Molecular Biology of the Cell

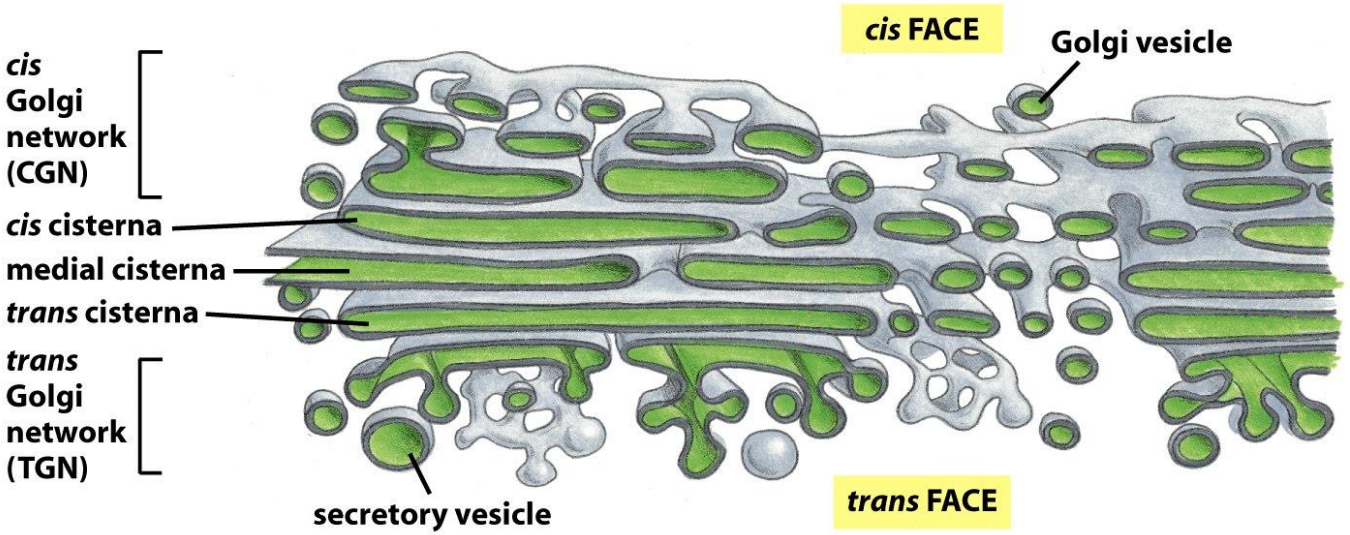
Chapter 13

Intracellular Vesicular Traffic

Golgi Apparatus

The Golgi Apparatus Consists of an Ordered Series of Compartments

- Because it could be selectively visualized by **silver stains**, the Golgi apparatus was one of the first organelles described by early light microscopists.
- It consists of a collection of flattened, membrane-enclosed compartments called **cisternae**, that somewhat resemble a **stack of pita breads**.
- Each Golgi stack typically consists of four to six cisternae, although some unicellular flagellates can have more than 20.
- During their passage through the Golgi apparatus, transported molecules undergo an ordered series of covalent modifications.
- Each Golgi stack has two distinct faces: a cis face (or entry face) and a trans face (or exit face).
- Both cis and trans faces are closely associated with **special compartments**, each composed of a network of interconnected tubular and cisternal structures: **the cis Golgi network (CGN) and the trans Golgi network (TGN)**.



The Golgi Apparatus Consists of an Ordered Series of Compartments

- The CGN is a collection of fused vesicular tubular clusters arriving from the ER.

- Proteins and lipids enter the cis Golgi network and exit from the trans Golgi network, bound for the cell surface or another compartment.

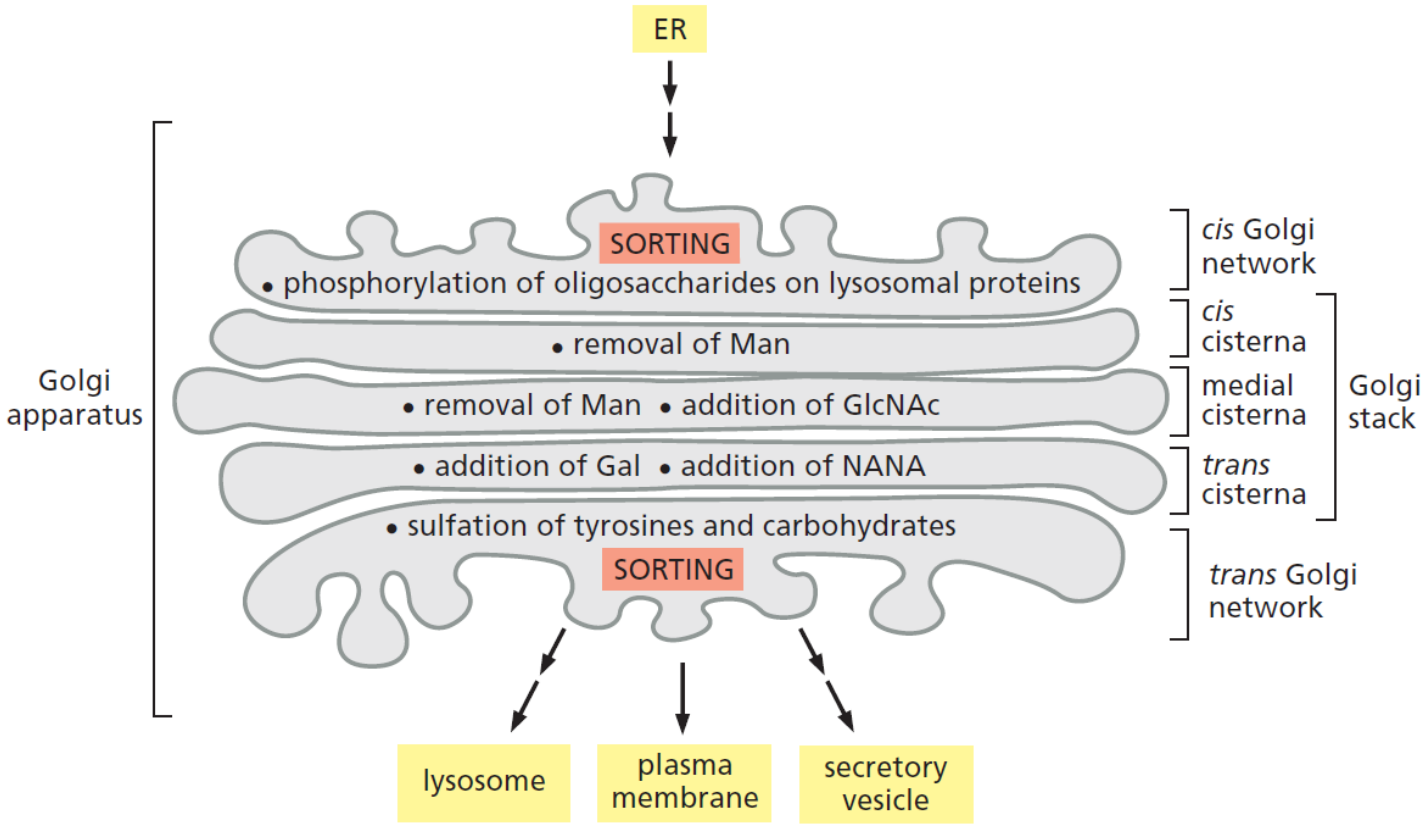
✓ **Both networks are important for protein sorting**: proteins entering the CGN can either move onward in the Golgi apparatus or be returned to the ER.
✓ Similarly, proteins exiting from the TGN move onward and are sorted according to their next destination: endosomes, secretory vesicles, or the cell surface. They also can be returned to an earlier compartment.
✓ Some membrane proteins are retained in the part of the Golgi apparatus where they function.

- A single species of N-linked oligosaccharide is attached en bloc to many proteins in the ER and then trimmed while the protein is still in the ER.
- The oligosaccharide intermediates created by the trimming reactions serve to help proteins fold and to help transport misfolded proteins to the cytosol for degradation in proteasomes.
- Thus, they play an important role in controlling the **quality of proteins exiting from the ER**.

The Golgi Apparatus Consists of an Ordered Series of Compartments

- Once these ER functions have been fulfilled, the cell reutilizes the oligosaccharides for new functions.
- This begins in the Golgi apparatus, which generates the **heterogeneous oligosaccharide structures seen in mature proteins**.
- After arrival in the CGN, proteins enter the first of the Golgi processing compartments (the cis Golgi cisternae). They then move to the next compartment (the medial cisternae) and finally to the trans cisternae, where glycosylation is completed.
- The lumen of the trans cisternae is thought to be continuous with the TGN, the place where **proteins are segregated into different transport packages and dispatched to their final destinations.**
- The oligosaccharide processing steps occur in an organized sequence in the Golgi stack, with each cisterna containing a characteristic mixture of processing enzymes.
- Proteins are modified in successive stages as they move from cisterna to cisterna across the stack, so that the stack forms a **multistage processing unit**.
- Investigators discovered the **functional differences** between the cis, medial, and trans subdivisions of the Golgi apparatus by localizing the enzymes involved in processing N-linked oligosaccharides in distinct regions of the organelle, both by physical fractionation of the organelle and by labeling the enzymes in electron microscope sections with antibodies.
- **The removal of mannose and the addition of N-acetylglucosamine**, for example, occur in the cis and medial cisternae, while **the addition of galactose and sialic acid** occurs in the trans cisterna and trans Golgi network.

Oligosaccharide processing in Golgi compartments



Processing enzymes are not restricted to a particular cisterna; instead, their distribution is graded across the stack, such that **early-acting enzymes** are present mostly in the cis Golgi cisternae and **later-acting enzymes** are mostly in the trans Golgi cisternae.

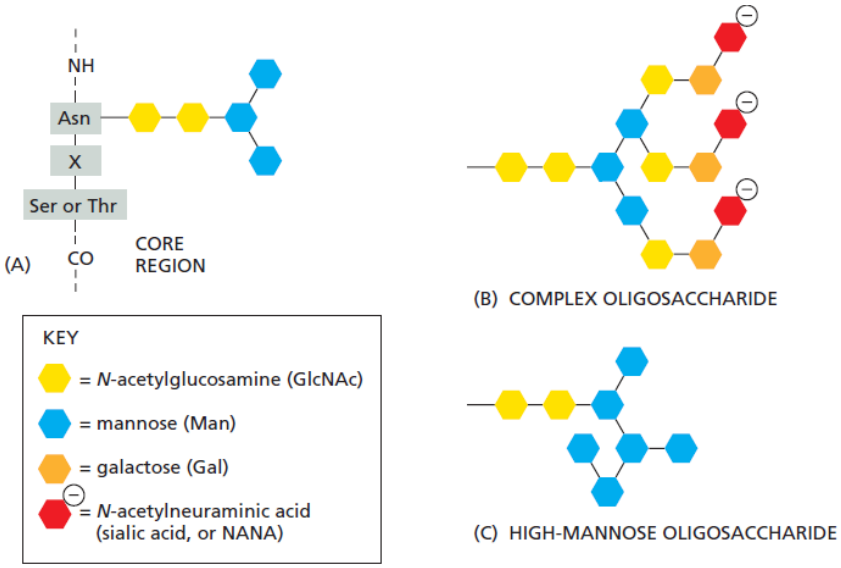
Man, mannose; GlcNAc, N-acetylglucosamine; Gal, galactose; NANA, N-acetylneuraminic acid (sialic acid).

Oligosaccharide Chains Are Processed in the Golgi Apparatus

- Whereas the ER lumen is full of soluble lumenal resident proteins and enzymes, **the resident proteins in the Golgi apparatus are all membrane bound, as the enzymatic reactions apparently occur entirely on membrane surfaces.**

- All of the **Golgi glycosidases** and **glycosyl transferases**, for example, are single-pass transmembrane proteins, many of which are organized in multienzyme complexes.

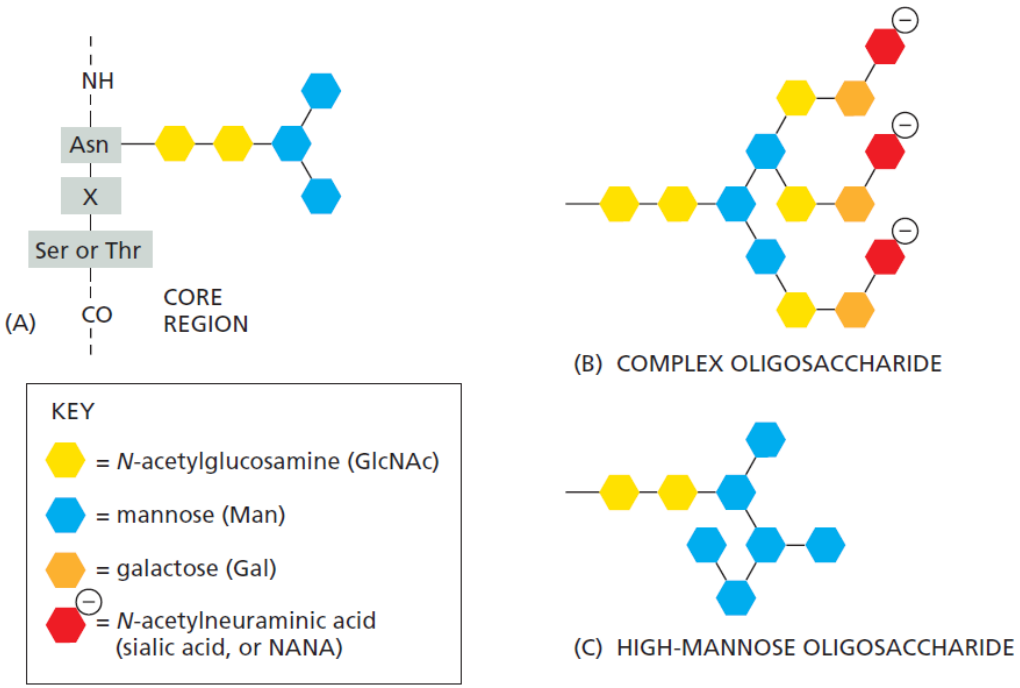
- Two broad classes of N-linked oligosaccharides, the **complex oligosaccharides** and the **high-mannose oligosaccharides**, are attached to mammalian glycoproteins.



(A) Both complex oligosaccharides and high-mannose oligosaccharides share a common core region derived from the original N-linked oligosaccharide added in the ER and typically containing two N-acetylglucosamines (GlcNAc) and three mannoses (Man).

✓ The three amino acids indicated in (A) constitute the sequence recognized by the oligosaccharyl transferase enzyme that adds the initial oligosaccharide to the protein. Ser, serine; Thr, threonine; X, any amino acid, except proline.

The two main classes of asparagine-linked (N-linked) oligosaccharides found in mature mammalian glycoproteins



(B) Each **complex oligosaccharide** consists of a core region, together with a terminal region that contains a variable number of copies of a special **trisaccharide unit (N-acetylglucosamine–galactose–sialic acid)** linked to the core mannoses.

- ✓ complex oligosaccharides are of special importance because they bear **a negative charge**.

Frequently, the terminal region is truncated and contains only GlcNAc and galactose (Gal) or just GlcNAc.

In addition, a fucose may be added, usually to the core GlcNAc attached to the asparagine (Asn). Thus, although the steps of processing and subsequent sugar addition are rigidly ordered, complex oligosaccharides can be heterogeneous. Moreover, although the complex oligosaccharide shown has three terminal branches, two and four branches are also common, depending on the glycoprotein and the cell in which it is made.

(C) **High-mannose oligosaccharides** are not trimmed back all the way to the core region and contain additional mannoses.

Oligosaccharide Chains Are Processed in the Golgi Apparatus

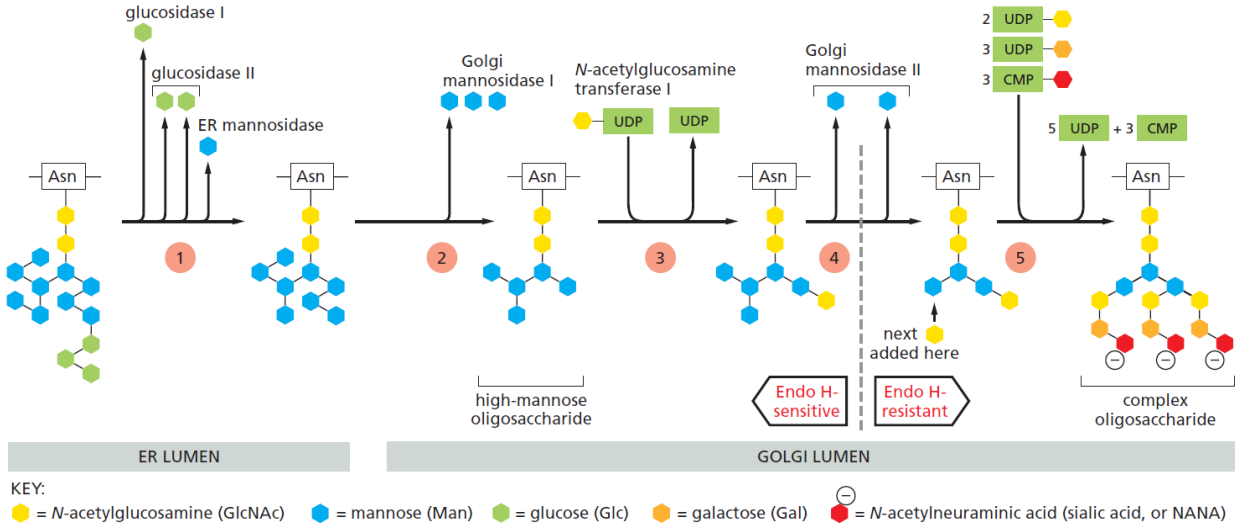
- Sometimes, both types are attached (in different places) to the same polypeptide chain.
- Whether a given oligosaccharide remains high-mannose or is processed depends largely on **its position in the protein**.

✓ If the oligosaccharide is **accessible to the processing enzymes in the Golgi apparatus**, it is likely to be converted to a complex form;

✓ if it is **inaccessible because its sugars are tightly held to the protein's surface**, it is likely to remain in a high-mannose form.

Oligosaccharide Chains Are Processed in the Golgi Apparatus

The processing that generates complex oligosaccharide chains follows the highly ordered pathway shown in Figure below:



- Beyond these commonalities in oligosaccharide processing that are shared among most cells, the products of the carbohydrate modifications carried out in the Golgi apparatus are highly complex and have given rise to a new field of study called **glycobiology**.

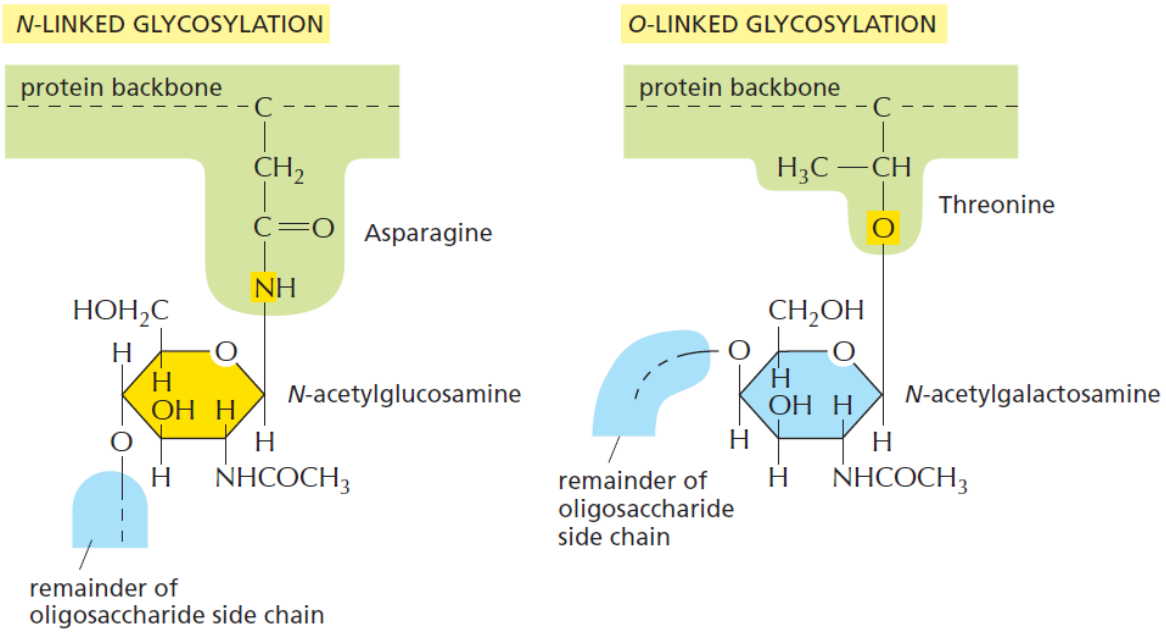
- The human genome, for example, encodes hundreds of different Golgi glycosyl transferases and many glycosidases, which are expressed differently from one cell type to another, resulting in a **variety of glycosylated forms of a given protein or lipid in different cell types and at varying stages of differentiation**, depending on the spectrum of enzymes expressed by the cell.
- The complexity of modifications is not limited to N-linked oligosaccharides but also occurs on O-linked sugars.

Proteoglycans Are Assembled in the Golgi Apparatus

- In addition to the N-linked oligosaccharide alterations made to proteins as they pass through the Golgi cisternae en route from the ER to their final destinations, many proteins are also modified in the Golgi apparatus in other ways.

- Some proteins have sugars added to the hydroxyl groups of selected serines or threonines, or, in some cases—such as **collagens**—to **hydroxylated proline and lysine side chains**.

- This O-linked glycosylation, like the extension of N-linked oligosaccharide chains, is catalyzed by a series of **glycosyl transferase enzymes** that use the sugar nucleotides in the lumen of the Golgi apparatus to add sugars to a protein one at a time.

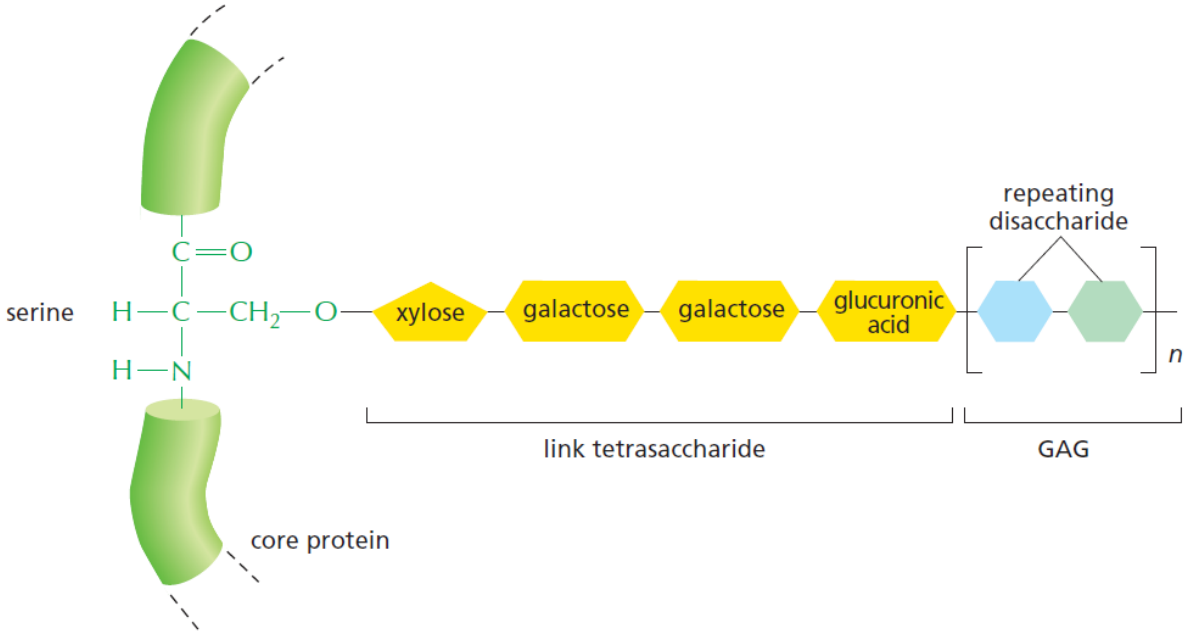


Proteoglycans Are Assembled in the Golgi Apparatus

- Usually, N-acetylgalactosamine is added first, followed by a variable number of additional sugars, ranging from just a few to 10 or more.

- The Golgi apparatus confers the heaviest O-linked glycosylation of all on mucins, the glycoproteins in mucus secretions, and on proteoglycan core proteins, which it modifies to produce proteoglycans.

- **This process involves the polymerization of one or more glycosaminoglycan (GAGs) chains (long, unbranched polymers composed of repeating disaccharide units;) onto serines on a core protein.**



Proteoglycans Are Assembled in the Golgi Apparatus

- Many proteoglycans are **secreted** and become components of the extracellular matrix, while others remain **anchored** to the extracellular face of the plasma membrane.
- Still others form a major component of slimy materials, such as the **mucus** that is secreted to form a protective coating on the surface of many epithelia.
- The sugars incorporated into glycosaminoglycans are heavily **sulfated** in the Golgi apparatus immediately after these polymers are made, thus adding a significant portion of their characteristically **large negative charge**.
- Some tyrosines in proteins also become sulfated shortly before they exit from the Golgi apparatus.
- In both cases, the sulfation depends on the sulfate donor **3′-phosphoadenosine-5′-phosphosulfate (PAPS)**, which is transported from the cytosol into the lumen of the trans Golgi network.