

cytoplasmic membrane

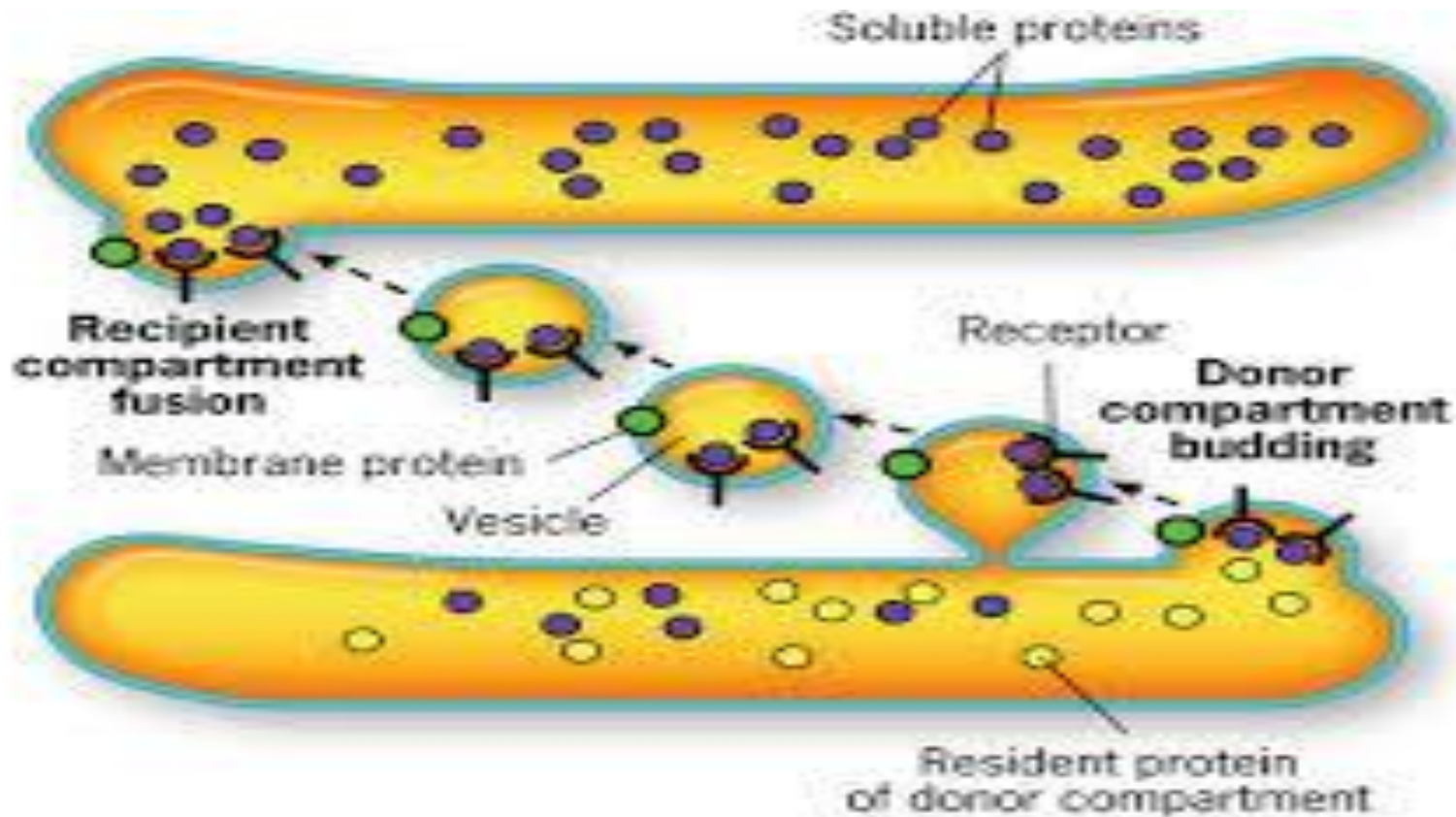
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Endomembrane System

- Endoplasmic reticulum
- Golgi complex
- Endosomes
- Lysosomes
- Vacuoles

An Overview of the Endomembrane System

The organelles of the endomembrane system are part of a dynamic, integrated network in which materials are shuttled back and forth from one part of the cell to another.



Pathways:

❑ Biosynthetic (or Secretory) Pathway

Moves proteins from their site of synthesis in the ER through the Golgi complex to the final destination

❑ Endocytic Pathway

Moves material in the opposite direction from the plasma membrane or extracellular space to the cell interior.

Types of Secretory Activities of Cells:

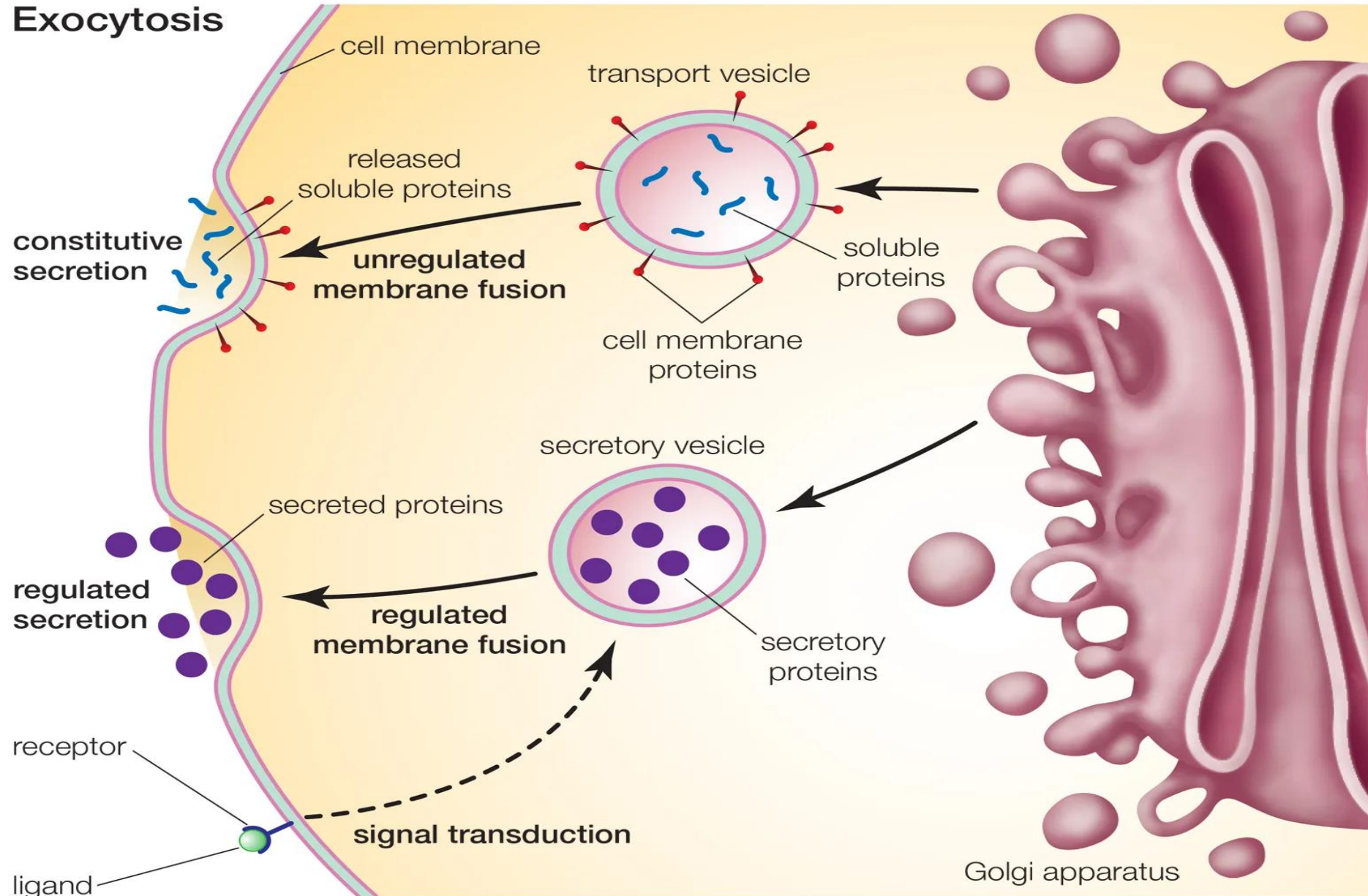
☐ Constitutive Secretion

Materials are transported in secretory vesicles from their sites of synthesis and discharged into the extracellular space in a continual matter.

☐ Regulated Secretion

Materials are stored as membrane-bound packages and discharged only in response to an appropriate stimulus.

Exocytosis



A FEW APPROACHES TO THE STUDY OF ENDOMEMBRANES

Determining the functions of cytoplasmic organelles required the development of new techniques and the execution of innovative experiments

Techniques:

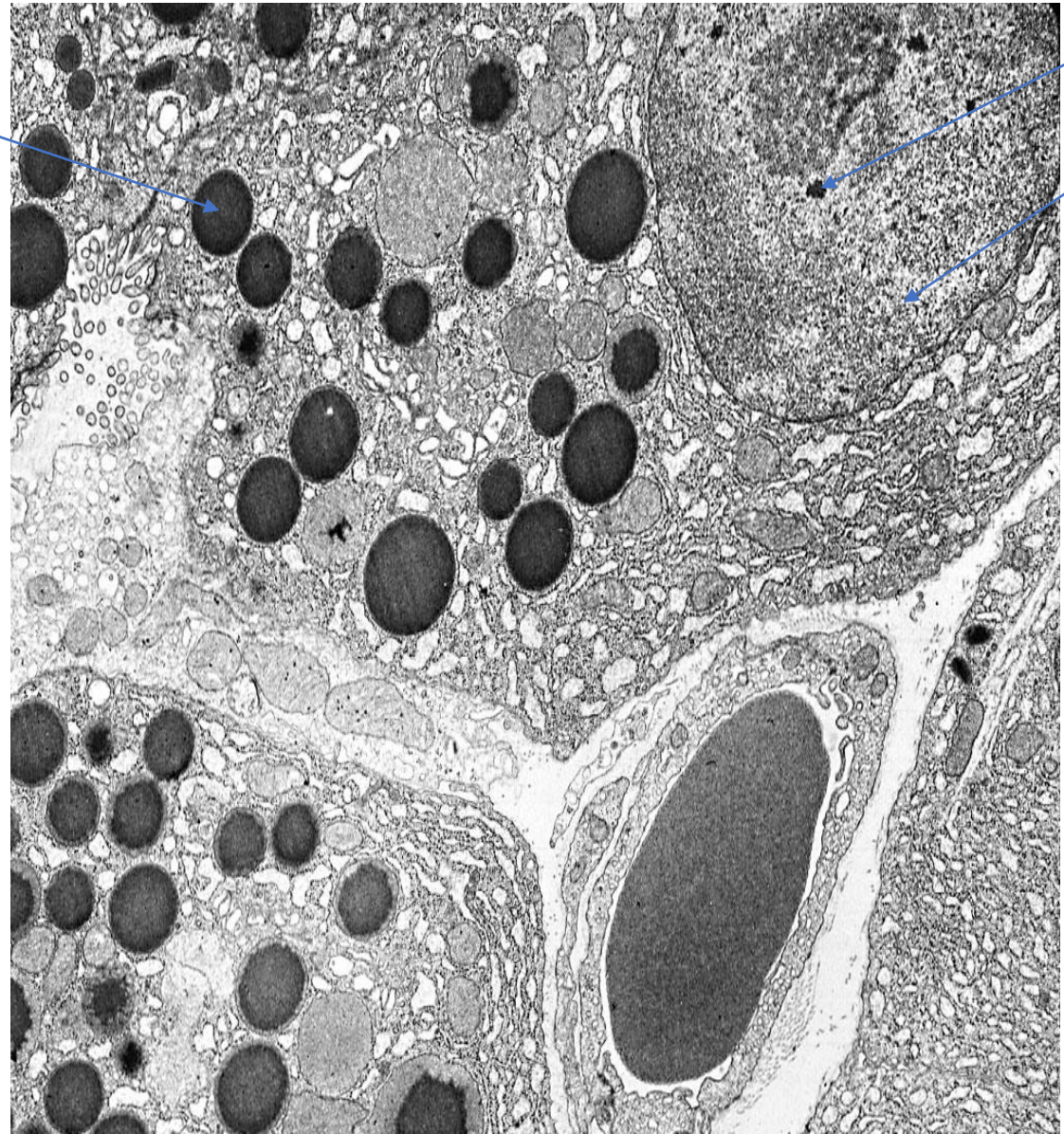
- Autoradiography
- Green Fluorescent Protein (GFP)
- Biochemical Analysis of Subcellular Fractions
- Cell-Free Systems
- Study of Mutant Phenotypes

Autoradiography

- ❑ Utilized by James Jamieson and George Palade of Rockefeller University
- ❑ Provides a means to visualize biochemical processes by allowing an investigator to determine the location of radioactively labeled materials within a cell
- ❑ Tissue sections containing radioactive isotopes are covered with a thin layer of photographic emulsion, which is exposed by radiation emanating from radioisotopes within the tissue
- ❑ Sites in the cells containing radioactivity are revealed under the microscope by silver grains in the overlying emulsion

Using this approach, the endoplasmic reticulum was discovered to be the site of synthesis of secretory proteins

Secretory granule



Silver grain

Rough ER

pulse-chase

- Pulse - refers to the brief incubation with radioactivity during which labeled amino acids are incorporated into protein
- Chase - refers to the period when the tissue is exposed to the unlabeled medium, a period during which additional proteins are synthesized using nonradioactive amino acids

- The longer the chase, the farther the radioactive proteins manufactured during the pulse will have traveled from their site of synthesis within the cell

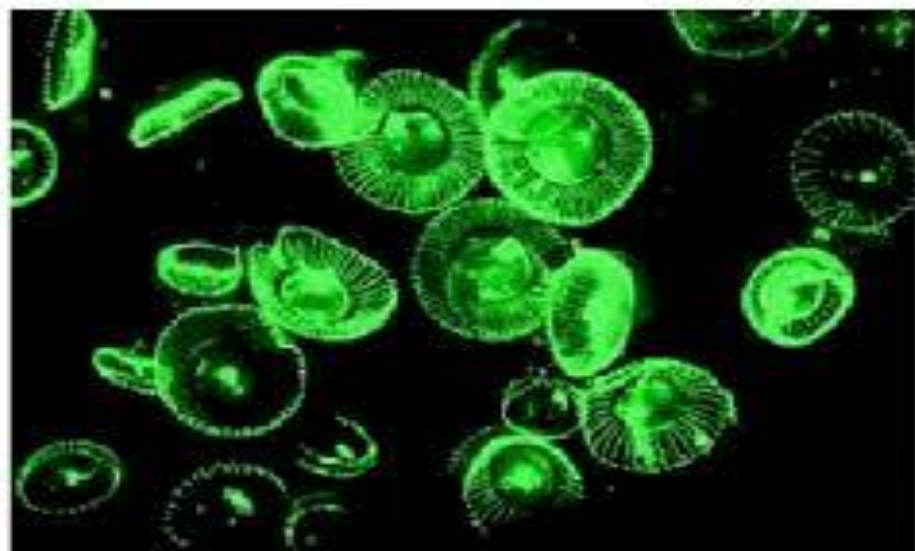
Green Fluorescent Protein (GFP)

- ❑ Utilizes a gene isolated from a jellyfish that encodes a small protein which emits a green fluorescent light
Fluorescent
- ❑ The use of GFP reveals the movement of proteins within a living cell

Viral genome contains
temperature-sensitive
VSVG gene fused to
GFP gene



+



Biochemical Analysis of Subcellular Fractions

Techniques to break up (homogenize) cells and isolate particular types of organelles were pioneered by Albert Claude and Christian De Duve.

Cell-Free Systems

- Did not contain whole cells
- Provided a wealth of information about complex processes that were impossible to study using intact cells
- Researchers have used cell-free systems to identify the roles of many of the proteins involved in membrane trafficking.

Study of Mutant Phenotypes

- Mutant = organism (or cultured cell) whose chromosomes contain one or more genes that encode abnormal proteins
- When a protein encoded by a mutant gene is unable to carry out its normal function, the cell carrying the mutation exhibits a characteristic deficiency

The Endoplasmic Reticulum (ER)

A system of tubules, cisternae, and vesicles that divides the cytoplasm into a luminal (or cisternal) space within the ER membranes and a cytosolic space outside the membranes.

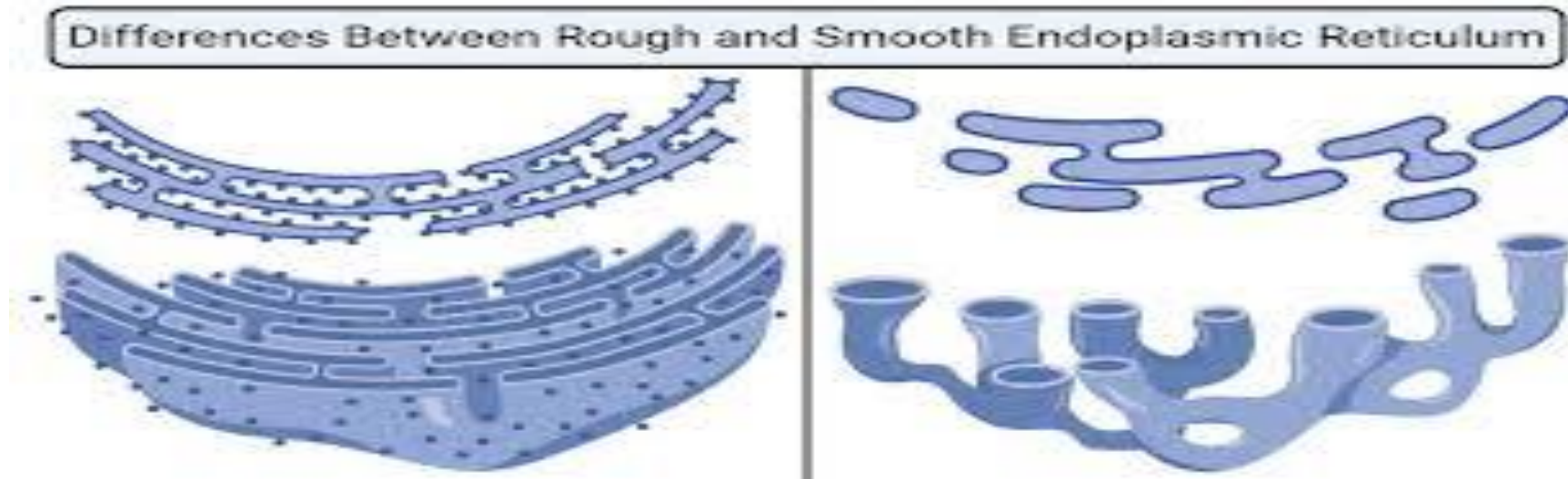
ER Subcompartments

❑ Rough Endoplasmic Reticulum (RER)

Occurs typically as flattened cisternae and whose membranes have attached ribosomes

❑ Smooth Endoplasmic Reticulum (SER)

Occurs typically as tubular compartments and whose membranes lack ribosomes



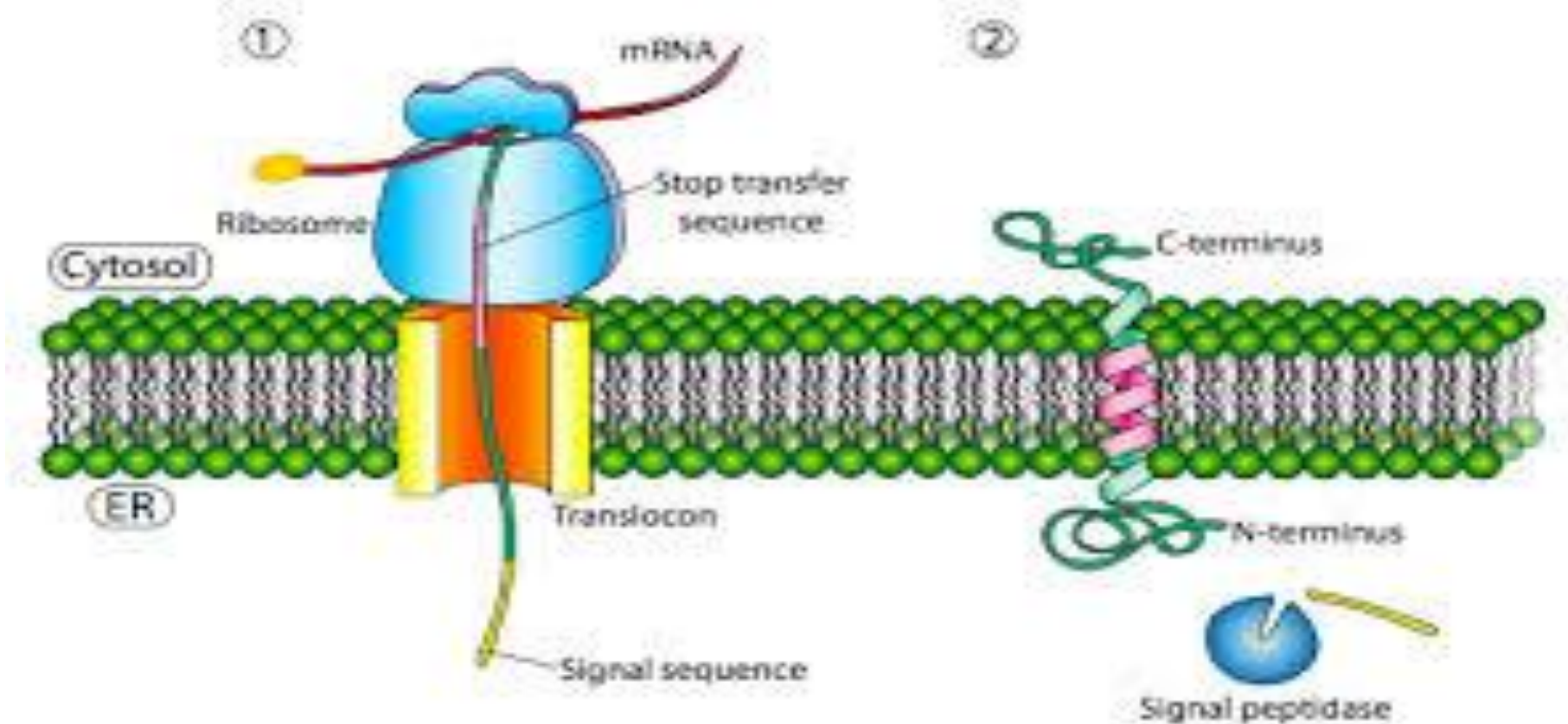
Smooth ER Functions:

- Synthesis of steroid hormones in the endocrine cells of the gonad and adrenal cortex
- Detoxification in the liver of a wide variety of organic compounds
- Sequestering calcium ions within the cytoplasm of cells

Rough ER Functions:

- Synthesis of secreted proteins, lysosomal proteins, and integral membrane proteins
- The RER is the starting point of the biosynthetic pathway: it is the site of synthesis of the proteins, carbohydrate chains, and phospholipids that journey through the membranous compartments of the cell.

Proteins to be synthesized on membrane-bound ribosomes of the RER are recognized by a hydrophobic signal sequence, which is usually situated near the N-terminus of the nascent polypeptide

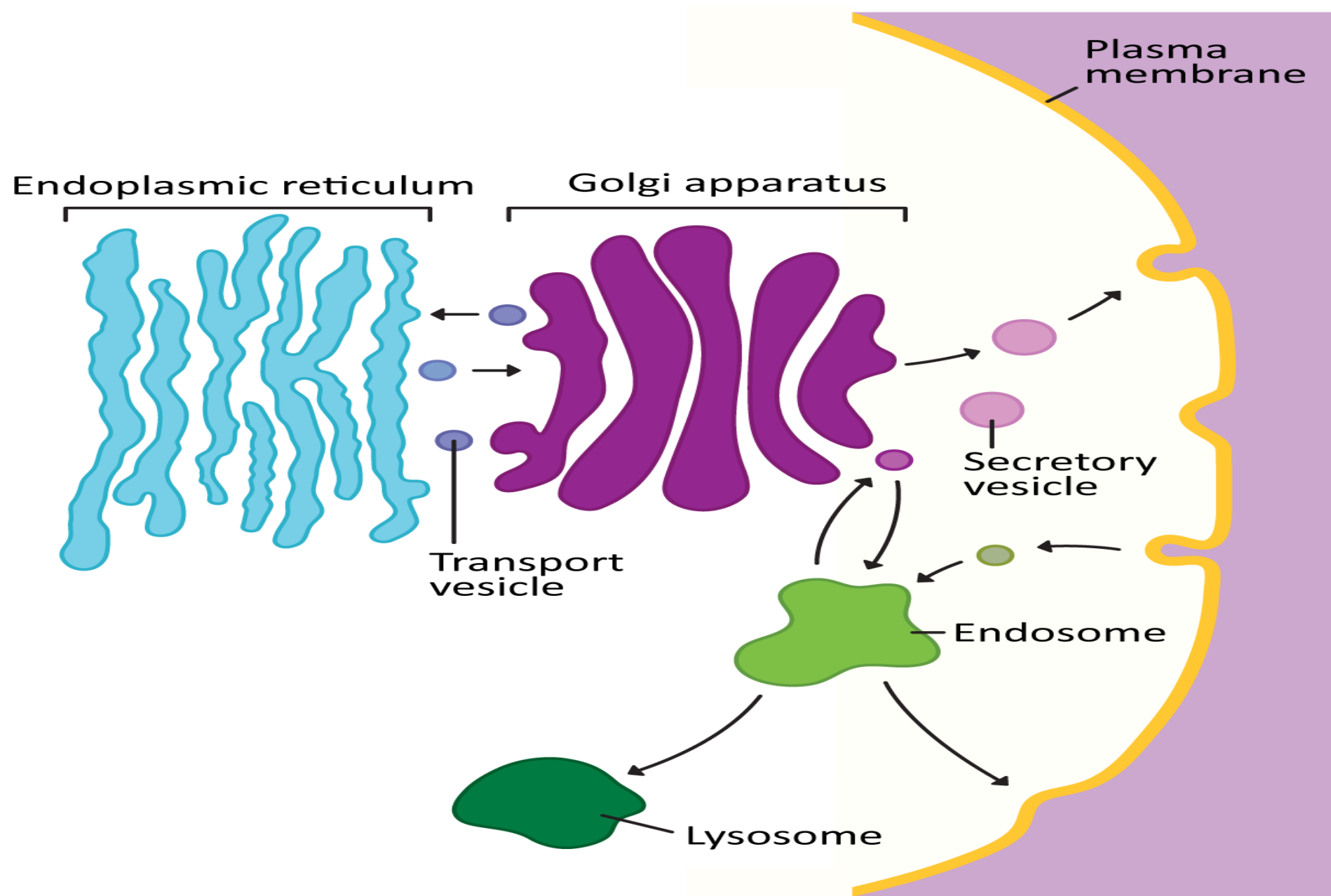


Glycosylation in the RER

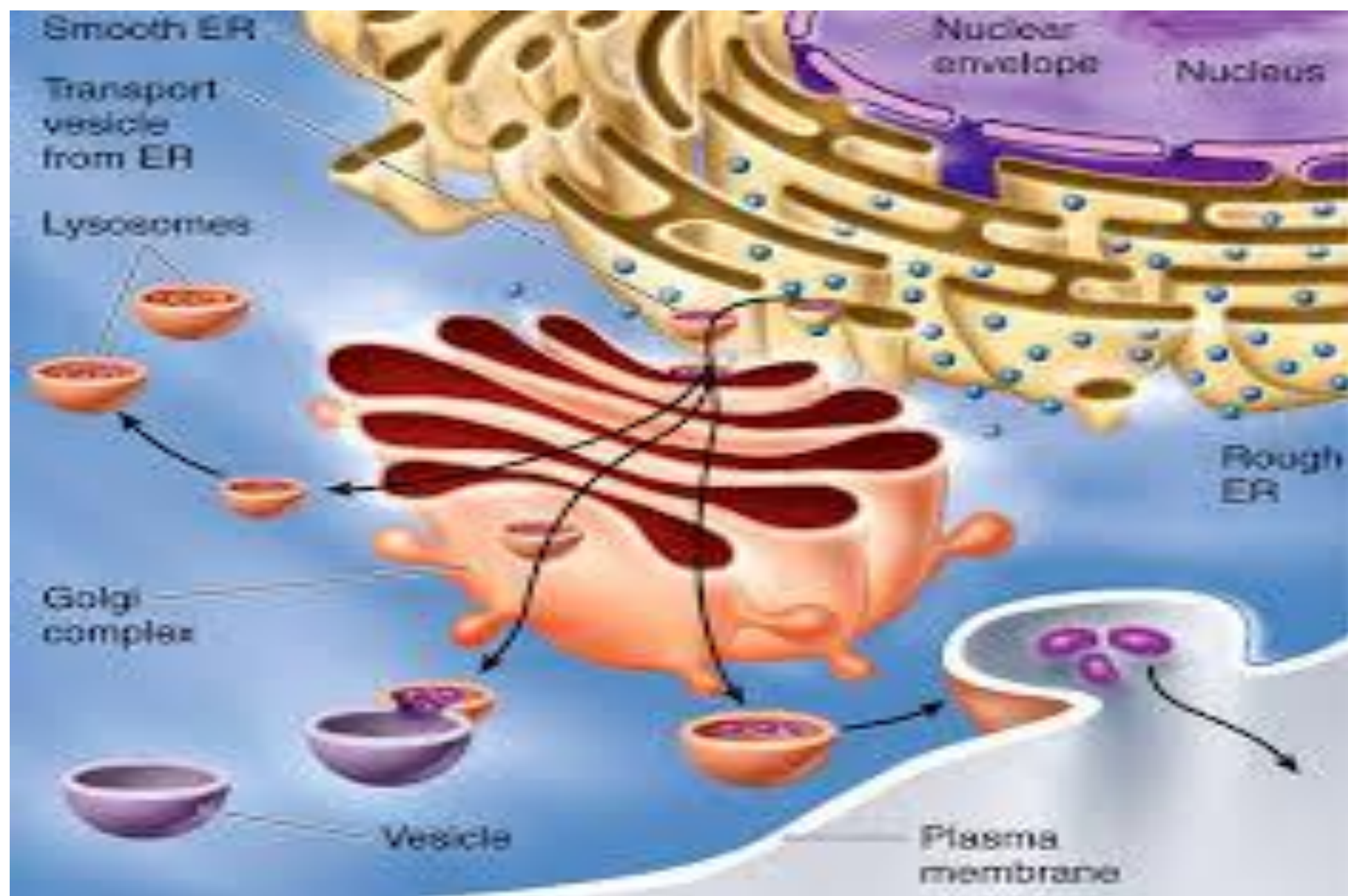
- The addition of sugars (glycosylation) to the asparagine residues of proteins begins in the RER and continues in the Golgi complex.
- The addition of sugars to an oligosaccharide chain is catalyzed by a family of membrane-bound enzymes called glycosyltransferases.

The Golgi Complex

- Consist primarily of flattened, dilated, membranous cisternae with dilated rims and associated vesicles and tubules.
- Functions as a processing plant, modifying the membrane components and cargo synthesized in the ER before it moves on to its target destination.
- Divided into several functionally distinct compartments arranged along an axis from the cis or entry face closest to the ER to the trans or exit face at the opposite end of the stack



- The cis-most face of the organelle is composed of an interconnected network of tubules referred to as the cis Golgi network (CGN)
- Function primarily as a sorting station that distinguishes between proteins to be shipped back to the ER and those that are allowed to proceed to the next Golgi station.
- The trans-most face of the organelle contains a distinct network of tubules and vesicles called the trans Golgi network (TGN).
- The TGN is a sorting station where proteins are segregated into different types of vesicles heading either to the plasma membrane or to various intracellular destinations.



- As the materials traverse the stack, sugars are added to the oligosaccharide chains by glycosyltransferases located in particular Golgi cisternae.
- The bulk of the Golgi complex consists of a series of large, flattened cisternae, which are divided into cis, medial, and trans cisternae.

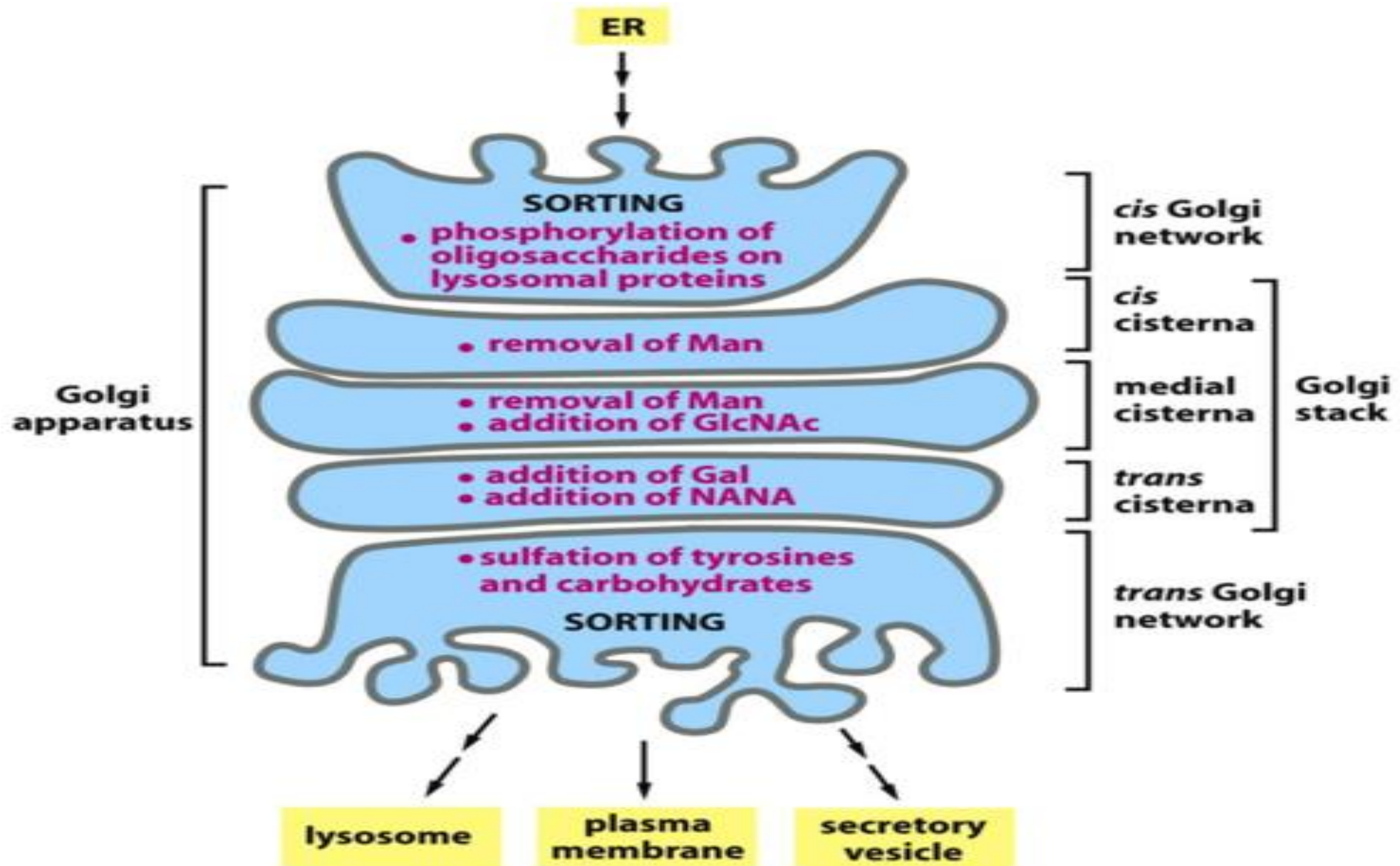


Figure 13-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Once the proteins reach the trans Golgi network (TGN) at the end of the stack, they are ready to be sorted and targeted for delivery to their ultimate cellular or extracellular destination.



THANK YOU FOR LISTEN