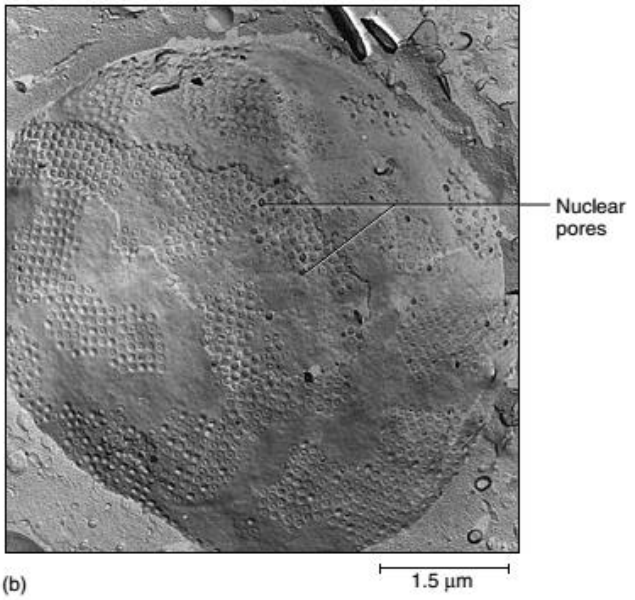
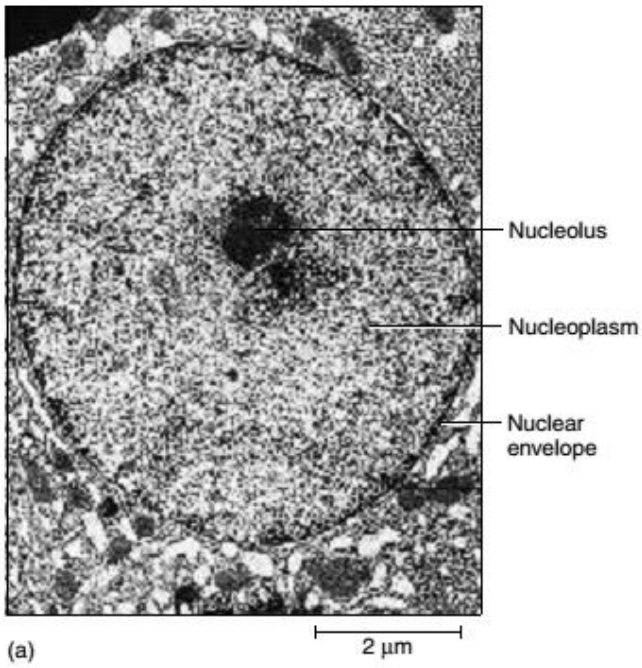
Nuclear structure and function

Nucleus is visible with the light microscope. It is usually **spherical** in shape and typically about **5 micrometers** in diameter. Most cells have a single nucleus, but there are exceptions. Mature red blood cells have none. A few cell types are multinucleate such as skeletal muscle cells. The material in the nucleus is called **nucleoplasm.** This includes **chromatin**, fine threadlike matter composed of **DNA** and **protein** and one or more dark-staining masses called **nucleoli** (singular, nucleolus). The nucleus contains the **genetic materials** to control the cell.





Nucleolus

The nucleolus is composed of **RNA** and **proteins. Nucleolus** is the site of most steps in **ribosome biogenesis**. Many **Nucleolar proteins** are involved in either **rRNA synthesis and modification** or **ribosome subunits assembly.** In addition, the nucleolus is involved in controlling the stability of the critical **cell-cycle regulator protein p53 (tumor suppressor protein).** Healthy cells keep p53 levels low by using **ubiquitylation.** Under certain types of stress, cells defend themselves by activating p53. They do this by having nucleolar proteins bind and inactivate the key factor that ubiquitylates p53.

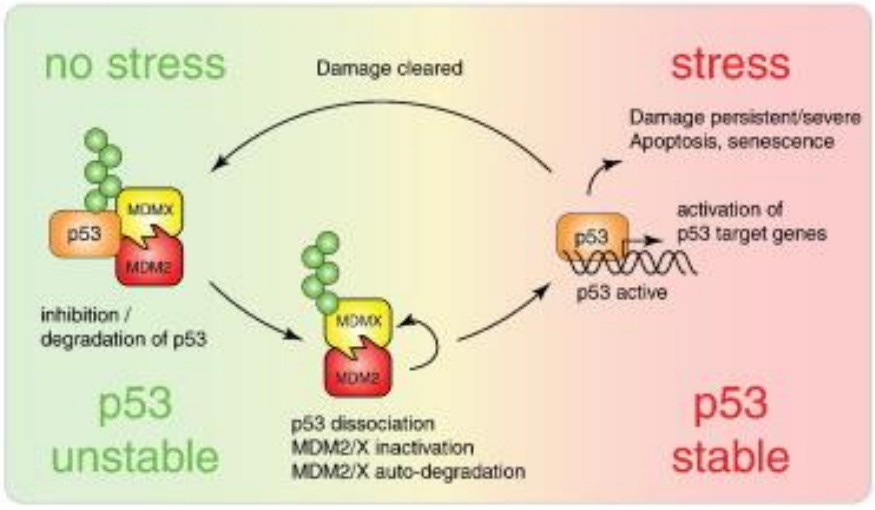


Figure: Feedback loop between p53 and MDM2/MDMX during stress response.

- In unstressed cells, p53 is kept inactive by both **transcriptional inhibition** and **proteasomal degradation**.
- Stress causes MDM2/MDMX to release p53 and increase **auto-degradation**. This leads to p53 accumulation, resulting in cell cycle arrest.
- If the stress can be resolved, the cells may return to the pre-stress state. Otherwise, p53 initiates **apoptosis**.

Nuclear envelope (Nuclear membranes)

Composition

With the TEM, the nucleus can be distinguished by the two-unit membranes surrounding it, which together form the **nuclear envelope**. The nuclear envelope is composed of **inner** and **outer nuclear membranes.** The outer nuclear membrane is continuous with the rough endoplasmic reticulum. The inner and outer nuclear membranes are separated by an approximately **luminal space (perinuclear space)** that is continuous with the lumen of the endoplasmic reticulum. A fibrous nuclear lamina of intermediate filaments supports the inner nuclear membrane in many eukaryotes. Nuclear lamina and other inner nuclear membrane proteins mediate interactions of the **envelope** with **chromatin**.

Functions

1. The nuclear envelope provides a **selective permeability barrier** between the nucleus and the cytoplasm.
2. The barrier keeps **pre-mRNA** in the nucleus until fully processed and licensed for export, so that only **mature mRNA** is delivered to ribosomes in the cytoplasm for translation into protein.
3. It also provides an additional level of **genetic protection and control** since the expression of certain genes is regulated by changes in the ability of factors to move between the cytoplasm and nucleus.

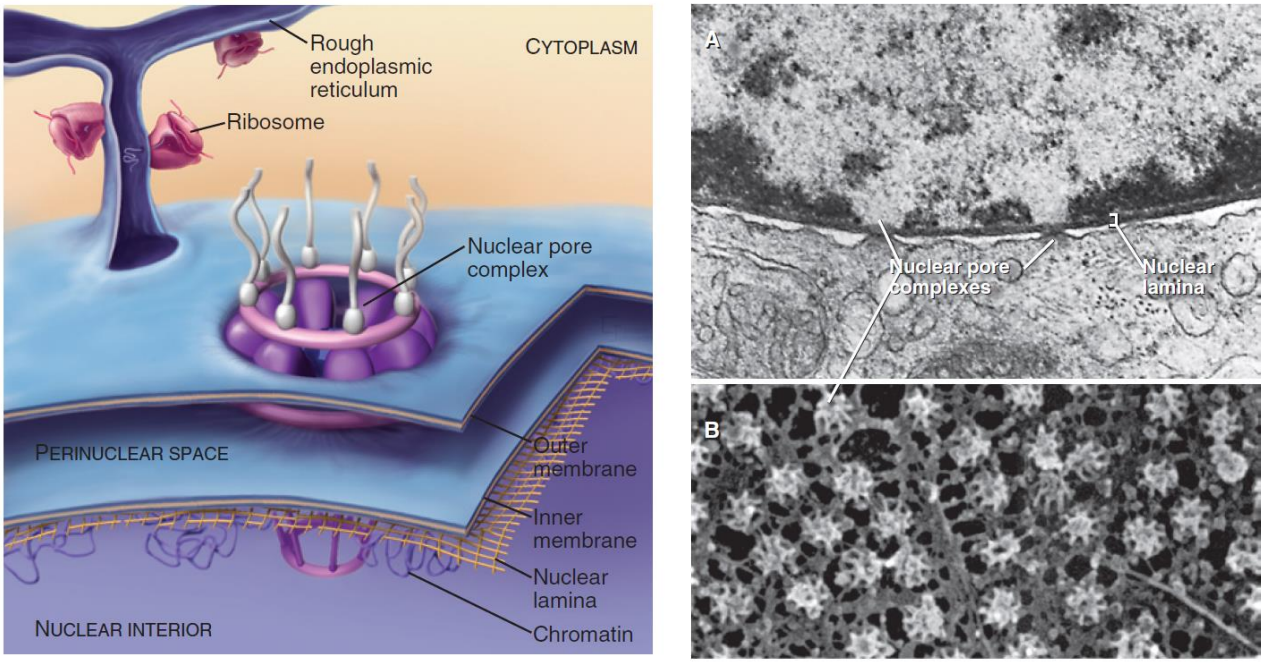


Figure: Nuclear envelope composition

Nuclear envelope defects lead to human diseases

Genetic defects in nuclear envelope proteins cause at least 20 disorders. The most dramatic of these is **Hutchinson-Gilford progeria syndrome**. Affected individuals are essentially normal at birth, but they appear to age rapidly and die.

Figure: Two young boys with the premature aging disorder Hutchinson-Gilford progeria.

Nuclear pore complexes (NPCs)

Nuclear pore complexes will bridge both nuclear membranes and provide the primary route for communication between the nucleus and cytoplasm. The nuclear envelope is perforated with **nuclear pores**, formed by a **ring of proteins**. Hundreds of molecules pass through the nuclear pores every minute. Coming into the nucleus are raw materials for DNA and RNA synthesis, enzymes that are made in the cytoplasm but function in the nucleus, and hormones that activate certain genes. Going the other way, RNA is made in the nucleus but leaves to perform its job in the cytoplasm.

1. **Cytoplasmic** and **nuclear rings**
2. **Spoke ring** that is associated with the pore membrane linking the inner and outer nuclear membranes.
3. The nuclear ring is anchored to the nuclear lamina.
4. Eight **filaments** project outward from both the nuclear and cytoplasmic rings.
5. The nuclear filaments are linked at their inner ends by a **terminal ring**. This structure is called the **nuclear basket.**

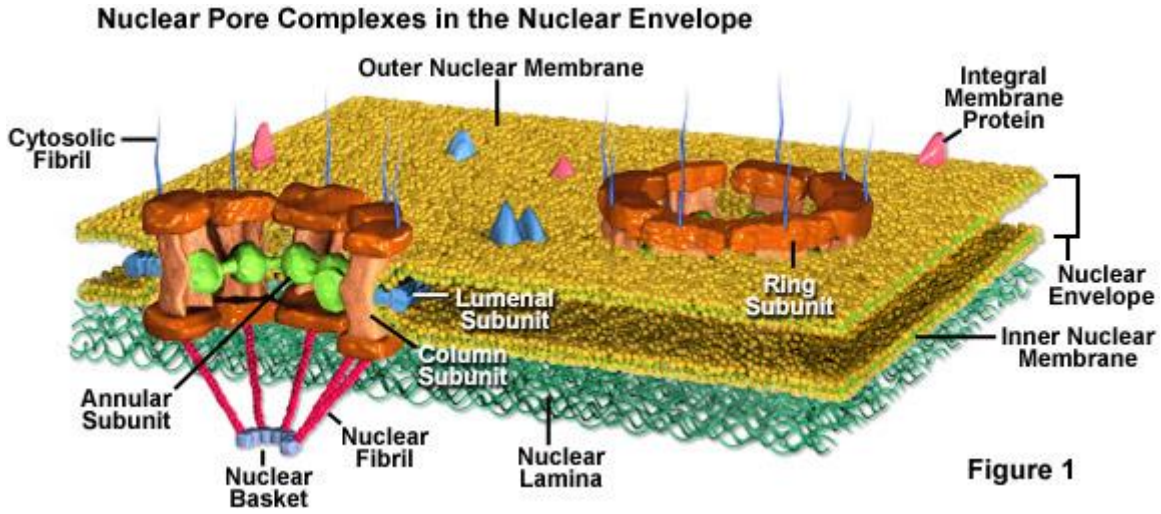


Figure 1

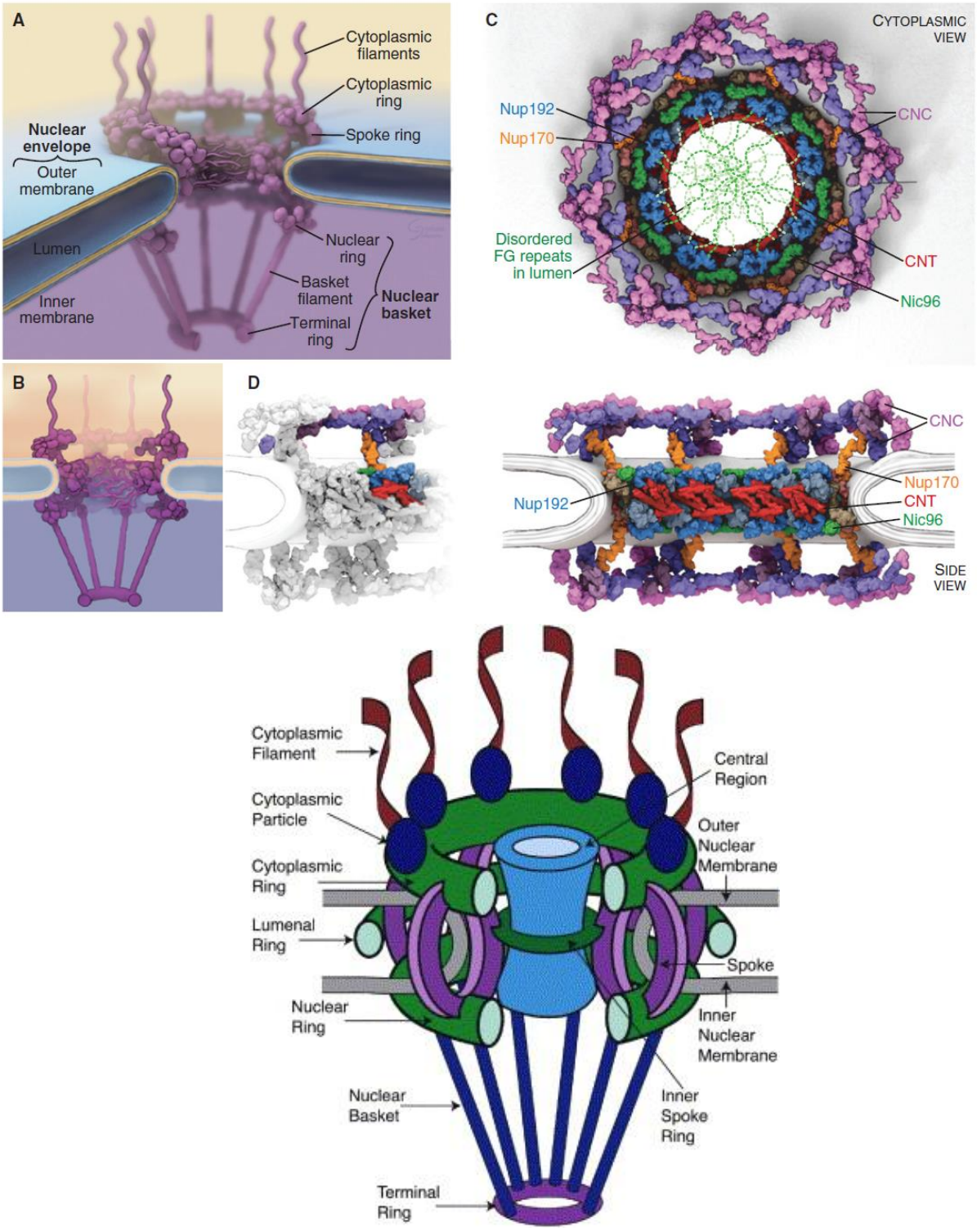
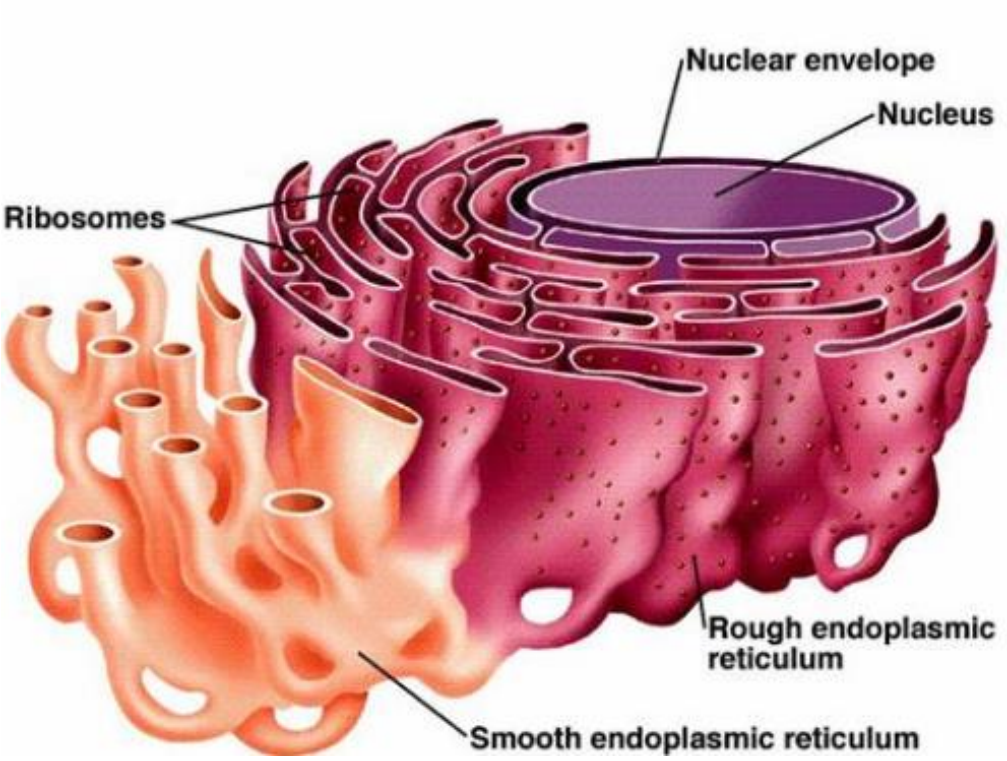


Figure: Nuclear pore complex

Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a network of membrane-enclosed channels that run throughout the cell. The ER is organized into a network of **branching tubules** and **flattened sacs** (cisternae) that extends throughout the cytosol. ER membrane is continuous with the outer nuclear membrane. The ER has **ER lumen**. The endoplasmic reticulum is divided into the **rough endoplasmic reticulum (RER)** and the **smooth endoplasmic reticulum (SER)**. The rough and smooth ER are not separated organelles, electron micrographs and studies in living cells show that their lumen spaces are connected.



Rough and Smooth ER

The basic difference is that the rough ER is covered with ribosomes, which give rough appearance in the electron microscope. The rough ER is where the cells make the proteins that will end up as integral membrane proteins in the plasma membrane, organelle proteins and proteins that the cell will export to the extracellular medium (such as the proteins of the extracellular matrix).

Smooth ER is without ribosomes. The function of the smooth ER varies from one tissue to another tissue. In the ovaries, testes, and the adrenal gland where steroid hormones are made; in the liver, it is the site of detoxification of foreign chemicals including drugs. Probably the most universal role of the smooth ER is the storage and sudden release of **calcium ions**. Calcium ions are pumped from the cytosol into the lumen of the smooth ER to more than 100 times the concentration found in the cytosol. Many stimuli can cause this calcium to be released back into the cytosol, where it activates many cell processes.

The rough ER membranes usually form large flattened sacs called cisternae, whereas smooth ER membranes generally form tubular structures. Cells involved in the biosynthesis of secretory proteins, such as cells producing digestive enzymes in pancreas, tend to have very prominent rough ER networks. On the other hand, cells producing steroid hormones, such as in the testis or ovary (gonads), contain extensive networks of smooth ER.

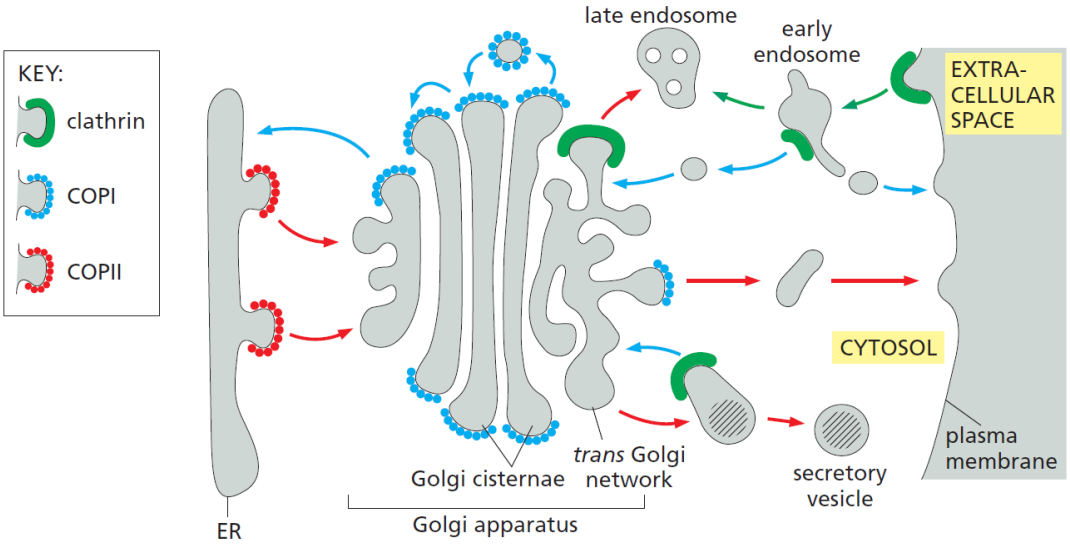
Intracellular membrane traffic

Within eukaryotic cells, transport vesicles continually bud off from one membrane and fuse with another, carrying membrane components and soluble molecules in lumen, which are referred to as cargo. For example, the secretory pathway leads outward from the ER toward the Golgi apparatus and cell surface.

There are various types of coated vesicles

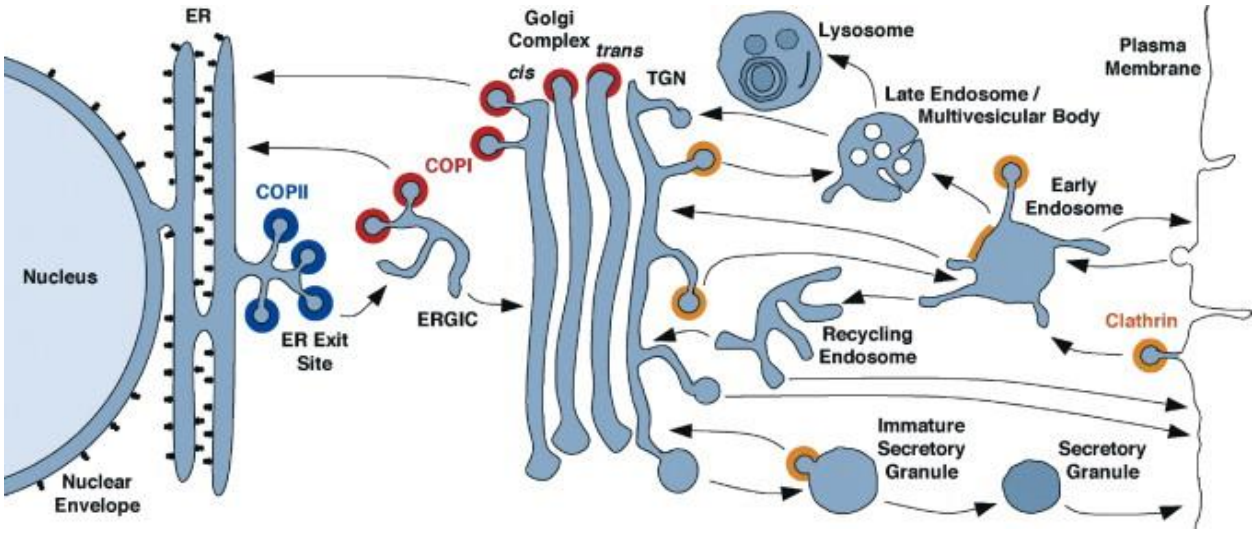
The transport vesicles bud off as coated vesicles. There are **three** types of coated vesicles, distinguished by their major coat proteins:

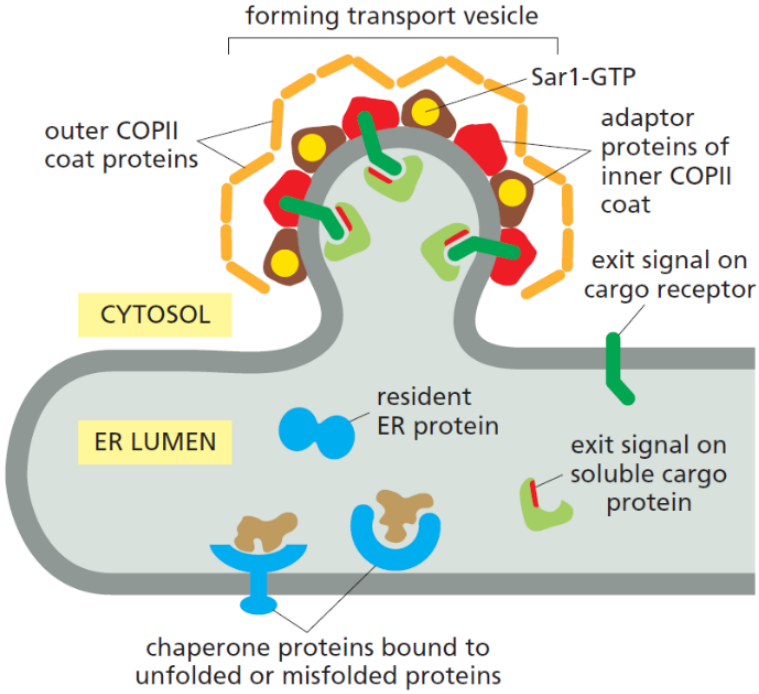
1. **Clathrin-coated vesicles.** Clathrin-coated vesicles mediate transport from the Golgi apparatus and from the plasma membrane.
2. **COPI-coated vesicles.** COPI-coated vesicles mediate transport from the Golgi cisternae.
3. **COPII-coated vesicles.** COPII-coated vesicles mediate transport from the ER.



Proteins Leave the ER in COPII-Coated Transport Vesicles

Proteins that have entered the ER and are destined for the Golgi apparatus are first packaged into COPII-coated transport vesicles. These vesicles bud from specialized regions of the ER called **ER exit sites,** whose membrane lacks bound ribosomes. Many membrane proteins are actively recruited into such vesicles, where they become concentrated. These cargo membrane proteins display **exit signals** on their cytosolic surface that **adaptor proteins** of the inner COPII coat recognize.

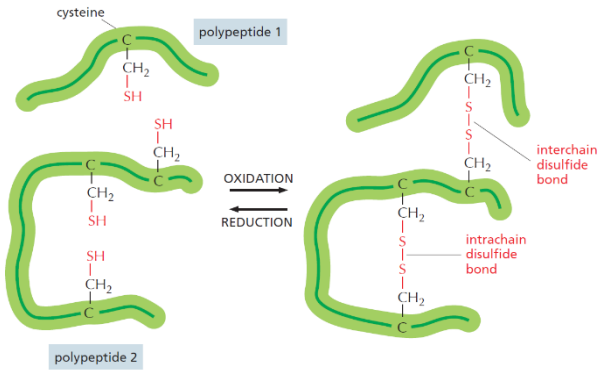




Protein modifications in the ER and Golgi apparatus

Proteins are modified in the ER

Most proteins that enter the ER are chemically modified there. For example, **disulfide bonds** are formed by the oxidation of pairs of **cysteine** side chains, a reaction catalyzed by an enzyme that resides in the ER lumen. The disulfide bonds help to **stabilize** the structure of proteins that will encounter **degradative enzymes** and changes in **pH outside** the cell.



Many of the proteins that enter the ER lumen are converted to **glycoproteins** by the covalent attachment of short, **branched oligosaccharide** side chains. This process of **glycosylation** is carried out by **glycosylating enzymes** present in the ER**.** The oligosaccharides on proteins can serve various functions, for example

1. Protect a protein from degradation
2. Hold protein in the ER until it is properly folded
3. Help to guide protein to the appropriate organelle by serving as a **transport signal**
4. When displayed on the **cell surface**, oligosaccharides can function in the **recognition** of one cell by another or **identification**.

Exit from the ER is controlled to ensure protein quality

Some proteins made in the ER are destined to function there. They are retained in the ER by a C-terminal sequence of **four amino acids** called an **ER retention signal.** But most proteins that enter the ER are destined for **other locations**; they are packaged into transport vesicles that bud from the ER and fuse with the **Golgi apparatus.** Exit from the ER is highly selective. Proteins that fail to **fold correctly**, and **dimeric** or **multimeric proteins** that do not **assemble properly**, are actively retained in the ER by binding to **chaperone proteins**. The chaperones hold these proteins in the ER until **proper folding** or **assembly** occurs. If proper folding and assembly still fail, the proteins are exported to the **cytosol**, where they are degraded by the **proteasome.** In this way, the ER controls the **quality of the proteins** that it **exports** to the Golgi apparatus.

Golgi apparatus

Golgi apparatus was discovered in the year 1898 by an Italian biologist Camillo Golgi. It was one of the first cellular organelles to be discovered and observed in detail due to its large size. The term Golgi apparatus was used in 1910 and in 1913 it first appeared in the scientific literature.

The newly synthesized proteins, found in the channels of the rough endoplasmic reticulum are moved to the Golgi body where they modify more and then modified molecules are enveloped in a part of the Golgi membrane and then the enveloped molecules leave the cell. The Golgi apparatus hence acts as the assembly factory of the cell where the raw materials are directed to the Golgi apparatus before being passed out from the cell.

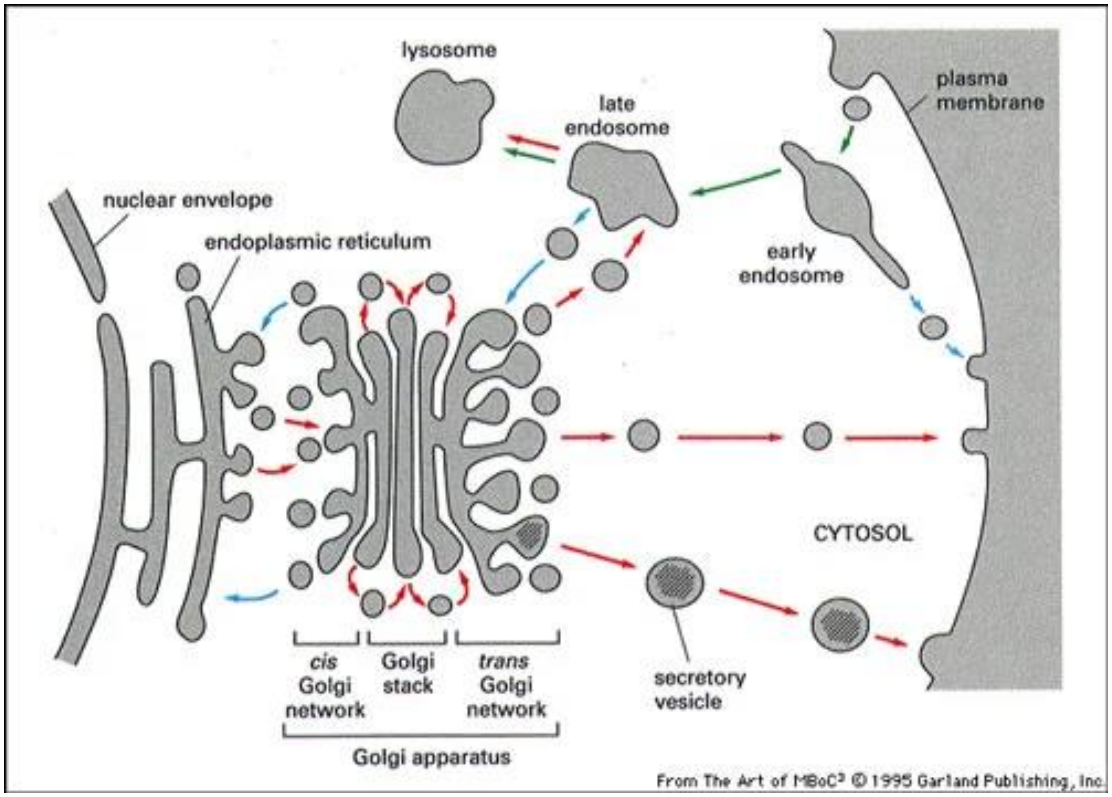
Golgi apparatus structure

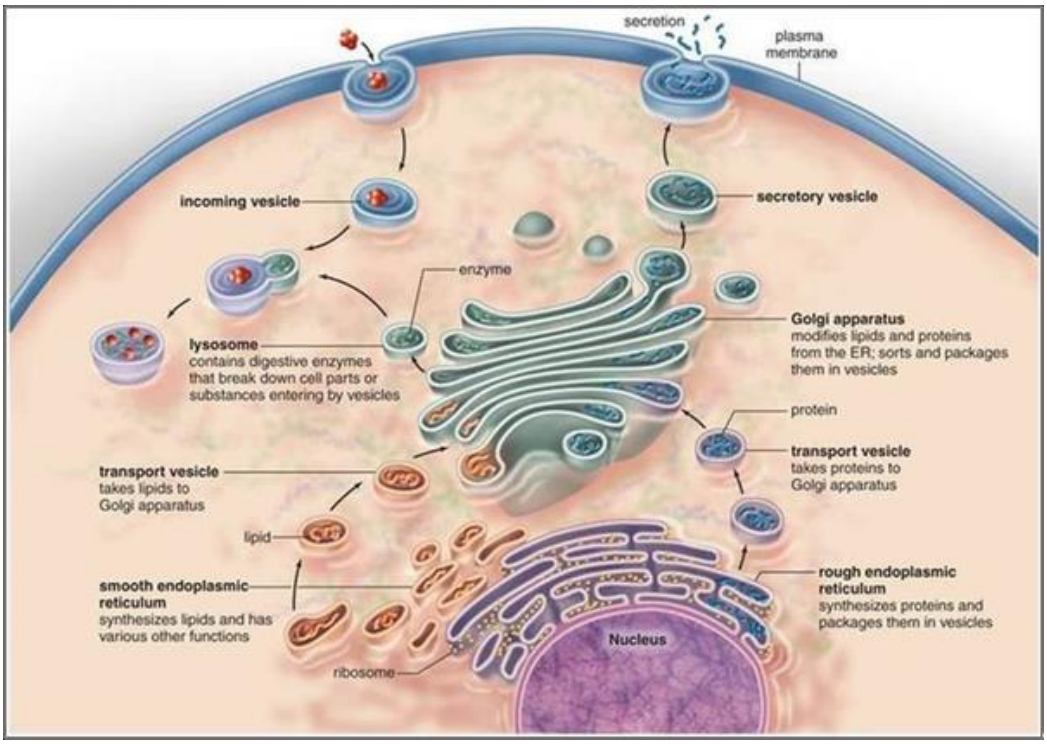
The Golgi apparatus is a major organelle in eukaryotic cells. They are membrane bound organelles, which are sac-like. With the aids of special staining techniques the Golgi bodies were seen as densely stained region of the cytoplasm under the optical microscope. Under the electron microscope the Golgi apparatus is seen to be composed of stacks of flattened structures, which contains numerous vesicles containing secretory granules. They are found in the cytoplasm of plant and animal cells. The Golgi complex is composed of stacks of membrane-bound structures; these structures are known as the **cisternae**. An individual stack of the cisternae is sometimes referred as **dictyosome**. In a typical animal cell, there are about **40** to **100 stacks**. In a stack there are about **four** to **eight** cisternae. Each cisternae is a disc enclosed in a membrane, it possess special enzymes of the Golgi which help to modify and transport of the modified proteins to their destination.

Golgi apparatus functions

1. The cell synthesizes a huge amount of variety of macromolecules. The main function of the Golgi apparatus is to modify, sort and package the macromolecules that are synthesized by the cells for secretion purposes or for use within the cell. It mainly modifies the proteins that are produced by the rough endoplasmic reticulum.
2. The enzymes in the cisternae have the ability to modify proteins by the addition of carbohydrates and phosphate by the process of glycosylation and phosphorylation respectively.
3. The Golgi complex is referred as post office where the molecules are packaged, labeled and sent to different parts of the cell.

4. They also create lysosomes.

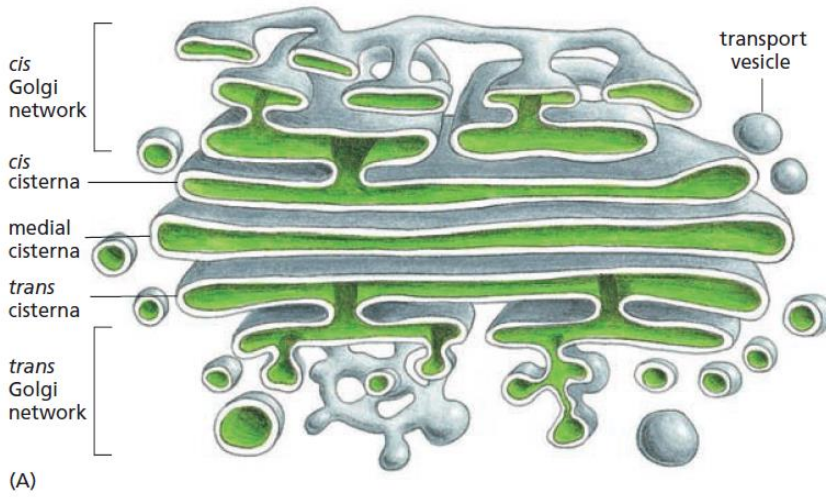


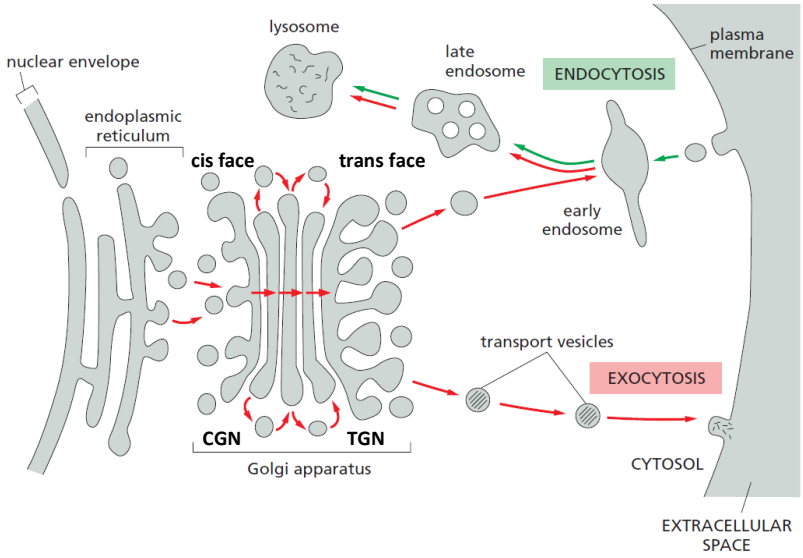


Proteins are further modified and sorted in the Golgi apparatus

Each Golgi stack has two distinct faces: ***cis* face** and ***trans* face**. The *cis* face is adjacent to the **ER**, while the *trans* face points toward the **plasma membrane**. The Golgi compartment closest to the ER is a network of flattened, membrane-bounded tubules referred to as the ***cis*-Golgi network (CGN).** The Golgi compartment closest to the plasma membrane is a network of flattened, membrane-bounded tubules referred to as the ***trans*-Golgi network (TGN).** The central sacs between the CGN and TGN comprise the medial cisternae of the Golgi stack, in which much of the **processing of proteins** occurs. Soluble proteins enter the CGN via **transport vesicles** derived from the **ER**. The proteins travel through the cisternae by means of transport vesicles that bud from one cisterna and fuse with the next and by a maturation process in which the Golgi cisternae themselves migrate through the Golgi stack. Proteins finally exit from the TGN in transport vesicles destined for either the **cell surface**, another **organelle** of the endomembrane system, or **secretion**.

Many of the oligosaccharide chains that are added to proteins in the ER undergo **further modifications** in the **Golgi apparatus**. On some proteins, more complex oligosaccharide chains are created by a highly ordered process in which sugars are added and removed by a series of enzymes that act in a sequence as the protein passes through the Golgi stack.

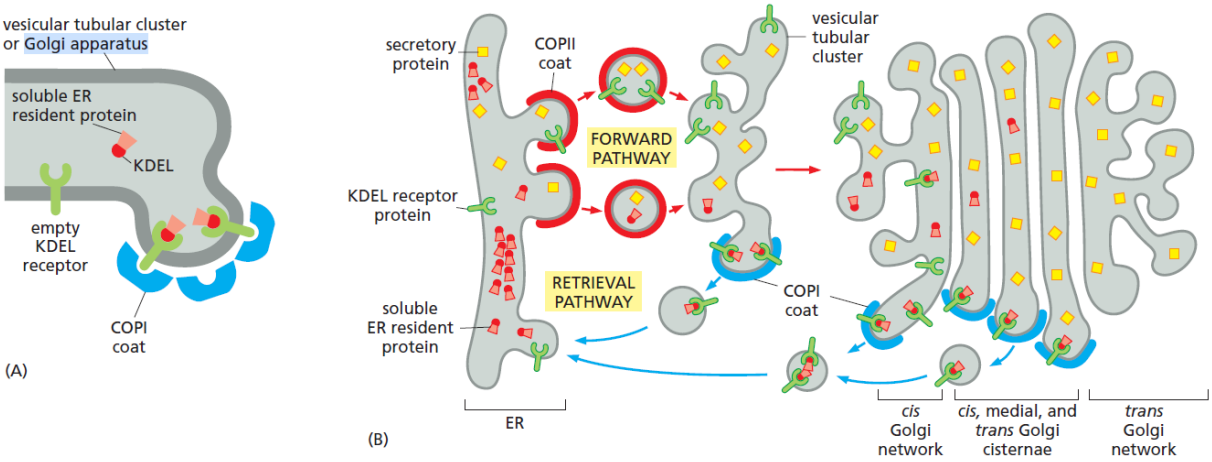




The retrieval pathway to the ER uses sorting signals

The retrieval pathway for returning escaped proteins back to the ER depends on ER retrieval signals. Resident **ER membrane proteins** contain signals that bind directly to COPI coats and are thus packaged into **COPI-coated transport vesicles** for retrograde delivery to the **ER**. Soluble ER resident proteins contain a short **ER retrieval signal**, which consists of a Lysine (K)-Aspartic acid (D)-Glutamic acid (E)-Leucine (L) (called the **KDEL sequence**). Soluble ER resident proteins must bind to specialized receptor proteins such as the **KDEL receptor**— a membrane protein that binds to the KDEL sequence and packages **any protein displaying** it

into COPI-coated **retrograde transport vesicles**. The **KDEL receptor** itself must cycle between the ER and the Golgi apparatus, and its **affinity** for the KDEL sequence must differ in these two compartments. The receptor has a **high affinity** for the KDEL sequence in the Golgi apparatus, but it has a **low affinity** for the KDEL sequence in the ER.



Lysosomes and peroxisomes

Endocytosis

Endocytosis is the general term describing vesicles that form at the plasma membrane, carrying material into the cell. Endocytosis is unique because it is the portal by which many physiologically important molecules are brought into the cell. In endocytosis a portion of the plasma membrane is invaginated and pinched off forming a membrane-bounded vesicle called an **endosome or phagosome**. Endocytosis requires energy in the form of ATP and therefore is active processes.

Fate of endosome and phagosome

Endosome and phagosome contain useful or harmful materials to the cell, the materials of the endosome and phagosome will lysed by action of enzymes within the lysosome. Therefore, Many extracellular particles and molecules ingested by cells end up in lysosomes.

Lysosomes

Lysosomes are membrane-enclosed organelles found in the cytoplasm of eukaryotic cells that contain enzymes capable of breaking down all types of biological polymers which include **proteins, nucleic acids, carbohydrates** and **lipids**. Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself. Lysosomes are organelles containing digestive enzymes (acid hydrolases). They are found in animal cells, while in plant cells the same roles are performed by the vacuole. Lysosomes were discovered by the Belgian cytologist Christian de Duve in 1955.

Occurrence

Lysosomes occur in animal cells and in plant cells as vacuoles. They are most abundant in cells, which are related with enzymatic reactions such as liver cells, pancreatic cells, kidney cells, spleen cells, leucocytes, macrophages etc.

Shape and size

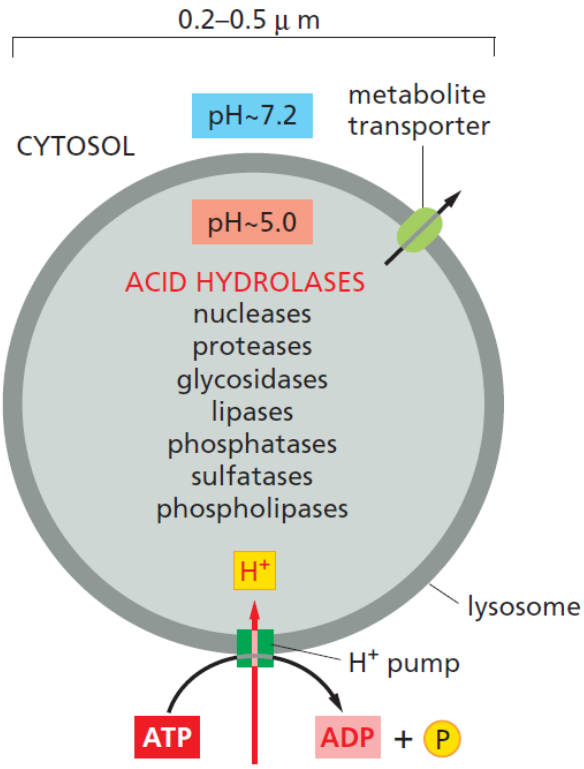
Lysosomes are usually spherical in shape. The size of the lysosomes usually ranges from 0.2 μm to 0.8 μm in diameter.

Contents and structures of lysosome

Lysosomes are single membrane bound organelle, which contains enzymes. The lysosomes may contain about **40 varieties** of enzymes. The lysosomal enzymes are classified into six main types namely **Nucleases, Proteases, glycosidases, lipases, phosphatases** and **sulphatases**. All of the enzymes of the lysosome are **acid hydrolases**, which are active at the **acidic pH** about 5 that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm. The requirement of these lysosomal hydrolases for acidic pH provides double protection against uncontrolled digestion of the contents of the cytosol; therefore, even if the lysosomal membrane were to break down, the released acid hydrolases would be inactive at the neutral pH of the cytosol. To maintain their acidic internal pH, lysosomes must actively concentrate H^+ ions (protons). This is accomplished by a **proton pump** in the lysosomal membrane, which actively transports protons into the lysosome from the cytosol. This pumping requires expenditure of energy in the form of ATP hydrolysis, since it maintains approximately a hundredfold higher H^+ concentration inside the lysosome.

In addition, the lysosomal membrane **transporters** allow the final products of the digestion of macromolecules, such as amino acids, sugars, and nucleotides, to be transferred to the cytosol

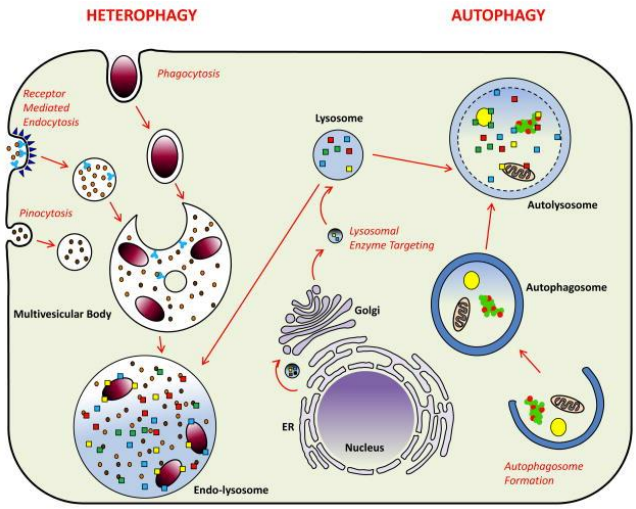
Figure: A lysosome contains a large variety of hydrolytic enzymes, which are only active under acidic conditions. The lumen of the lysosome is maintained at an acidic pH by an ATP-driven H^+ pump in the membrane that hydrolyzes ATP to pump H^+ into the lumen.

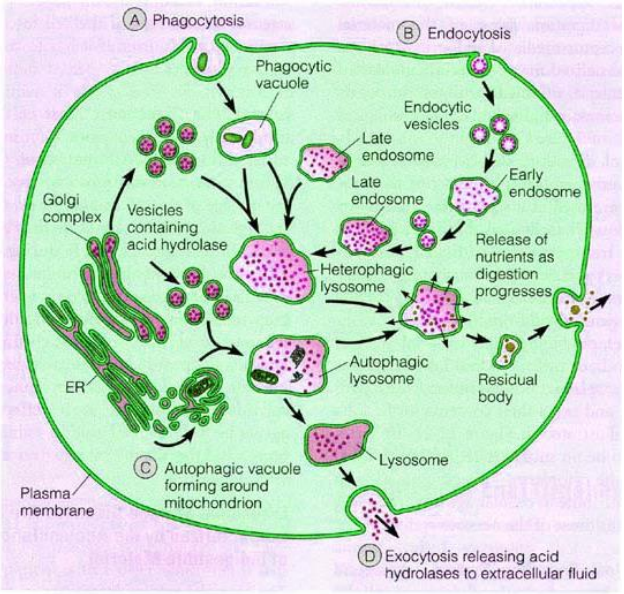


Lysosomes and intracellular digestion

The extracellular particles are taken up, in a process called phagocytosis (cellular eating), into phagosomes, which fuse with lysosomes. Also, the extracellular fluid and macromolecules are taken up, in a process called endocytosis (pinocytosis, cellular drinking), into smaller endocytic vesicles, which deliver their contents to lysosomes via endosomes.

Cells have an additional pathway that supplies materials to lysosomes, which called autophagy. The autophagy is used to degrade old parts of the cell: the cell literally eats itself. The process involves the surrounding of the organelle such as mitochondria with a double membrane, creating an autophagosome, which then fuses with a lysosome. Autophagy of organelles and cytosolic proteins increases when eukaryotic cells are starved.





What are

1. Primary lysosomes
2. Secondary lysosomes
3. Autolysosomes
4. Residual bodies?

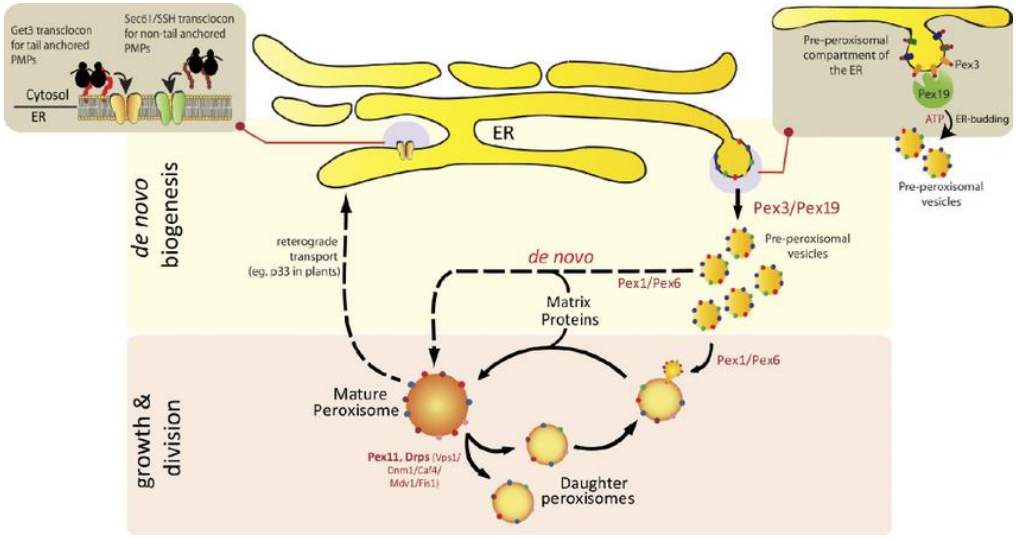
Peroxisomes

Peroxisomes are small (0.1–1 μm), single-membrane-enclosed organelles that contain enzymes involved in a variety of **metabolic reactions**. Peroxisomes have two sub-compartments, an internal matrix and an outer membrane. They contain a wide variety of enzymes that can catalyze the O_2-dependent oxidation of substrates with the production of **hydrogen peroxide**. Peroxisomes frequently contain the **catalase** enzyme, which breaks down hydrogen peroxide into oxygen and water.

$2H_2O_2 \rightarrow 2\ H_2O + O_2$

The majority of peroxisomal proteins (enzymes) are synthesized on free ribosomes and translocated across the peroxisomal membrane.

They are found in virtually eukaryotic cells and basically multiply by division. Most new peroxisomes are formed by the growth and division of preexisting peroxisomes. However, recent evidence suggests that there might also be a ***de novo* pathway** for peroxisome biogenesis.



Functions of peroxisomes

1. Peroxisomes contain more than 50 different enzymes that play critical roles in several metabolic pathways. They carry out oxidation reactions leading to the production of **hydrogen peroxide (H_2O_2).** Because hydrogen peroxide is harmful to the cell, peroxisomes also contain the **catalase** enzyme, which **decomposes** hydrogen peroxide by converting it to **water.**
2. A variety of substrates are broken down by such oxidative reactions in peroxisomes, including uric acid, amino acids, purines, methanol, and fatty acids.
3. Peroxisomes are also involved in **biosynthesis of lipids**. In animal cells, cholesterol is synthesized in peroxisomes as well as in the **endoplasmic reticulum (ER).**
4. In the **liver**, peroxisomes are also involved in the **synthesis of bile acids**, which are derived from cholesterol.