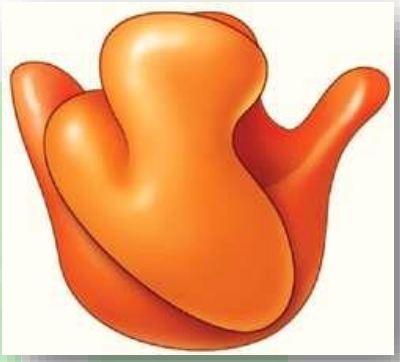
Ribosomes, Endoplasmic reticulum (ER) and Golgi body

LEC. 4 | COMPONENTS OF THE CELL (Pt.3)

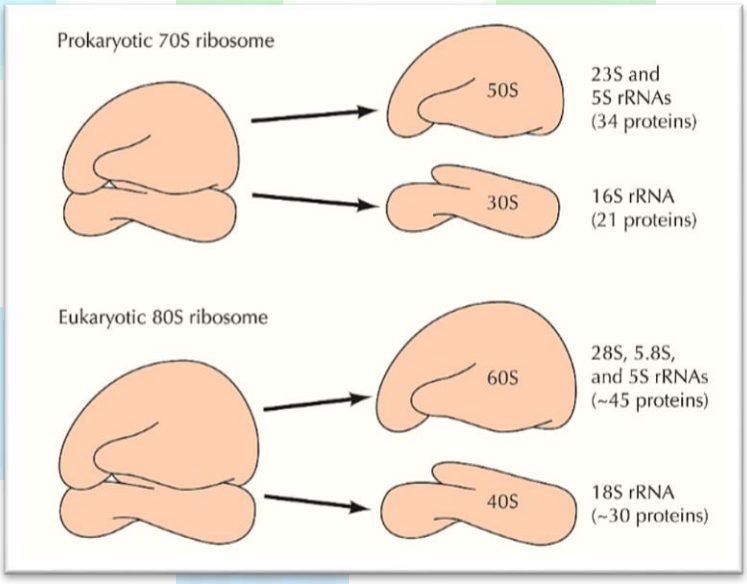
The Structure and Function of the RIBOSOMES, ENDOPLASMIC RETICULUM (ER) AND GOLGI BODY

INTRODUCTION

The RIBOSOME



The ribosome is composed of two subunits that work together to carry out mRNA-directed polypeptide synthesis. This process involves a highly dynamic interplay of two ribosomal subunits with each other and numerous cellular factors. Our understanding of protein biosynthesis is most advanced for bacteria which contain **70S** ribosomes composed of a small (**30S**) and a large (**50S**) subunit. The activity of the ribosome involves initiation, elongation, termination and recycling step. The ribosome adopts many different functional states during each of the above steps. Understanding the complicated details of translation, therefore, requires, in addition to biochemical data, high resolution structures of each of the functional states of the ribosome. Our understanding of ribosomal structure has proceeded from the early reconstructions of the shapes of the two interacting subunits, to the current atomic- resolution structures of the prokaryotic **70S** ribosome and of its large and small subunits captured in various functional states. Our intention is to present in this review how our knowledge about the ribosome's structure evolved, starting from its discovery until nowadays.



Each subunit is made of one or more ribosomal RNAs (rRNAs) and many ribosomal proteins (r-proteins). The small subunit (**30S** in bacteria and archaea, **40S** in eukaryotes) has the decoding function, whereas the large subunit (**50S** in bacteria and archaea, **60S** in eukaryotes) catalysis the formation of peptide bonds, referred to as the peptidyl-

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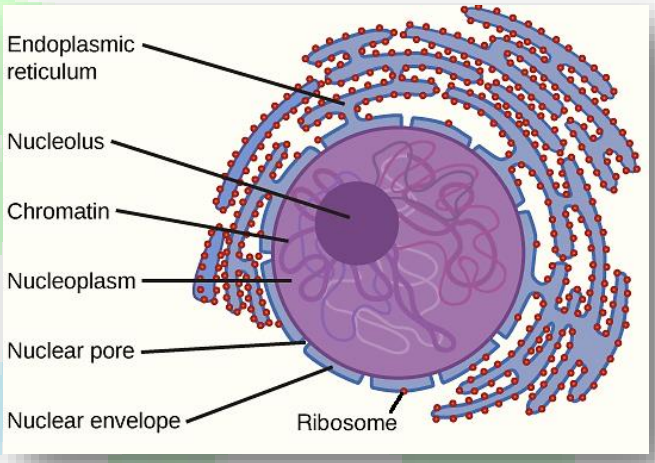
transferase activity. The bacterial (and archaeal) small subunit contains the **16S** rRNA and 21 r-proteins (*Escherichia coli*), whereas the eukaryotic small subunit contains the 18S rRNA and 32 r-proteins (*Saccharomyces cerevisiae*; although the numbers vary between species). The bacterial large subunit contains the **5S** and **23S** rRNAs and 34 r-proteins (*E. coli*), with the eukaryotic large subunit containing the **5S**, **5.8S** and 25S/28S rRNAs and 46 r-proteins (*S. cerevisiae;* again, the exact numbers vary between species).

It started from the mitochondria …

The beginnings of the long and continuous discovery of the ribosomes lie in an excellent work with cell fractionation in the 1930s and 1940s performed by Albert Claude, the 1974 Nobel Prize laureate in Physiology or Medicine. The particular components of the cell were first seen in 1941 but were not recognized yet. By means of newly developed high-speed centrifugation, the cytoplasm no longer appeared as never ending space full of unknown substances, but as a powerful space in which the unknown substances showed up, waiting to be isolated, purified and characterized. The subcellular fragments could be obtained by many scientists by rubbing cells in a mortar, and further subjection to multiple cycles of sedimentations, washings and resuspensions. In addition to the nucleus, which was the most prominent feature of eukaryotic cell, mitochondria were also visualized in such way. In fact, mitochondria were detected under the light microscope as early as 1894, but despite extensive investigation by microscopy in the course of the following 50 years, no progress was achieved in this field. Finally, in 1940s, the staining properties of mitochondria led to the conclusion that they contained ribonucleic acids and thus put them as an object of new studies.



THE FUNCTION OF RIBOSOMES

Ribosomes are small particles, 20-30 nm in diameter, consisting of **ribosomal RNA** (**rRNA**) complexed with protein forming **ribonucleoprotein**. Each ribosome is composed of a large and a small subunit. Ribosomes exist either freely in the cytoplasm or attached to the membrane of the RER. Their presence in the cytoplasm is signified by **cytoplasmic basophilia** (also called ergastoplasm), which is due to the affinity of basic stains for the RNA. Cytoplasmic basophilia is particularly prominent in cells synthesizing large amounts of protein (e.g., plasma cells synthesizing antibodies). In the cytoplasm,

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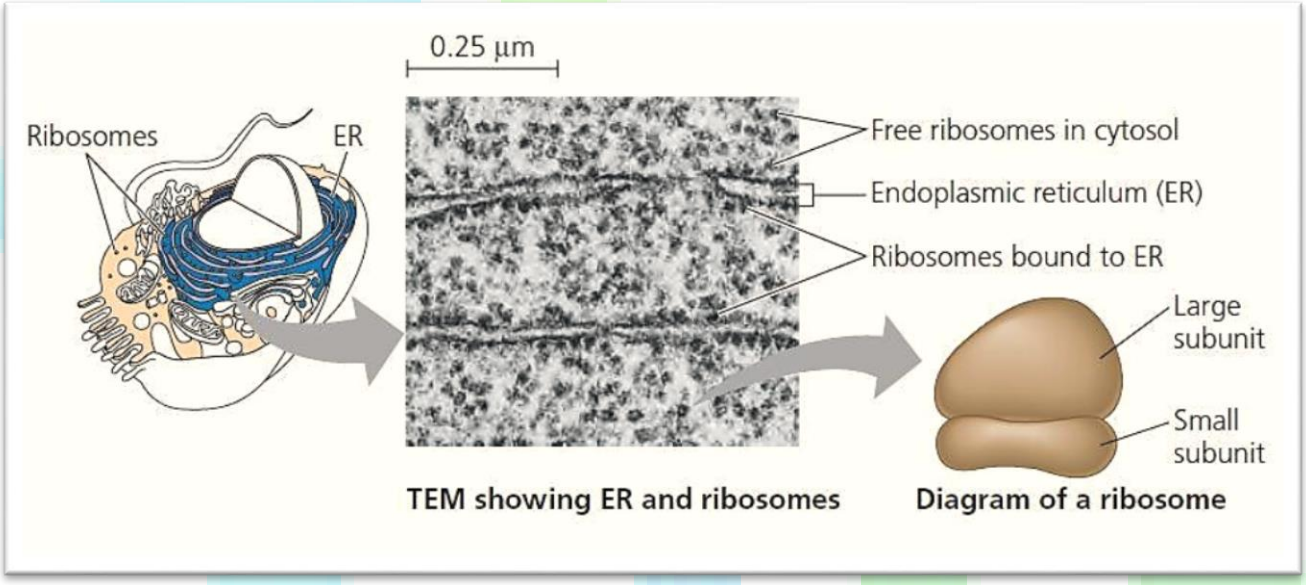
ribosomes may be found both singly and in groups called **polysomes**. Polysomes represent the visible manifestation of protein translation, in which several ribosomes have become attached to a single molecule of **messenger RNA** (**mRNA**). The ribosomes provide a stable site on the mRNA molecule for the sequential linkage of **amino acids**, carried by **transfer RNA** (**tRNA**), to the growing polypeptide chain. Because the length of



the mRNA molecule determines the length of the polypeptide chain being synthesized, the size of the polysome provides a good index to the size of the polypeptide. Proteins destined for the cytoplasm are synthesized on free polysomes. However, hydrolytic enzymes or proteins destined for secretion are synthesized in the RER.

ENDOPLASMIC RETICULUM (ER)

Endoplasmic reticulum (ER) and Golgi body are single membrane bound structures. The membrane has the same structure (lipid-protein) as the plasma membrane but ribosomes do not have membranes Ribosomes are involved in synthesis of substances in the cell, Golgi bodies in secreting and the ER in transporting and storing the products. These three organelles operate together.

The concurrent development of methods for differential centrifugation of liver homogenates led to identification of a cell fraction consisting of 50 to 300 nm particles that were called microsomes (Claude, 1943).

This fraction rapidly became a focus of investigative attention because it was found to contain the bulk of the cytoplasmic ribonucleoprotein and therefore was thought to be involved in protein synthesis. In his pioneering application of the electron microscope to the study of thinly spread tissue culture cells, Porter (1945) observed a system of delicate branching and anastomosing strands that formed a lace-like network

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throughout the cytoplasm. This endoplasmic reticulum was considered to be a new cell organelle (Porter and Thompson, 1947). Also noted in these preparations were small vesicles 50 to 200 nm in diameter, sometimes connected in rows and sometimes entirely separate. It was suggested that this vesicular component corresponded to Claude's "microsomes."

Endoplasmic reticulum (ER)	Golgi body	Ribosomes
Structure		
A network of membranes with thickness between 50 - 60A°. It is of two types' rough endoplasmic reticulum (RER) i.e. when ribosomes are attached to it and Smooth-endoplasmic reticulum (SER) when no ribosomes are present. Throughout the cytoplasm and is in contact with the cell membrane as well as the nuclear membrane.	Is a stack of membranous sacs of the same thickness as ER. Exhibit great diversity in size and shape. In animal cells present around the nucleus, 3 to 7 in number. In plant cells, many and present scattered throughout the cell called dictyosomes.	Spherical about 150 - 250 Å in diameter, made up of large molecules of RNA and proteins (ribonucleo proteins) Present either as free particles in cytoplasm or attached to ER. Also found stored in nucleolus inside the nucleus. 80S types found in eukaryotes and 70S in prokaryotes (Svedberg unit of measuring ribosomes).
Function		
Provides internal framework, compartment and reaction surfaces, transports enzymes and other materials throughout the cell. RER is the site for protein synthesis and SER for steroid synthesis, stores carbohydrates.	Synthesis and secretion as enzymes, participates in transformation of membranes to give rise to other membrane structure such as lysosome, acrosome, and dictyosomes, synthesize wall element like pectin, mucilage.	Site for protein synthesis.

NOTES ENDOPLASMIC RETICULUM:

1. The endomembrane system; smooth and rough ER
2. ER growth and microtubules
3. Interconnections between ER stacks
4. Co-translational translocation
5. Protein sorting
6. Lipid modification and glycosylation in the ER

Smooth ER functions

1. Synthesis of steroid hormones (e.g. cells of the gonads and endocrine glands)
2. Detoxification of a variety of organic compounds in the liver (e.g. barbiturates and ethanol)
3. Release of glucose from glycogen by Glucose 6- phosphate
4. Sequestration and regulated release of calcium ions (e.g. skeletal muscle cells—sarcoplasmic reticulum)

Rough ER functions

1. Synthesis of secreted and membrane bound proteins

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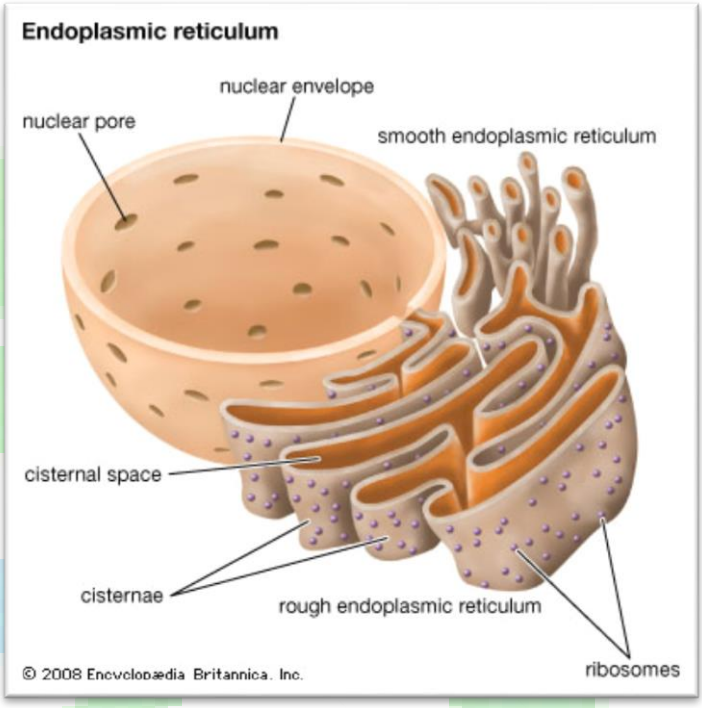
2. Post-translational modification of membrane proteins (e.g. glycosylation and lipid modification)
3. Membrane biosynthesis

The Rough Endoplasmic Reticulum



The RER is an intracellular membrane system that functions to sequester enzymatic reactions and their products from the rest of the cell. It consists of interconnected, flattened membranous sacs called **cisternae**, which are in direct continuity with the outer membrane of the nuclear envelope and with the smooth ER. In the electron microscope, the RER is seen as a series of parallel unit membranes studded with ribosomes. The synthesis of proteins that are destined to be segregated within the RER starts in the cytoplasm on a polysome. Such proteins contain an initial **signal sequence** of amino acids, which binds to a **signal recognition particle**, which in turn binds to a receptor on the RER membrane. The growing polypeptide chain then passes through a channel in the ribosome and enters the lumen of the RER, where the signal sequence is cleaved from the polypeptide via **signal peptidase**. Once inside, the polypeptide undergoes conformational changes to prevent its passage out of the RER. If destined to be a glycoprotein, the polypeptide acquires core sugars here. The completed protein is then transferred from the RER to the Golgi apparatus by means of membranous **Transport Vesicles** for further modifications. In the absence of a signal sequence, the polypeptide is not sequestered in the RER and remains in the cytoplasm. Regardless of whether the protein is synthesized free in the cytoplasm or sequestered within the RER, it must be properly folded to be functional, a process guided by **Chaperone Proteins**. Proteins that are not properly folded or defective in some other way are "tagged" with proteins called **Ubiquitin** and degraded by **Proteasomes**, which are small, cylinder-shaped complexes of proteolytic enzymes.

THE GOLGI APPARATUS

The Golgi consists of a stacked series of flattened, membranous **saccules** that are interconnected by a complex network of anastomosing tubules. It is polarized into a convex **forming face (cis face)** and a concave **maturing face (trans face)**. Transfer vesicles

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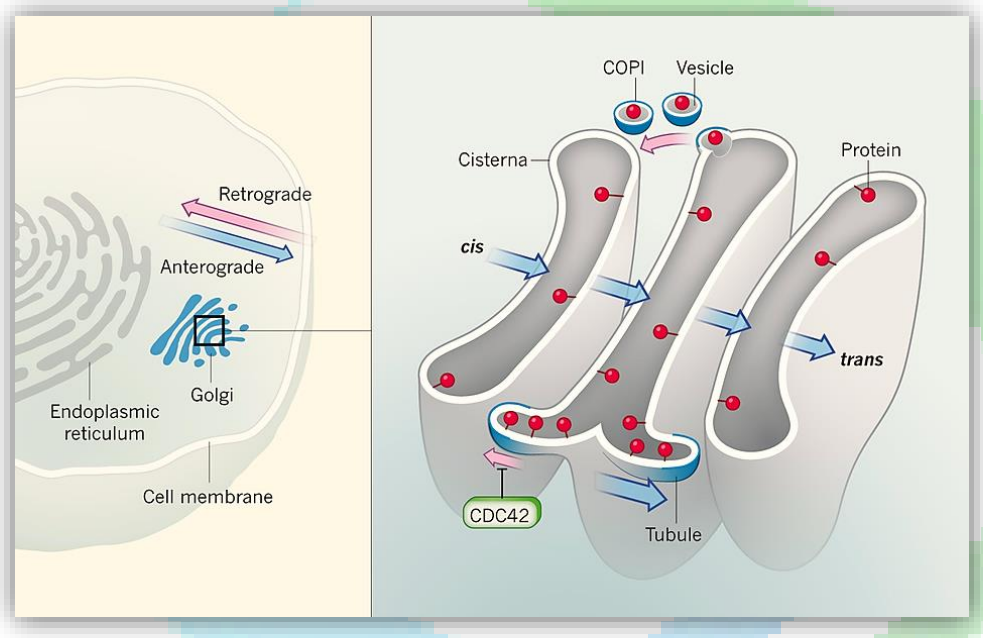
from the RER fuse with the saccule of the forming face, and after suitable processing within the Golgi, the membrane-bound product emerges from the maturing face.

The name behind the apparatus

The Golgi apparatus is the only cell organelle to be named after a scientist. The visible characteristics of the organelle were first reported by Camillo Golgi (1843-1926) at a meeting of the Medical Society of Pavia on 19 April 1898 when he named it the 'internal reticular apparatus'.

Functions of the Golgi

1. Post-translational modification of proteins



- ☒ **Glycosylation;** Membrane-bound enzymes add terminal sugars to glycoproteins (core sugars were added in the RER).
- ☒ **Sulfation:** Membrane-bound enzymes add sulfate groups to proteins.
- ☒ **Phosphorylation:** Membrane-bound enzymes add phosphate groups to proteins.
- ☒ **Proteolysis:** Cleavage of some precursor proteins, e.g., prohormones

2. Sorting and packaging of modified proteins

Most proteins processed by the Golgi are either secretory proteins for export or hydrolytic enzymes for cell use. These two kinds of proteins are segregated and packaged separately by the Golgi.

Secretory proteins are seen emerging from the maturing face contained in a membranous dilation termed a **pro-secretory granule**. The pro-secretory granule buds off to become a **condensing vacuole**, which, after the removal of fluid, is termed a

secretory granule or **secretory vesicle**. Secretory granules containing digestive enzymes are specifically referred to as **zymogen granules**. Under the appropriate conditions, the secretory granule moves to the cell surface and fuses with the membrane, thereby releasing its contents to the outside. This **Ca++- dependent process** is called **exocytosis** or **secretion**. There are two kinds of secretion:

3. Constitutive secretion

Secretory products are produced and released continuously.

4. Regulated secretion

Secretory products are released in response to specific stimuli.

Hydrolytic enzymes are similarly packaged in membrane-bound vesicles called **lysosomes**. Within the Golgi, hydrolytic enzymes are "tagged" with **mannose-6-phosphate (MSP)**, which diverts them into a separate pathway for lysosome production. These MSP-tagged enzymes are packaged into **clathrin-coated vesicles** and transported to a low-pH membranous compartment called the **late endosome**, from which the mature lysosomes arise.

Golgi apparatus, also called Golgi complex or Golgi body, membrane-bound **organelle** of **eukaryotic** cells (cells with clearly defined nuclei) that is made up of a series of flattened, stacked pouches called cisternae. The Golgi apparatus is responsible for transporting, modifying, and packaging **proteins** and **lipids** into **vesicles** for delivery to targeted destinations. It is located in the **cytoplasm** next to the **endoplasmic reticulum** and near the **cell nucleus**. While many types of **cells** contain only one or several Golgi apparatus, **plant** cells can contain hundreds.

In general, the Golgi apparatus is made up of approximately four to eight cisternae, although in some single-celled organisms it may consist of as many as 60 cisternae. The cisternae are held together by matrix proteins, and the whole of the Golgi apparatus is supported by cytoplasmic **microtubules**. The apparatus has three primary compartments, known generally as "cis" (cisternae nearest the endoplasmic reticulum), "medial" (central layers of cisternae), and "trans" (cisternae farthest from the endoplasmic reticulum). Two networks, the cis Golgi network and the trans Golgi network, which are made up of the outermost cisternae at the cis and trans faces, are responsible for the essential task of sorting proteins and lipids that are received (at the cis face) or released (at the trans face) by the organelle.

The proteins and lipids received at the cis face arrive in clusters of fused vesicles. These fused vesicles migrate along microtubules through a special trafficking compartment, called the vesicular-tubular cluster, which lies between the endoplasmic reticulum and the Golgi apparatus. When a vesicle cluster fuses with the cis membrane, the contents are delivered into the lumen of the cis face cisterna. As proteins and lipids progress from the cis face to the trans face, they are modified into functional molecules and are

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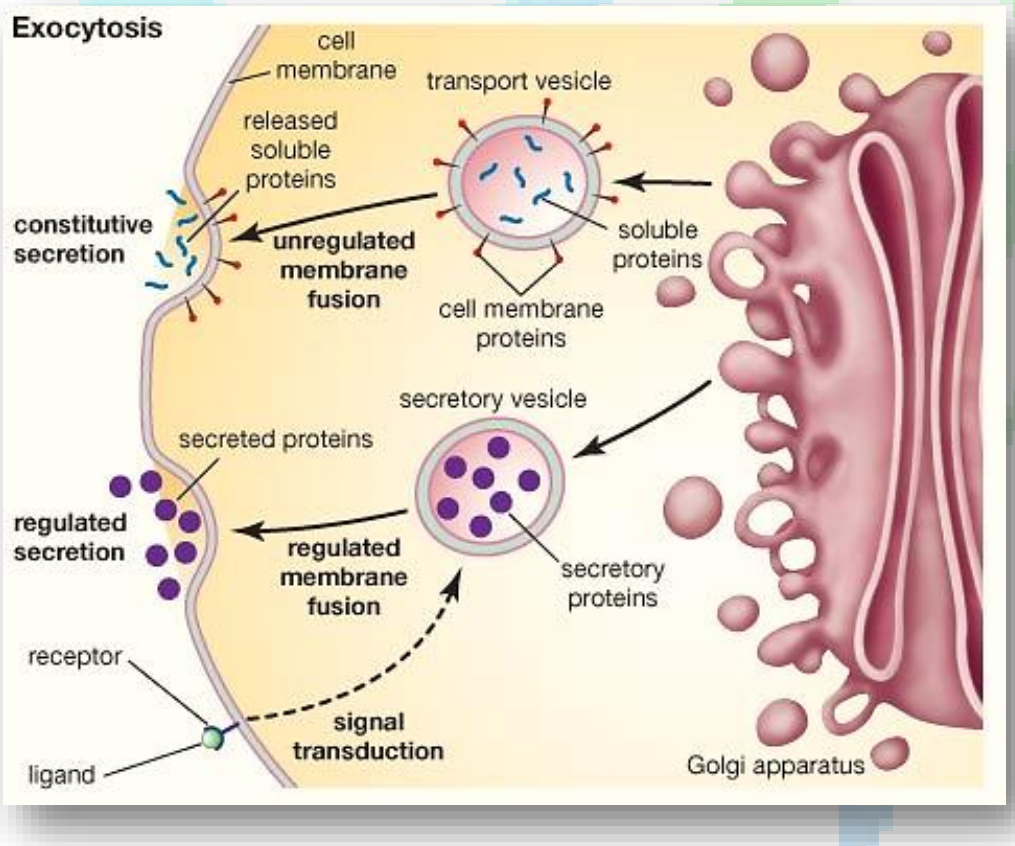
marked for delivery to specific intracellular or extracellular locations. Some modifications involve cleavage of **oligosaccharide** side chains followed by attachment of different sugar moieties in place of the side chain. Other modifications may involve the addition of **fatty acids** or **phosphate** groups (**phosphorylation**) or the removal of **monosaccharides**. The different **enzyme**-driven modification reactions are specific to the compartments of the Golgi apparatus. For example, the removal of mannose moieties occurs primarily in the cis and medial cisternae, whereas the addition of **galactose** or **sulfate** occurs primarily in the trans cisternae. In the final stage of transport through the Golgi apparatus, modified proteins and lipids are sorted in the trans Golgi network and are packaged into vesicles at the trans face. These vesicles then deliver the molecules to their target destinations, such as **lysosomes** or the **cell membrane**. Some molecules, including certain soluble proteins and secretory proteins, are carried in vesicles to the **cell** membrane for **exocytosis** (release into the extracellular environment). The exocytosis of secretory proteins may be regulated, whereby a **ligand** must bind to a **receptor** to trigger vesicle fusion and **protein secretion**.



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The way in which proteins and lipids move from the cis face to the trans face is of some debate, and today there exist two models, with quite different perceptions of the Golgi apparatus, competing to explain this movement. The vesicular transport model stems from initial studies that identified vesicles in association with the Golgi apparatus. This model is based on the idea that vesicles bud off and fuse to cisternae membranes, thus moving molecules from one cisterna to the next; budding vesicles can also be used to transport molecules back to the endoplasmic reticulum. A vital element of this model is that the cisternae themselves are stationary. In contrast, the cisternal maturation model depicts the Golgi apparatus as a far more dynamic organelle than does the vesicular transport model. The cisternal maturation model indicates that cis cisternae move forward and mature into trans cisternae, with new cis cisternae forming from the fusion of vesicles at the cis face. In this model, vesicles are formed but are used only to transport molecules back to the endoplasmic reticulum.

The Golgi apparatus was observed in 1897 by Italian cytologist Camillo Golgi. In Golgi's early studies of nervous tissue, he had established a staining technique that he referred to as *reazione nera*, meaning "black reaction"; today it is known as the Golgi stain. In this technique nervous tissue is fixed with potassium dichromate and then suffused with silver nitrate. While examining neurons that Golgi stained using his black reaction, he identified an "internal reticular apparatus." This structure became known as the Golgi apparatus, though some scientists questioned whether the structure was real and attributed the find to free-floating particles of Golgi's metal stain. In the 1950s, however, when the electron microscope came into use, the existence of the Golgi apparatus was confirmed.