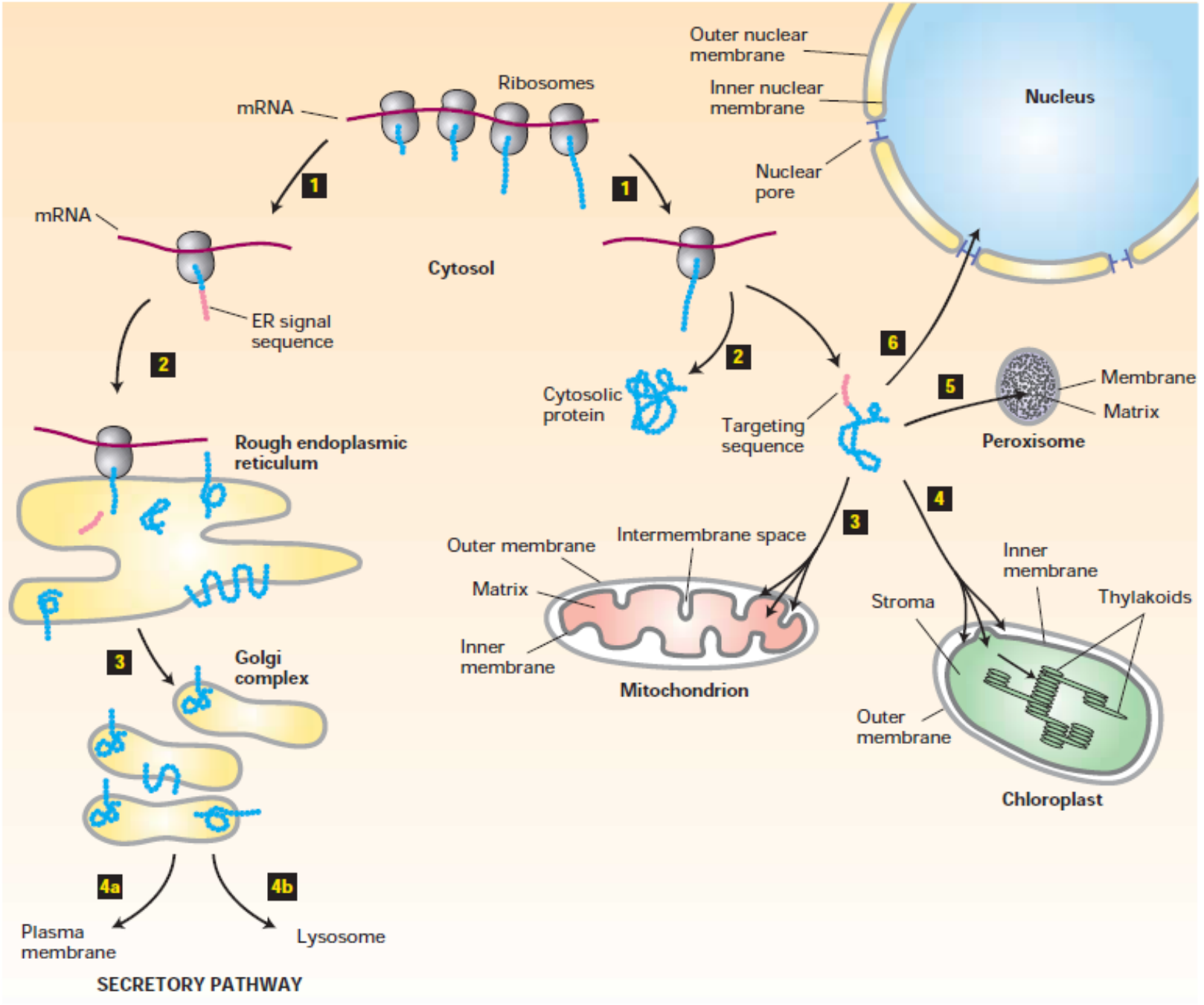
Lecture 10

Protein targeting



Protein modifications

Membrane and soluble secretory proteins synthesized on the rough ER undergo four principal modifications before they reach their final destinations:

(1) addition and processing of carbohydrates *(glycosylation)* in the ER and Golgi,
(2) Formation of disulfide bonds in the ER,
(3) proper folding of polypeptide chains and assembly of multisubunit proteins in the ER, and
(4) specific proteolytic cleavages in the ER, Golgi, and secretory vesicles.

Protein modification - glycosylation

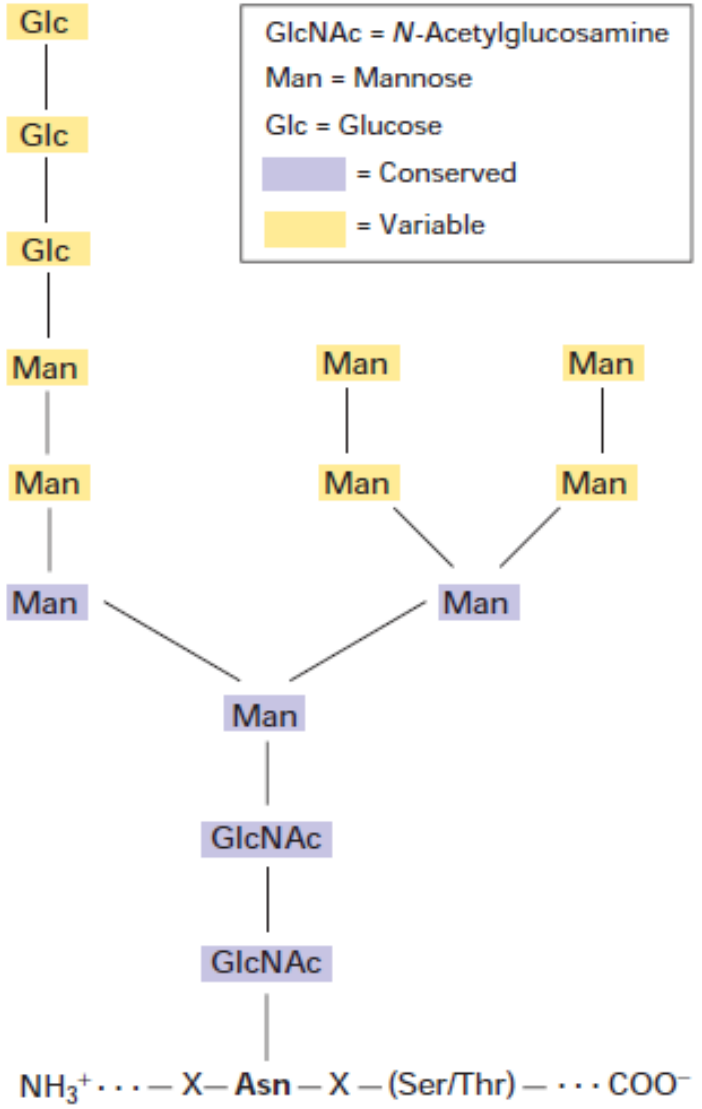
One or more carbohydrate chains are added to the vast majority of proteins that are synthesized on the rough ER;
glycosylation is the principal chemical modification to most of these proteins.

Carbohydrate chains in glycoproteins may be attached to the hydroxyl group in serine and threonine residues or to the amide nitrogen of asparagine.

These are referred to as *O-linked* and *N-linked oligosaccharides*, respectively.

The entire 14-residue precursor is transferred to the asparagine residue on a nascent polypeptide as it emerges into the ER lumen

Only asparagine residues in the tripeptide sequences Asn-X-Ser and Asn-X-Thr (where X is any amino acid except proline) are substrates for *oligosaccharyl transferase,* the enzyme that catalyzes this reaction.



Functions

- to fold properly in the ER
- confer stability
- cell-cell adhesion

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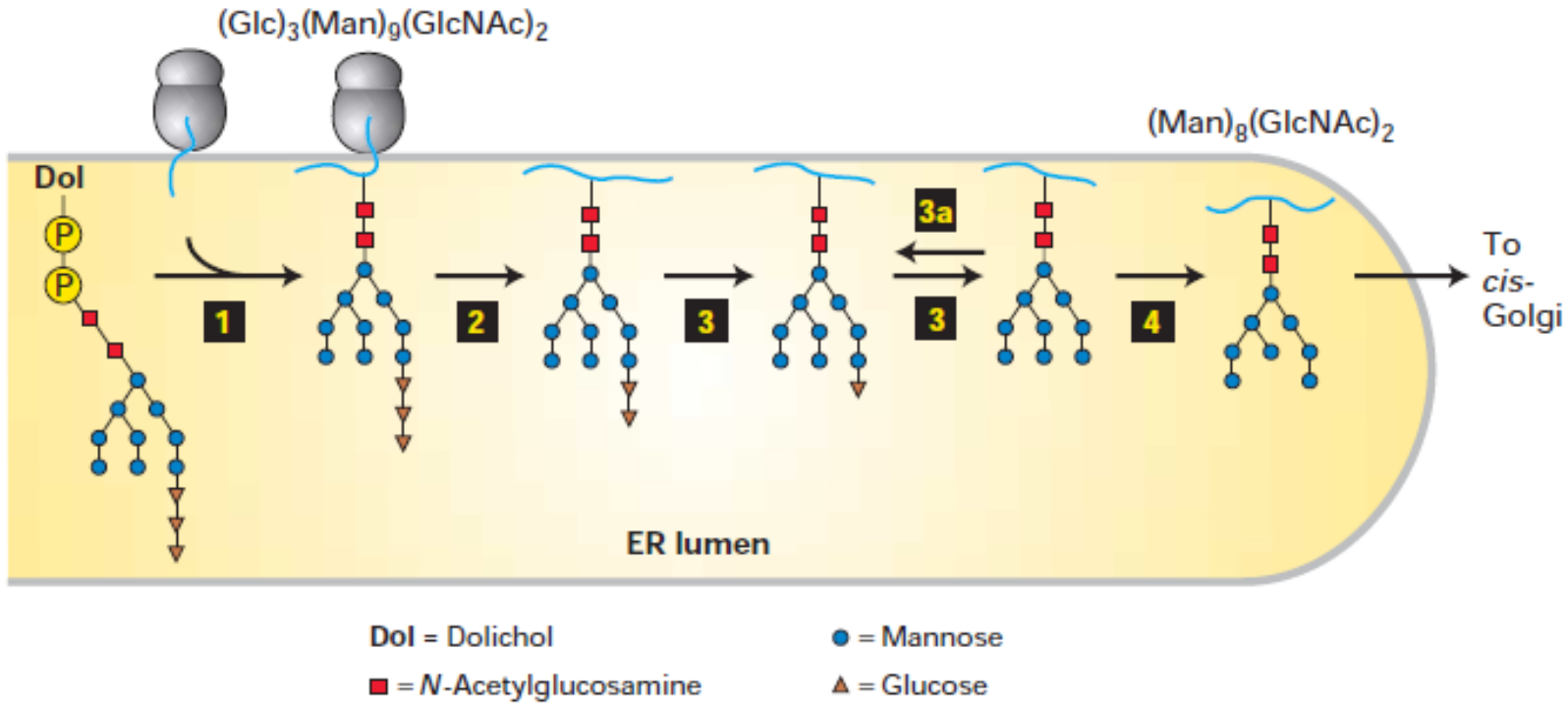


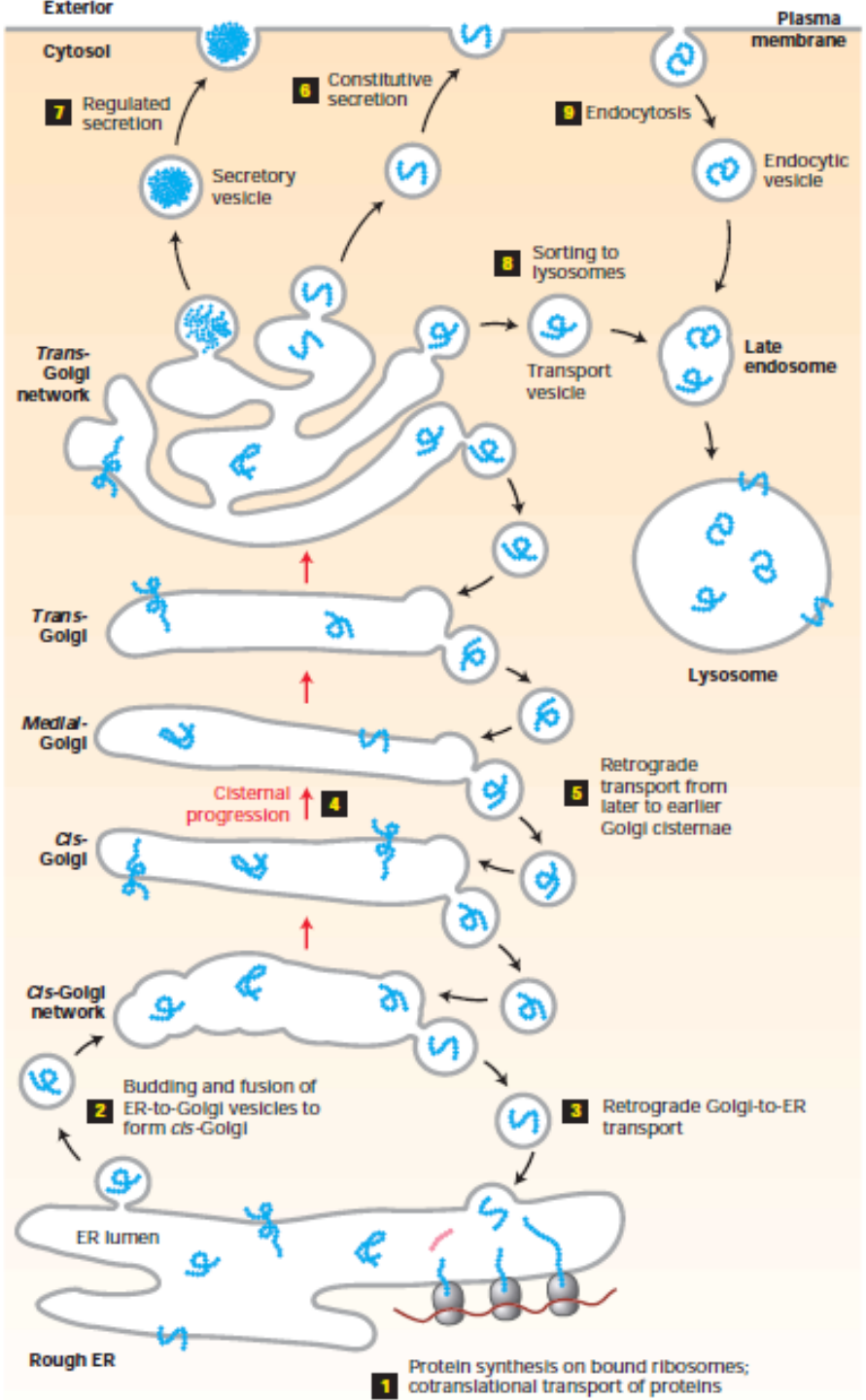
A second general sorting process applies to proteins that initially are targeted to the ER membrane, thereby entering the **secretory pathway.**

These proteins include not only soluble and membrane proteins that reside in the ER itself but also proteins that are secreted from the cell, enzymes and other resident proteins in the lumen of the Golgi complex and lysosomes, and integral proteins in the membranes of these organelles and the plasma membrane.

Transport vesicles that collect "*cargo" proteins* in buds arising from the membrane of one compartment and then deliver these cargo proteins to the next compartment by fusing with the membrane of that compartment.

Anterograde (forward-moving) transport vesicles.

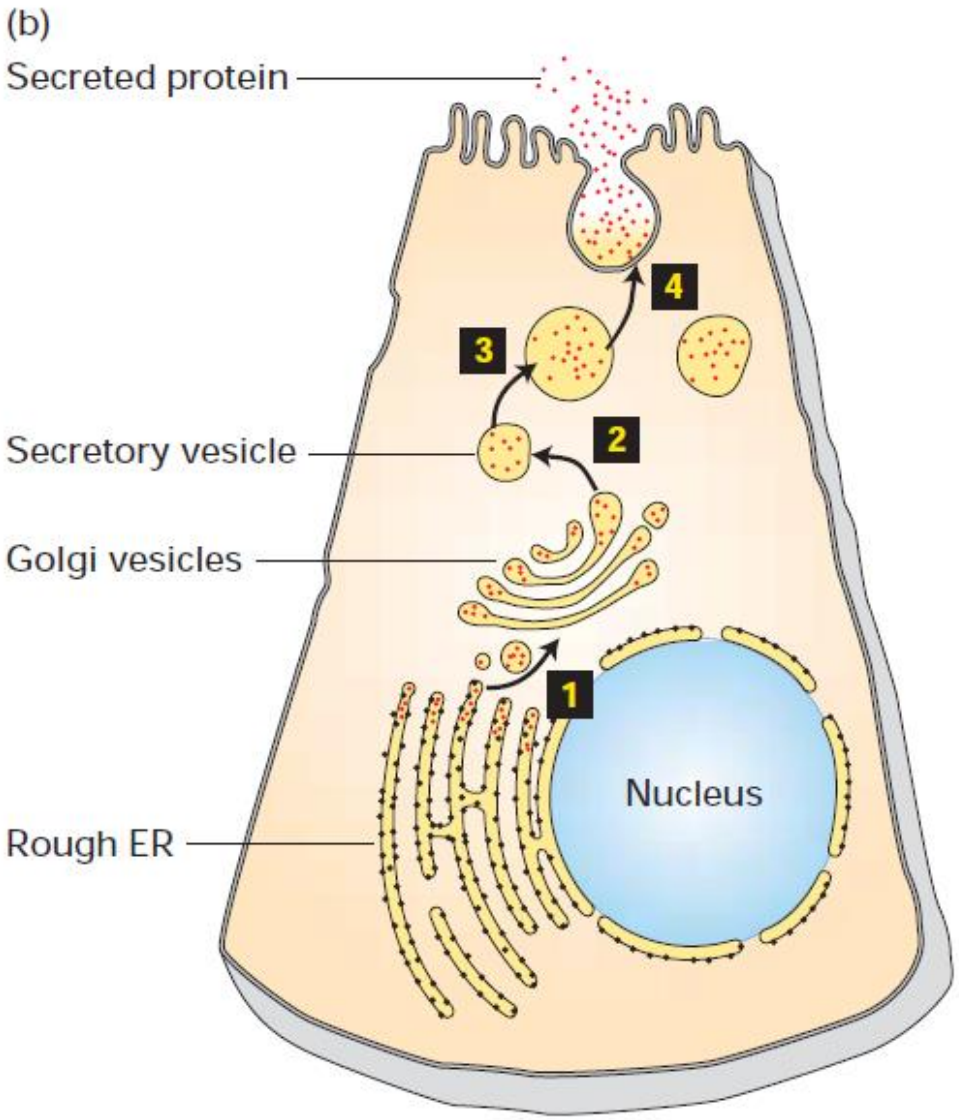
retrograde (backward-moving) transport vesicles.



Exocytosis
Secretory vesicles
Vesicles that fuse with Lysosome

Endocytosis

Secreted proteins



A typical secretory cell tracing the pathway followed by a protein (small red dots) to be secreted.

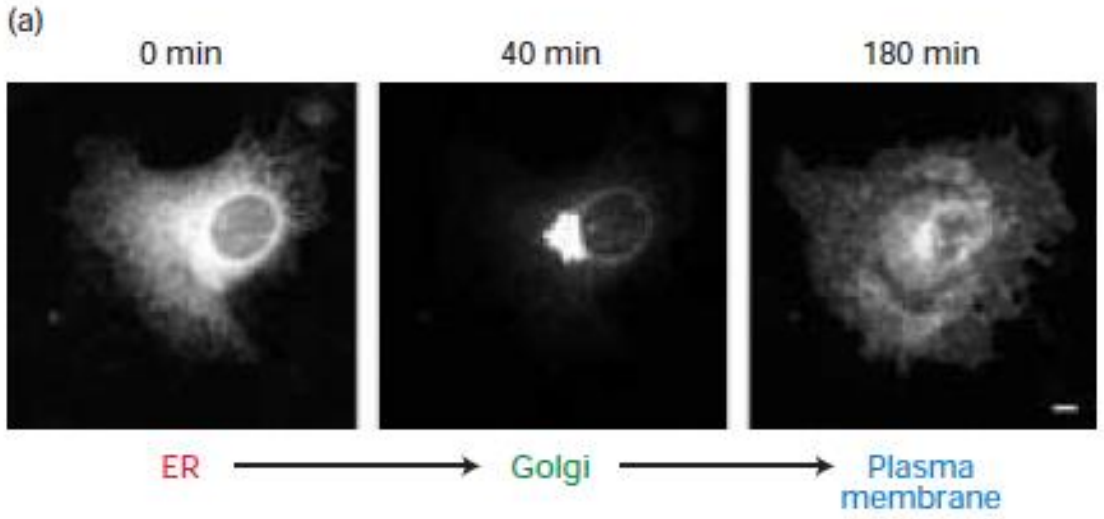
Immediately after their synthesis on ribosomes (blue dots) of the rough ER, secreted proteins are found in the lumen of the rough ER.

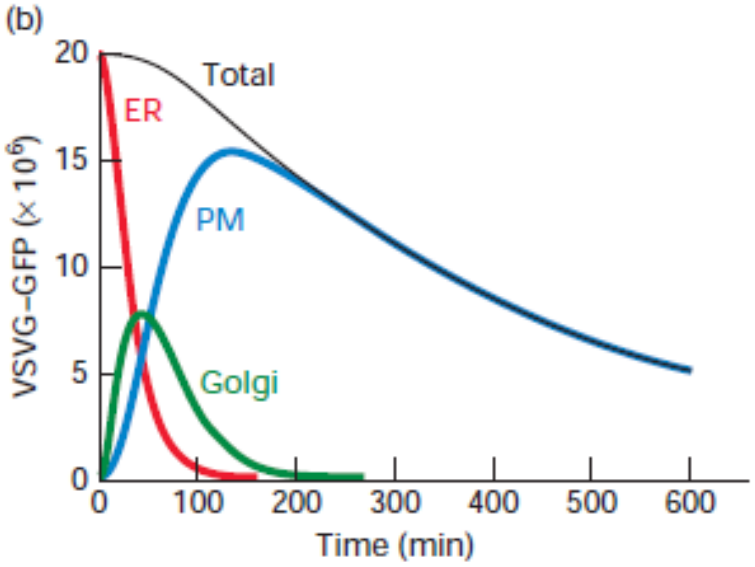
Transport vesicles bud off and carry these proteins to the Golgi complex (1), where the proteins are concentrated and packaged into immature secretory vesicles (2).

These vesicles then coalesce to form larger mature secretory vesicles that lose water to the cytosol, leaving an almost crystalline mixture of secreted proteins in the lumen(3).

After these vesicles accumulate under the apical surface, they fuse with the plasma membrane and release their contents (**exocytosis**) in response to appropriate hormonal or nerve stimulation (4).

Protein transport through the secretory pathway can be visualized by fluorescence microscopy of cells producing a GFP-tagged membrane protein.





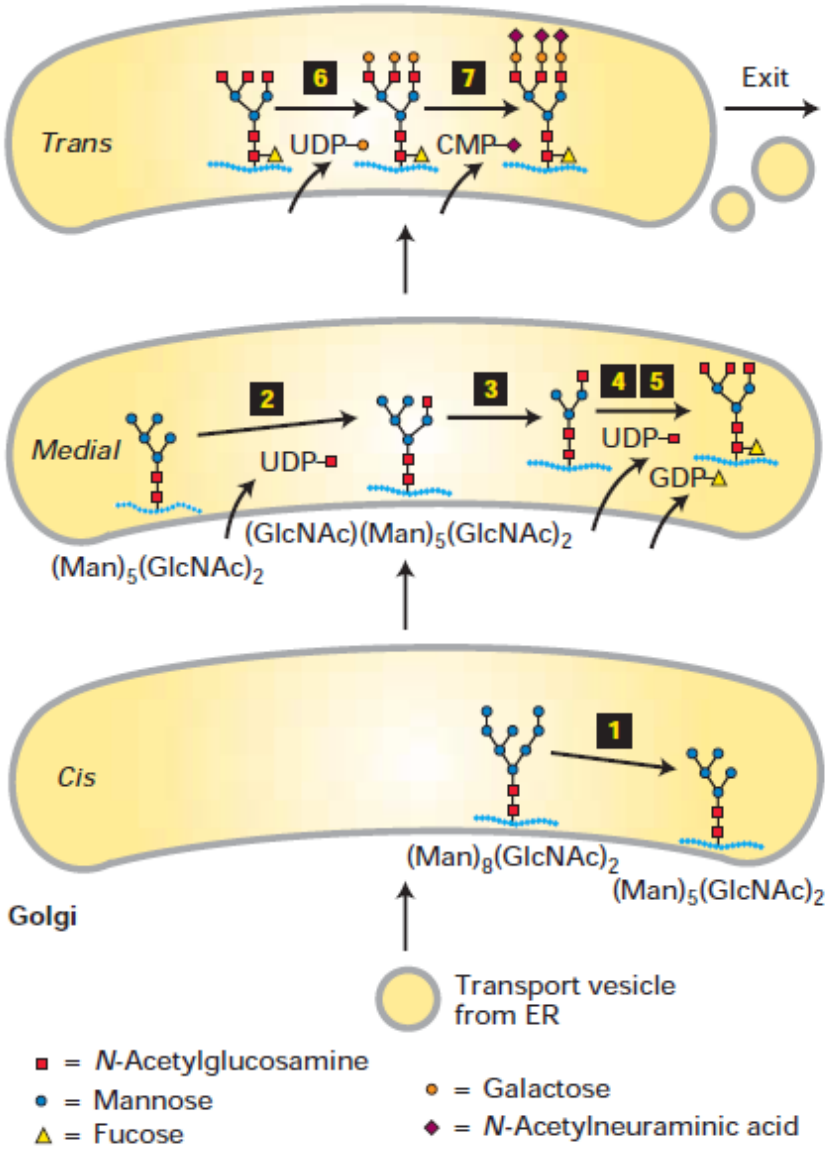
A second way to follow the transport of secretory proteins takes advantage of modifications to their carbohydrate side chains that occur at different stages of the secretory pathway.

Many secretory proteins leaving the ER contain one or more copies of the ***N*-linked oligosaccharide** $Man_8(GlcNAc)_2$, which are synthesized and attached to secretory proteins in the ER

As a protein moves through the Golgi complex, different enzymes localized to the *cis*-, *medial*-, and *trans*- Golgi cisternae catalyze an ordered series of reactions to these core $Man_8(GlcNAc)_2$ chains.

For instance, glycosidases that reside specifically in the *cis*-Golgi compartment sequentially trim mannose residues off of the core oligosaccharide to yield a “trimmed” form $Man_5(GlcNAc)_2$

Processing of *N*-linked oligosaccharide chains on glycoproteins within *cis*-, *medial*-, and *trans*-Golgi cisternae in vertebrate cells.

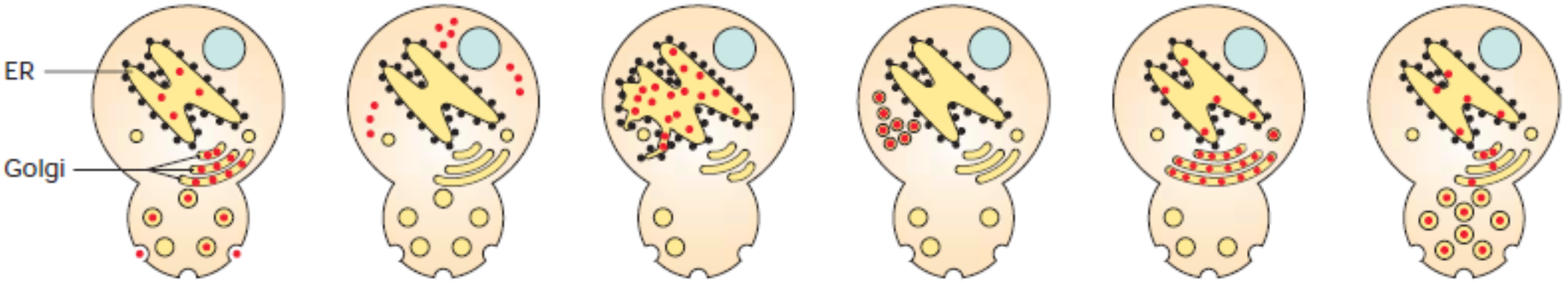


Removal of three mannose residues in the *cis*-Golgi

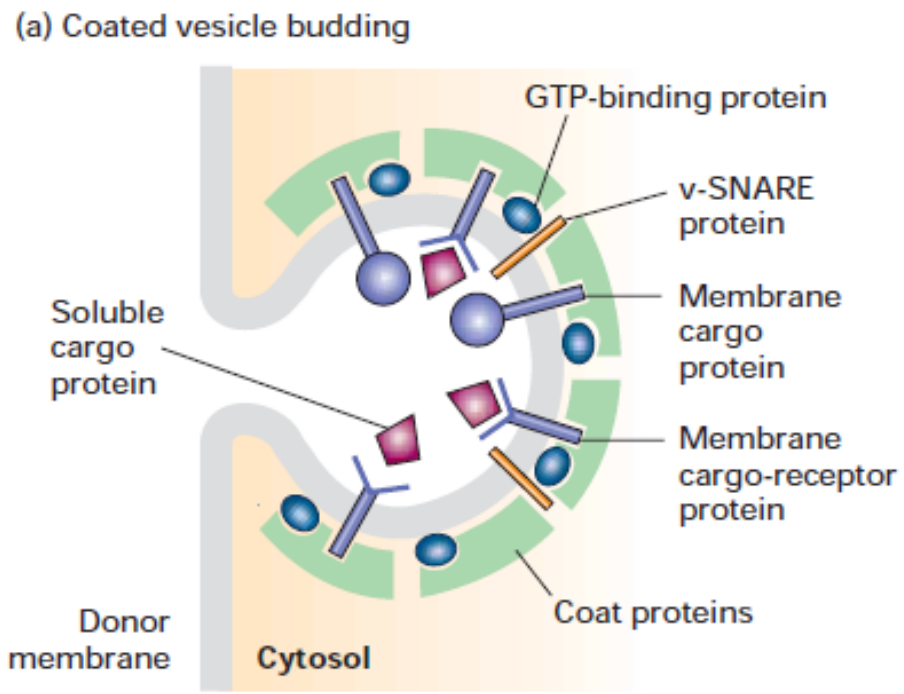
Three GlcNAc residues are added in the medial-Golgi
two more mannose residues are removed
and a single fucose is added

Processing is completed in the *trans*-Golgi by addition of three galactose residues and finally by linkage of an *N*-acetylneuraminic acid residue to each of the galactose residues

Secretory mutants identified using yeast



		Class A	Class B	Class C	Class D	Class E
Fate of secreted proteins	Normal secretion	Accumulation in the cytosol	Accumulation in rough ER	Accumulation in ER-to-Golgi transport vesicles	Accumulation in Golgi	Accumulation in secretory vesicles
Defective function		Transport into the ER	Budding of vesicles from the rough ER	Fusion of transport vesicles with Golgi	Transport from Golgi to secretory vesicles	Transport from secretory vesicles to cell surface

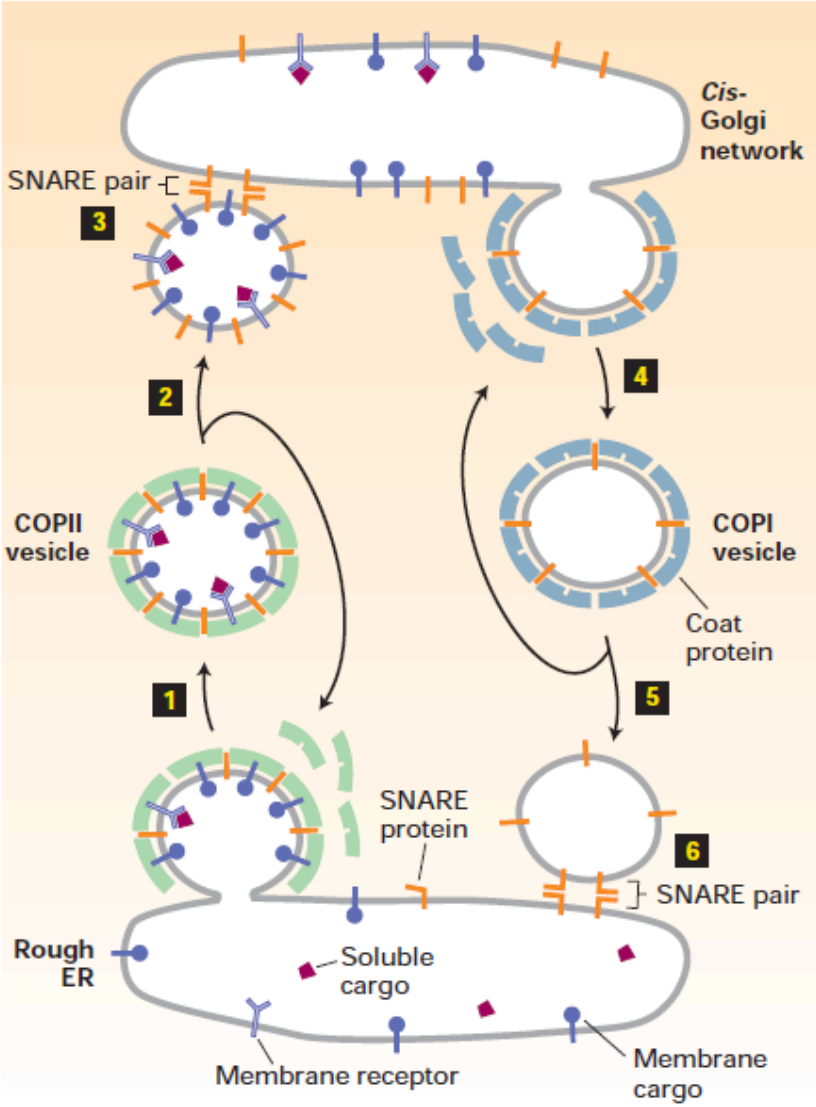


- **COPII** vesicles transport proteins from the rough ER to the Golgi.

- **COPI** vesicles mainly transport proteins in the retrograde direction between Golgi cisternae and from the *cis*-Golgi back to the rough ER.

- **Clathrin** vesicles transport proteins from the plasma membrane (cell surface) and the *trans*-Golgi network to late endosomes.

In addition to sculpting the curvature of a donor membrane, the vesicle coat also functions in selecting specific proteins as cargo.



Sheer abundance of some proteins like chaperones causes them to be continuously loaded passively into vesicles destined for the *cis*-Golgi.

The transport of these soluble proteins back to the ER, mediated by COPI vesicles, prevents their eventual depletion

Most soluble ER-resident proteins carry a Lys-Asp-Glu-Leu (KDEL in the one-letter code) sequence at their C-terminus.