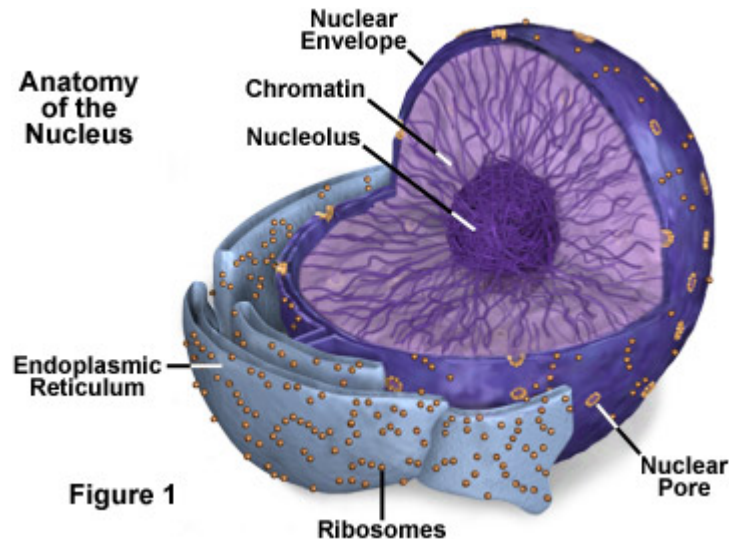


## The Cell Nucleus

The nucleus is a highly specialized organelle that serves as the information processing and administrative center of the cell. This organelle has two major functions: it stores the cell's hereditary material, or DNA, and it coordinates the cell's activities, which include growth, intermediary metabolism, protein synthesis, and reproduction (cell division).



Only the cells of advanced organisms, known as **eukaryotes**, have a nucleus. Generally there is only one nucleus per cell, but there are exceptions, such as the cells of slime molds and the **Siphonales** group of algae. Simpler one-celled organisms (**prokaryotes**), like the bacteria and cyanobacteria, don't have a nucleus. In these organisms, all of the cell's information and administrative functions are dispersed throughout the cytoplasm.

The spherical nucleus typically occupies about 10 percent of a eukaryotic cell's volume, making it one of the cell's most prominent features. A double-layered membrane, the nuclear envelope, separates the contents of the nucleus from the cellular cytoplasm. The envelope is riddled with holes called nuclear pores that allow specific types and sizes of molecules to pass back and forth between the nucleus and the cytoplasm. It is also attached to a network of tubules and sacs, called the endoplasmic reticulum, where protein synthesis occurs, and is usually studded with ribosomes (see Figure 1).

The semifluid matrix found inside the nucleus is called nucleoplasm. Within the nucleoplasm, most of the nuclear material consists of chromatin, the less condensed form of the cell's DNA that organizes to form chromosomes during mitosis or cell division. The nucleus also contains one or more nucleoli, organelles that synthesize protein-producing macromolecular assemblies called ribosomes, and a variety of other smaller components, such as Cajal bodies, **GEMS** (Gemini of coiled bodies), and interchromatin granule clusters.

**Chromatin and Chromosomes** - Packed inside the nucleus of every human cell is nearly 6 feet of DNA, which is divided into 46 individual molecules, one for each chromosome and each about 1.5 inches long. Packing all this material into a microscopic cell nucleus is an

extraordinary feat of packaging. For DNA to function, it can't be crammed into the nucleus like a ball of string. Instead, it is combined with proteins and organized into a precise, compact structure, a dense string-like fiber called chromatin.

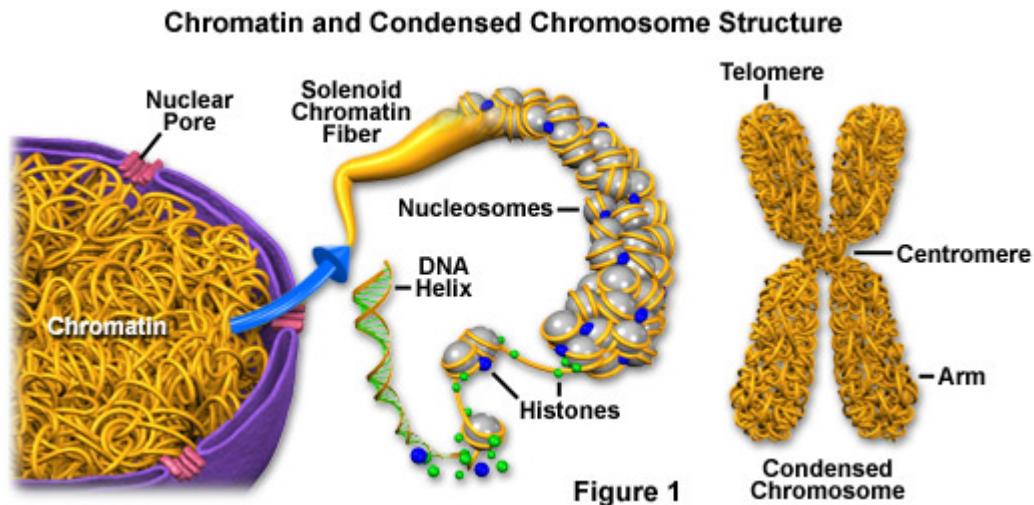
**The Nucleolus** - The nucleolus is a membrane-less organelle within the nucleus that manufactures ribosomes, the cell's protein-producing structures. Through the microscope, the nucleolus looks like a large dark spot within the nucleus. A nucleus may contain up to four nucleoli, but within each species the number of nucleoli is fixed. After a cell divides, a nucleolus is formed when chromosomes are brought together into nucleolar organizing regions. During cell division, the nucleolus disappears. Some studies suggest that the nucleolus may be involved with cellular aging and, therefore, may affect the senescence of an organism.

**The Nuclear Envelope** - The nuclear envelope is a double-layered membrane that encloses the contents of the nucleus during most of the cell's lifecycle. The space between the layers is called the perinuclear space and appears to connect with the rough endoplasmic reticulum. The envelope is perforated with tiny holes called nuclear pores. These pores regulate the passage of molecules between the nucleus and cytoplasm, permitting some to pass through the membrane, but not others. The inner surface has a protein lining called the nuclear lamina, which binds to chromatin and other nuclear components. During mitosis, or cell division, the nuclear envelope disintegrates, but reforms as the two cells complete their formation and the chromatin begins to unravel and disperse.

**Nuclear Pores** - The nuclear envelope is perforated with holes called nuclear pores. These pores regulate the passage of molecules between the nucleus and cytoplasm, permitting some to pass through the membrane, but not others. Building blocks for building DNA and RNA are allowed into the nucleus as well as molecules that provide the energy for constructing genetic material.

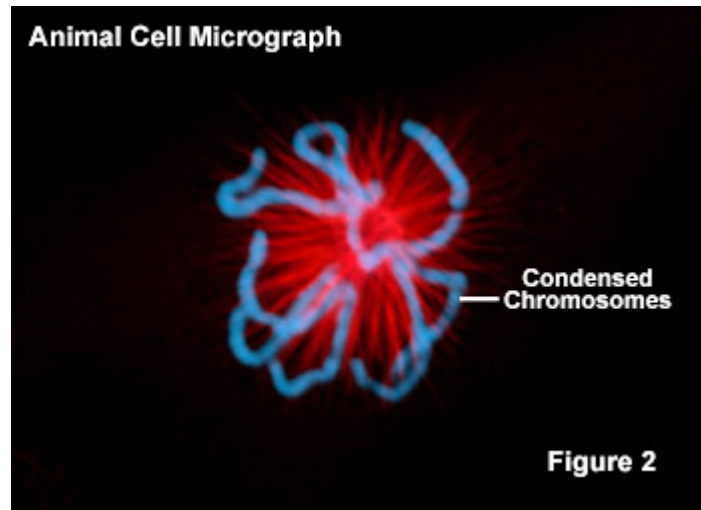
### **Chromatin and Chromosomes**

Packed inside the nucleus of every human cell is nearly 6 feet of DNA, which is subdivided into 46 individual molecules, one for each chromosome and each about 1.5 inches long. Collecting all this material into a microscopic cell nucleus is an extraordinary feat of packaging. For DNA to function when necessary, it can't be haphazardly crammed into the nucleus or simply wound up like a ball of string. Consequently, during interphase, DNA is combined with proteins and organized into a precise, compact structure, a dense string-like fiber called chromatin, which condenses even further into chromosomes during cell division.



Each DNA strand wraps around groups of small protein molecules called **histones**, forming a series of bead-like structures, called **nucleosomes**, connected by the DNA strand (as illustrated in Figure 1). Under the microscope, uncondensed chromatin has a "beads on a string" appearance. The string of nucleosomes, already compacted by a factor of six, is then coiled into an even denser structure known as a **solenoid** that compacts the DNA by a factor of 40. The solenoid structure then coils to form a hollow tube. This complex compression and structuring of DNA serves several functions. The overall negative charge of the DNA is neutralized by the positive charge of the histone molecules, the DNA takes up much less space, and inactive DNA can be folded into inaccessible locations until it is needed.

There are two basic types of chromatin. **Euchromatin** is the genetically active type of chromatin involved in transcribing RNA to produce proteins used in cell function and growth. The predominant type of chromatin found in cells during interphase, euchromatin is more diffuse than the other kind of chromatin, which is termed **heterochromatin**. The additional compression of heterochromatin is thought to involve various proteins in addition to the histones, and the DNA it contains is thought to be genetically inactive. Heterochromatin tends to be most concentrated along chromosomes at certain regions of the structures, such as the centromeres and telomeres. Genes typically located in euchromatin can be experimentally **silenced** (not expressed) by relocating them to a heterochromatin position.

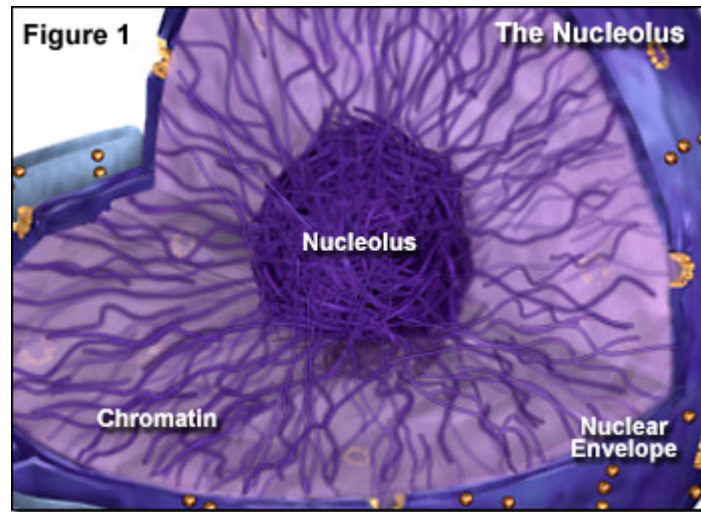


Throughout the life of a cell, chromatin fibers take on different forms inside the nucleus. During interphase, when the cell is carrying out its normal functions, the chromatin is dispersed throughout the nucleus in what appears to be a tangle of fibers. This exposes the euchromatin and makes it available for the transcription process. When the cell enters metaphase and prepares to divide, the chromatin changes dramatically. First, all the chromatin strands make copies of themselves through the process of DNA replication. Then they are compressed to an even greater degree as they undergo a 10,000-fold compaction into specialized structures for reproduction, the chromosomes (see Figure 2). As the cell divides to become two cells, the chromosomes separate, giving each cell a complete copy of the genetic information contained in the chromatin.

The number of chromosomes within the nuclei of an organism's cells is a species-specific trait. Human diploid cells (those that are not gametes) characteristically exhibit 46 chromosomes, but this number can be as low as 2, as is the case for some ants and roundworms, or more than a thousand, as exemplified by the Indian fern (*Ophioglossum reticulatum*), which has 1,260 chromosomes. Accordingly, the number of chromosomes a species has does not correlate to the complexity of the organism.

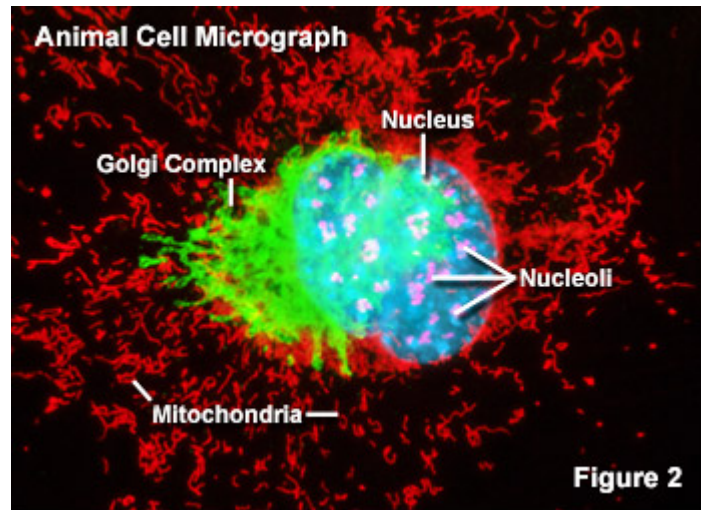
### The Nucleolus

The nucleolus is a prominent sub-nuclear structure that is not bound by a membrane and resides within the nuclear matrix. Though known to exist since the eighteenth century, the primary function of the nucleolus was not discovered until the 1960s. It is now been determined that nucleoli manufacture the subunits that combine to form ribosomes, the cell's protein-producing factories. Accordingly, the size of nucleoli depends upon the ribosomal requirements of the type of cell in which they are found. In cells that produce large amounts of protein, and thus call for significant numbers of ribosomes, the size of the nucleolus is considerable, sometimes occupying as much as 25 percent of the total volume of the nucleus.



Through the microscope, the nucleolus appears like a large dark spot within the nucleus (see Figure 2). Eukaryotic cells often contain a single nucleolus, but several are also possible. The exact number of nucleoli is fixed among members of the same species. Each diploid cell in the human body features only one nucleolus, though immediately after cell division ten tiny nucleoli appear before they coalesce into a single, large nucleolus. This is because nucleoli are formed at certain chromosome sites usually referred to as nucleolus organizer regions (**NORs**), and two copies of five different human chromosomes contain NORs. The DNA found at chromosomal NORs encodes the genes for ribosomal RNA (**rRNA**). At the onset of mitosis, the single nucleolus present in a human cell disappears, and subsequent to the process, the formation of the new nucleolus, which is created from the ten smaller nucleolus-like structures that develop from the NORs, can be observed.

The nucleolus is comprised of granular and fibrillar components, as well as an ill-defined matrix, in addition to DNA. The granular material consists of ribosomal subunits that have already been formed but have not yet matured and are waiting to be exported to the cytoplasm. The threadlike fibrillar part of a nucleolus is predominantly composed of rRNA molecules and associated proteins that have joined together to form fibrils. It has not yet been determined exactly how the various components of the nucleolus are secured together and organized.



Illustrated in Figure 2 is a fluorescence digital image of a Swiss mouse embryo fibroblast cell stained with fluorescent probes targeting the nucleus (blue), mitochondria network (red), Golgi complex (green) and nucleoli (magenta) to demonstrate the proximity of these structures. The nucleoli of this cell were targeted with an antibody to fibrillar, a small nuclear ribonucleoprotein (**SnRNP**) involved in ribosomal RNA processing and centralized within the sub-organelle. Fibrillar has a well conserved amino acid sequence that enables the protein to serve as an excellent marker for nucleoli in a wide variety of species.

In recent years, scientists have been industriously investigating a possible connection between the nucleolus and cell senescence (aging). Most evidence for such a link thus far has come from studies with yeast cells, some of which suggest that cumulative damage to ribosomal RNA genes and fragmentation of the nucleolus may be central to the aging process. Support for the theory has also come from genetic research focused upon Werner syndrome, an inherited disease characterized by premature aging, but additional studies are needed to more precisely understand any possible role the nucleolus plays in senescence.

### **The Nuclear Envelope**

The nuclear envelope is a double-layered membrane that encloses the contents of the nucleus during most of the cell's lifecycle. The outer nuclear membrane is continuous with the membrane of the rough endoplasmic reticulum (**ER**), and like that structure, features numerous ribosomes attached to the surface. The outer membrane is also continuous with the inner nuclear membrane since the two layers are fused together at numerous tiny holes called nuclear pores that perforate the nuclear envelope. These pores regulate the passage of molecules between the nucleus and cytoplasm, permitting some to pass through the membrane, but not others. The space between the outer and inner membranes is termed the **perinuclear space** and is connected with the lumen of the rough ER.

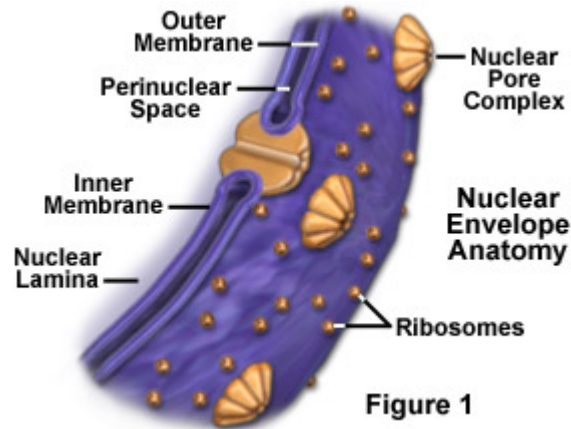


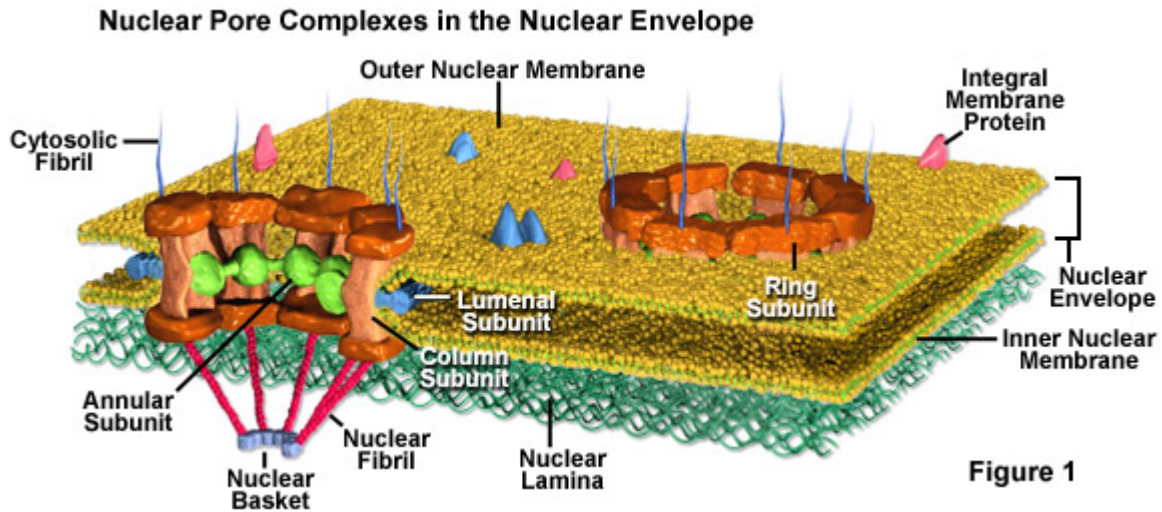
Figure 1

Structural support is provided to the nuclear envelope by two different networks of intermediate filaments. Along the inner surface of the nucleus, one of these networks is organized into a special mesh-like lining called the **nuclear lamina**, which binds to chromatin, integral membrane proteins, and other nuclear components. The nuclear lamina is also thought play a role in directing materials inside the nucleus toward the nuclear pores for export and in the disintegration of the nuclear envelope during cell division and its subsequent reformation at the end of the process. The other intermediate filament network is located on the outside of the outer nuclear membrane and is not organized in such a systemic way as the nuclear lamina.

The amount of traffic that must pass through the nuclear envelope on a continuous basis in order for the eukaryotic cell to function properly is considerable. RNA and ribosomal subunits must be constantly transferred from the nucleus where they are made to the cytoplasm, and histones, gene regulatory proteins, DNA and RNA polymerases, and other substances required for nuclear activities must be imported from the cytoplasm. An active mammalian cell can synthesize about 20,000 ribosome subunits per minute, and at certain points in the cell cycle, as many as 30,000 histones per minute are required by the nucleus. In order for such a tremendous number of molecules to pass through the nuclear envelope in a timely manner, the nuclear pores must be highly efficient at selectively allowing the passage of materials to and from the nucleus.

### Nuclear Pores

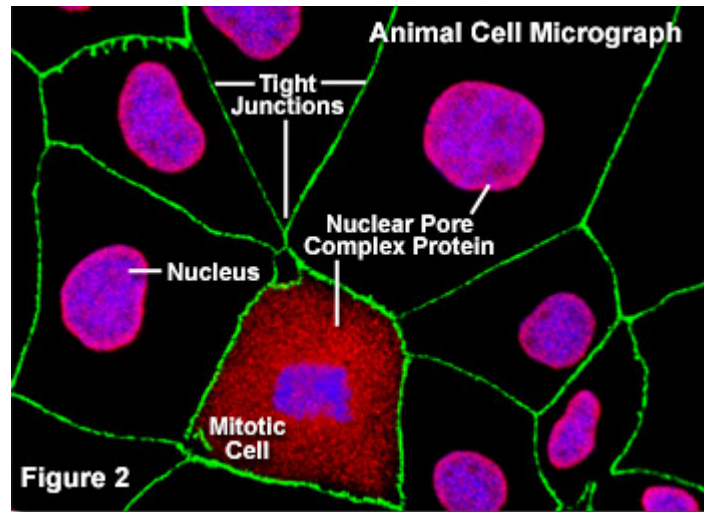
The nuclear envelope is perforated with tiny holes known as nuclear pores, which were first discovered in the mid-twentieth century. These pores regulate the passage of molecules between the nucleus and cytoplasm, permitting some to pass through the membrane, but not others. Building blocks for manufacturing DNA and RNA are some of the materials that are allowed into the nucleus, as are molecules that provide the energy for constructing genetic material. Ribosomal subunits, which are built in nucleosomes, are a prime example of materials that must be allowed to leave the nucleus and enter the cytoplasm.



Nuclear pores are fully permeable to small molecules up to the size of the smallest proteins, but form a barrier keeping most large molecules out of the nucleus. Yet, some larger proteins, such as histones, are granted admission into the nucleus despite the fact that the pores should be too small to let them through. It is generally thought that the elaborate protein structure called the **nuclear pore complex** (see Figure 1) that surrounds each pore plays a key role in allowing the active transport of a select set of large molecules into and out of the nucleus.

The nuclear pore complex is comprised of several subunits. Surrounding the inside of the pore is a nonmembranous material organized into an annulus that extends spoke-like structures toward the center of the small channel. The actual pore wall is predominantly comprised of columnar subunits, and lumenal subunits, with the help of transmembrane proteins, anchor the entire pore complex into the nuclear envelope. Also, tiny fibrils usually extend from both sides of the complex and congregate into basket-like configurations on the nuclear side of the complex. The proteinaceous composition of these fibrils is different on opposing sides of the structure.

In addition to their role in nuclear transport, nuclear pores are important as sites where the outer membrane and inner membrane of the nuclear envelope are fused together. Due to this fusion, the membranes can be considered continuous with one another although they have different biochemical characteristics and can function in distinctive ways. Since the outer nuclear membrane is also continuous with the membrane of the endoplasmic reticulum (**ER**), both it and the inner nuclear membrane can exchange membranous materials with the ER. This capability enables the nuclear envelope to grow bigger or smaller when necessary to accommodate the dynamic contents of the nucleus.

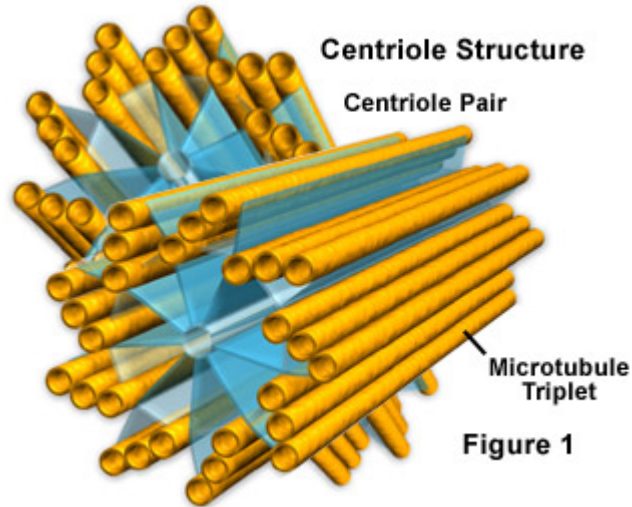


Illustrated in Figure 2 is a fluorescence digital image of an adherent culture of Madin-Darby canine kidney cells (MDCK line) stained with fluorescent probes targeting the nucleus (blue), nuclear pore complex proteins (red), and the tight junctions formed between epithelial cells (green) to demonstrate the proximity of these structures. The nuclear pores of these cells were targeted with a wide-spectrum polyclonal antibody to a large family of nuclear pore complex proteins, which serves as a useful tool for studying the morphology and composition of the nucleus and nuclear envelope. The antibody mixture is also useful in studying changes in the nuclear structure during mitosis and meiosis (note the mitotic cell in the lower central portion of Figure 2).

Pore density varies greatly and tends to be greatest among cells that are highly activate and differentiated, such as liver cells. A typical mammalian cell features approximately 3,000 to 4,000 pores along its nuclear envelope. The oocytes of certain amphibians, however, have such large nuclei and such a density of pores that the nuclear envelope of one of the cells may contain more than ten million pores. Consequently, these oocytes have been heavily utilized in studies of nuclear pore complexes and nuclear transport.

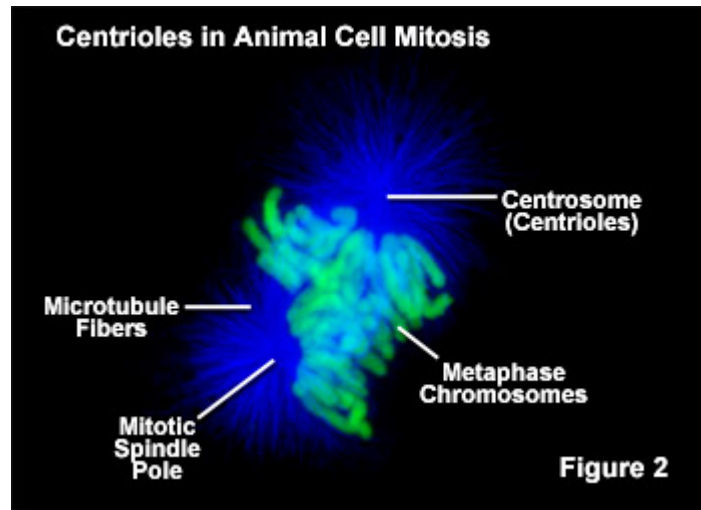
### Centrioles

Found only in animal cells, these paired organelles are typically located together near the nucleus in the **centrosome**, a granular mass that serves as an organizing center for microtubules. Within the centrosome, the centrioles are positioned so that they are at right angles to each other, as illustrated in Figure 1. Each centriole is made of nine bundles of microtubules (three per bundle) arranged in a ring.



Centrioles play a notable role in cell division. During interphase of an animal cell, the centrioles and other components of the centrosome are duplicated, though scientists are not yet sure how this duplication takes place. At first the two pairs of centrioles remain in close proximity to each other, but as mitosis initiates, the original centrosome divides and the pairs are split up so that one set of centrioles is located in each of the new microtubule-organizing centers. These new centers radiate microtubules in star-shaped clusters known as **asters**. As the asters move to opposing poles of the cells, the microtubules, with the help of the centrioles, become organized into a spindle-shaped formation that spans the cell (see Figure 2). These spindle fibers act as guides for the alignment of the chromosomes as they separate later during the process of cell division.

Though centrioles play a role in the mitosis of animal cells, plant cells are able to reproduce without them. Researchers have, therefore, been very interested in determining exactly how important the organelles really are. Studies have shown that certain animal cells, particularly female gametes (oocytes), can successfully divide even when their centrioles are destroyed. Some investigators have also found, however, that the absence of centrioles in animal cells is associated with an increased number of divisional errors and substantial delays in the mitotic process, especially before chromosome segregation. Consequently, it has been suggested that centrioles evolved as a refinement of the cell, making mitosis a much more efficient and less error-prone process.



In cells that feature cilia or flagella, **basal bodies**, which exhibit the same structural form as centrioles, are present. These assemblies are located, however, near the cell surface at the base of each cilium or flagellum, rather than in the centrosome near the nucleus. Basal bodies are anchored in their cytoplasmic locations by what is called a **rootlet system** in the cell. In some organisms, such as the unicellular *Chlamydomonas*, basal bodies change their location and are functionally converted to centrioles before the mitotic process.

### Cilia and Flagella

Cilia and flagella are motile cellular appendages found in most microorganisms and animals, but not in higher plants. In multicellular organisms, cilia function to move a cell or group of cells or to help transport fluid or materials past them. The respiratory tract in humans is lined with cilia that keep inhaled dust, smog, and potentially harmful microorganisms from entering the lungs. Among other tasks, cilia also generate water currents to carry food and oxygen past the gills of clams and transport food through the digestive systems of snails. Flagella are found primarily on gametes, but create the water currents necessary for respiration and circulation in sponges and coelenterates as well. For single-celled eukaryotes, cilia and flagella are essential for the locomotion of individual organisms. Protozoans belonging to the phylum **Ciliophora** are covered with cilia, while flagella are a characteristic of the protozoan group **Mastigophora**.

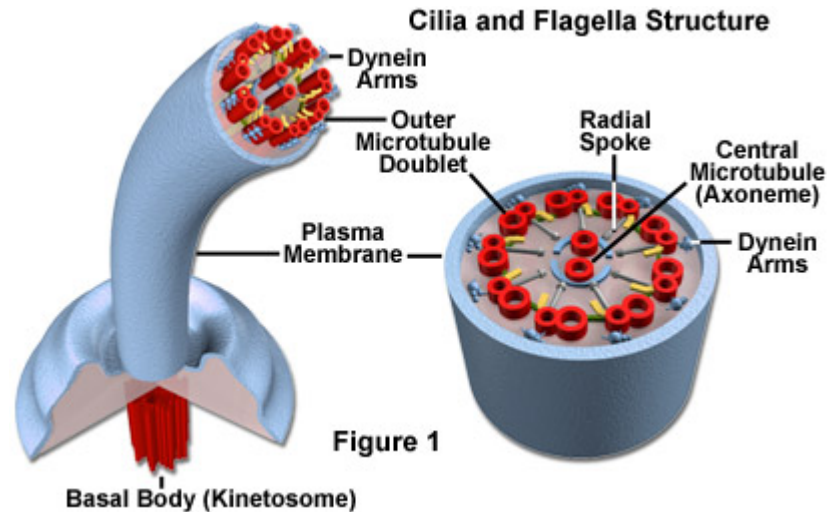


Figure 1

In eukaryotic cells, cilia and flagella contain the motor protein dynein and microtubules, which are composed of linear polymers of globular proteins called **tubulin**. The core of each of the structures is termed the **axoneme** and contains two central microtubules that are surrounded by an outer ring of nine **doublet** microtubules. One full microtubule and one partial microtubule, the latter of which shares a tubule wall with the other microtubule, comprise each doublet microtubule (see Figure 1). Dynein molecules are located around the circumference of the axoneme at regular intervals along its length where they bridge the gaps between adjacent microtubule doublets.

A plasma membrane surrounds the entire axoneme complex, which is attached to the cell at a structure termed the **basal body** (also known as a **kinetosome**). Basal bodies maintain the basic outer ring structure of the axoneme, but each of the nine sets of circumferential filaments is composed of three microtubules, rather than a doublet of microtubules. Thus, the basal body is structurally identical to the centrioles that are found in the centrosome located near the nucleus of the cell. In some organisms, such as the unicellular *Chlamydomonas*, basal bodies are locationally and functionally altered into centrioles and their flagella resorbed before cell division.

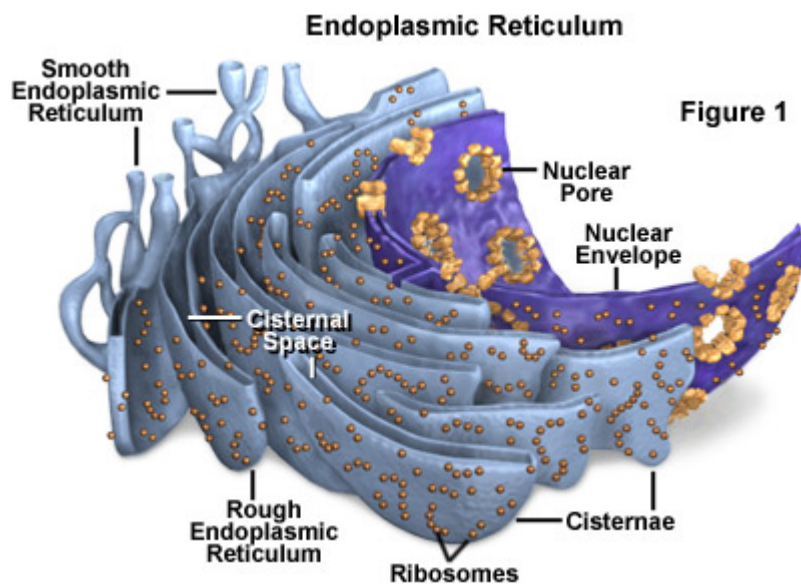
Eukaryotic cilia and flagella are generally differentiated based on size and number: cilia are usually shorter and occur together in much greater numbers than flagella, which are often solitary. The structures also exhibit somewhat different types of motion, though in both cases movement is generated by the activation of dynein and the resultant bending of the axoneme. The movement of cilia is often described as whip-like, or compared to the breast stroke in swimming. Adjacent cilia move almost simultaneously (but not quite), so that in groups of cilia, wave-like patterns of motion occur. Flagella, however, exhibit a smooth, independent undulatory type of movement in eukaryotes. Prokaryotic flagella, which have a completely different structure built from the protein **flagellin**, move in a rotating fashion powered by the basal motor.

Defects in the cilia and flagella of human cells are associated with some notable medical problems. For example, a hereditary condition known as Kartagener's syndrome is caused by problems with the dynein arms that extend between the microtubules present in the axoneme, and is characterized by recurrent respiratory infections related to the inability of cilia in the

respiratory tract to clear away bacteria or other materials. The disease also results in male sterility due to the inability of sperm cells to propel themselves via flagella. Damage to respiratory cilia may also be acquired rather than inherited and is most commonly linked to smoking cigarettes. Bronchitis, for instance, is often triggered by a build-up of mucus and tar in the lungs that cannot be properly removed due to smoking-related impairment of cilia.

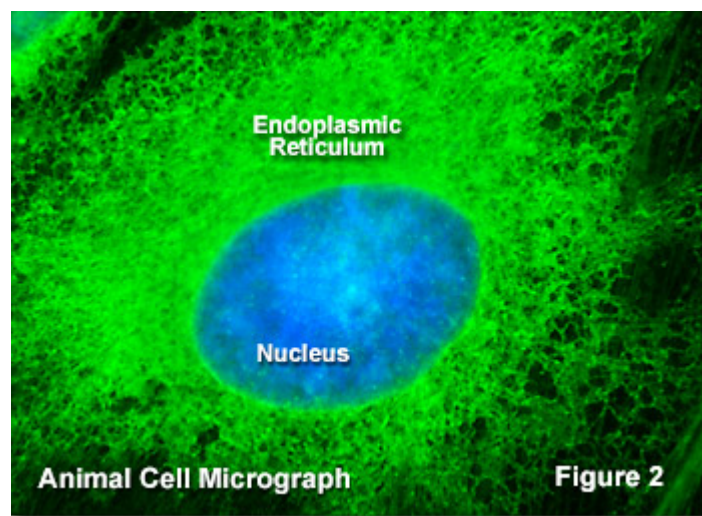
## The Endoplasmic Reticulum

The endoplasmic reticulum (**ER**) is a network of flattened sacs and branching tubules that extends throughout the cytoplasm in plant and animal cells. These sacs and tubules are all interconnected by a single continuous membrane so that the organelle has only one large, highly convoluted and complexly arranged **lumen** (internal space). Usually referred to as the endoplasmic reticulum cisternal space, the lumen of the organelle often takes up more than 10 percent of the total volume of a cell. The endoplasmic reticulum membrane allows molecules to be selectively transferred between the lumen and the cytoplasm, and since it is connected to the double-layered nuclear envelope, it further provides a pipeline between the nucleus and the cytoplasm.



The endoplasmic reticulum manufactures, processes, and transports a wide variety of biochemical compounds for use inside and outside of the cell. Consequently, many of the proteins found in the cisternal space of the endoplasmic reticulum lumen are there only transiently as they pass on their way to other locations. Other proteins, however, are targeted to constantly remain in the lumen and are known as endoplasmic reticulum **resident proteins**. These special proteins, which are necessary for the endoplasmic reticulum to carry out its normal functions, contain a specialized retention signal consisting of a specific sequence of amino acids that enables them to be retained by the organelle. An example of an important endoplasmic reticulum resident protein is the chaperone protein known as **BiP** (formally: the chaperone immunoglobulin-binding protein), which identifies other proteins that have been improperly built or processed and keeps them from being sent to their final destinations.

There are two basic kinds of endoplasmic reticulum morphologies: rough and smooth. The surface of rough endoplasmic reticulum is covered with ribosomes, giving it a bumpy appearance when viewed through the microscope. This type of endoplasmic reticulum is involved mainly with the production and processing of proteins that will be exported, or secreted, from the cell. The ribosomes assemble amino acids into protein units, which are transported into the rough endoplasmic reticulum for further processing. These proteins may be either transmembrane proteins, which become embedded in the membrane of the endoplasmic reticulum, or water-soluble proteins, which are able to pass completely through the membrane into the lumen. Those that reach the inside of the endoplasmic reticulum are folded into the correct three-dimensional conformation, as a flattened cardboard box might be opened up and folded into its proper shape in order to become a useful container. Chemicals, such as carbohydrates or sugars, are added, then the endoplasmic reticulum either transports the completed proteins to areas of the cell where they are needed, or they are sent to the Golgi apparatus for further processing and modification.



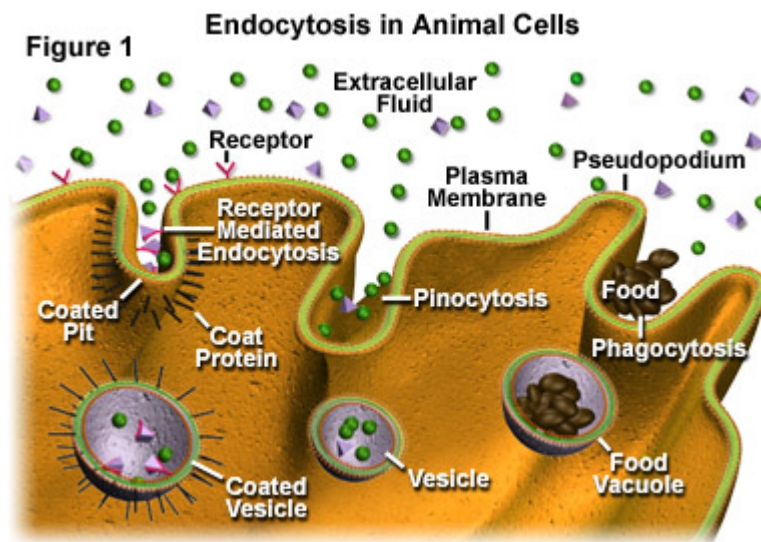
Most proteins exported from the endoplasmic reticulum exit the organelle in vesicles budded from the smooth portion, which has a more even appearance than rough endoplasmic reticulum when viewed through the electron microscope because of the lack of ribosomes. The smooth endoplasmic reticulum in most cells is much less extensive than the rough endoplasmic reticulum and is sometimes alternatively termed **transitional**. Smooth endoplasmic reticulum is chiefly involved, however, with the production of lipids (fats), building blocks for carbohydrate metabolism, and the detoxification of drugs and poisons. Therefore, in some specialized cells, such as those that are occupied chiefly in lipid and carbohydrate metabolism (brain and muscle) or detoxification (liver), the smooth endoplasmic reticulum is much more extensive and is crucial to cellular function. Smooth endoplasmic reticulum also plays a role in various cellular activities through its storage of calcium and involvement in calcium metabolism. In muscle cells, smooth endoplasmic reticulum releases calcium to trigger muscle contractions. Presented in Figure 2 is a fluorescence digital image taken through the microscope of the endoplasmic reticulum network in a bovine (cow) pulmonary artery endothelial cell grown in culture.

It is the rough endoplasmic reticulum that is directly continuous with the nuclear envelope (as illustrated in Figure 1), which is also studded with ribosomes, and the two organelles are thought

to have evolved simultaneously in ancient cells. Due to their physical membranous connection, the lumen of the endoplasmic reticulum and the space between the layers of the nuclear envelope comprise a single compartment. Accordingly, the nucleus has direct access to proteins (many of which are produced by the ribosomes upon its surface) and other materials present in the endoplasmic reticulum lumen, so that transport vesicles are not needed to obtain them. The close association between the endoplasmic reticulum and the nucleus also enables the organelles to share information in a very efficient manner. For instance, if the endoplasmic reticulum begins to undergo functional problems and unfolded proteins accumulate within the organelle, which can be extremely hazardous to the cell, the organelle quickly sends a signal to the nucleus (as well as to the cytoplasm). The nucleus responds by slowing ribosomal translation through a several-step process, thereby giving the endoplasmic reticulum extra time to catch up on its protein folding, thus maintaining cellular health.

### Endosomes and Endocytosis

Endosomes are membrane-bound vesicles, formed via a complex family of processes collectively known as **endocytosis**, and found in the cytoplasm of virtually every animal cell. The basic mechanism of endocytosis is the reverse of what occurs during exocytosis or cellular secretion. It involves the invagination (folding inward) of a cell's plasma membrane to surround macromolecules or other matter diffusing through the extracellular fluid. The encircled foreign materials are then brought into the cell, and following a pinching-off of the membrane (termed **budding**), are released to the cytoplasm in a sac-like vesicle. The size of vesicles varies, and those larger than 100 nanometers in diameter are typically referred to as **vacuoles**.

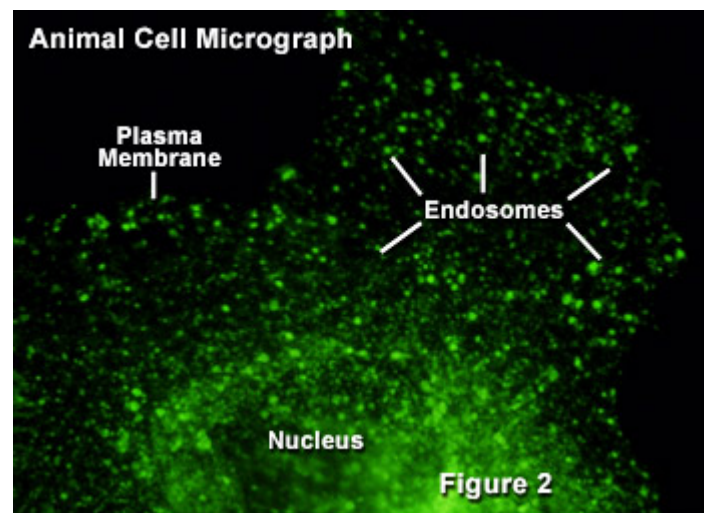


Three primary mechanisms of endocytosis that are exhibited by a typical cell are illustrated in Figure 1. On the far left of the figure, **receptor mediated endocytosis**, which is the most specifically-targeted form of the endocytic process, is presented. Through receptor mediated endocytosis, active cells are able to take in significant amounts of particular molecules (**ligands**) that bind to receptor sites extending from the cytoplasmic membrane into the extracellular fluid surrounding the cell. These receptor sites are commonly grouped together along coated pits in the membrane, which are lined on their cytoplasmic surface with a bristle-like collection of coat

proteins. The coat proteins are thought to play a role in enlarging the pit and forming a vesicle. Note, as shown in Figure 1, vesicles produced via receptor mediated endocytosis may internalize other molecules in addition to ligands, though the ligands are usually brought into the cell in higher concentration.

A less specific mechanism of endocytosis is **pinocytosis**, which is illustrated in the central section of Figure 1. By means of pinocytosis, a cell is able to ingest droplets of liquid from the extracellular fluid. All solutes found in the droplets outside of the cell may become encased in the vesicles formed via this process, with those present in the greatest concentration in the extracellular fluid also becoming the most concentrated in the membranous sacs. Pinocytic vesicles tend to be smaller than vesicles produced by other endocytic processes.

The final type of endocytosis, termed **phagocytosis** (see Figure 1), is probably the most well-known manner in which a cell may import outside materials. In many school science labs, children observe amoebas under the microscope and watch the single-celled organisms eat by stretching out **pseudopodia** and encircling any food particles they find in their paths. This engulfment and subsequent packaging of the particles into vesicles, which are usually large enough to be correctly referred to as vacuoles, is phagocytosis. Though commonly associated with amoebas, phagocytosis is practiced by many organisms. In most multicellular animals, phagocytic cells chiefly function in bodily defense rather than as a means to gain nourishment. For example, leukocytes in the human body often phagocytose protozoa, bacteria, dead cells, and similar materials in order to help stave off infections or other problems.



Once freed into the cytoplasm, several small vesicles produced via endocytosis may come together to form a single entity. This endosome generally functions in one of two ways. Most commonly, endosomes transport their contents in a series of steps to a lysosome, which subsequently digests the materials. In other instances, however, endosomes are used by the cell to transport various substances between different portions of the external cell membrane. This latter function is particularly important among epithelial cells, such as those that compose the outer layer of the skin, because they exhibit polarity (one side of the cell is different from the other side). Illustrated in Figure 2 is a fluorescence digital image of a single African green

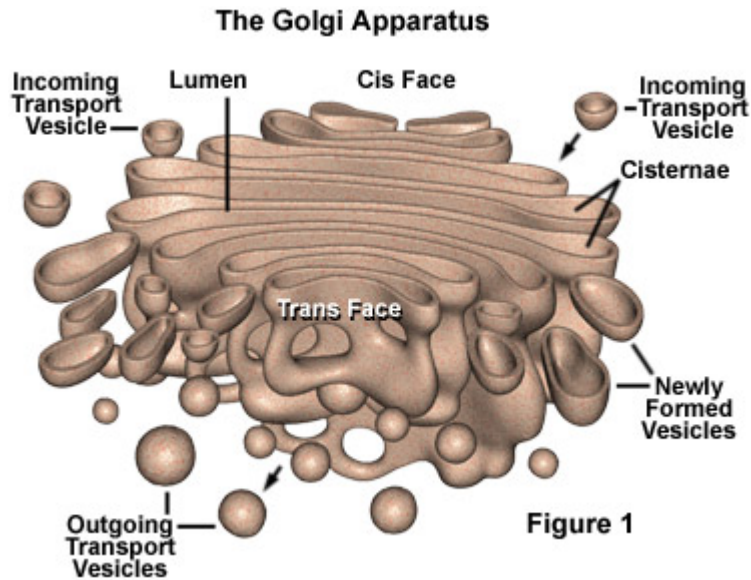
monkey kidney fibroblast cell (**CV-1** line) transfected with a fluorescent protein fused to a targeting amino acid sequence for endosomes (green). The nucleus, plasma membrane, and endosome components are marked in the figure.

An endosome that is destined to transfer its contents to a lysosome generally goes through several changes along its way. In its initial form, when the structure is often referred to as an early endosome, the specialized vesicle contains a single compartment. Over time, however, chemical changes in the vesicle take place and the membrane surrounding the endosome folds in upon itself in a way that is similar to the invagination of the plasma membrane. In this case, however, the membrane is not pinched off. Consequently, a structure with multiple compartments, termed a multivesicular endosome, is formed. The multivesicular endosome is an intermediate structure in which further chemical changes, including a significant drop in pH level, take place as the vesicle develops into a late endosome.

Though late endosomes are capable of breaking down many proteins and fats, a lysosome is needed to fully digest all of the materials they contain. Frequently the contents of late endosomes are conveyed to a lysosome through fusion (joining together) of their membranes. Under some circumstances, late endosomes are able to further mature into lysosomes through additional chemical and structural modifications, in which case fusion is not necessary for digestion to be completed.

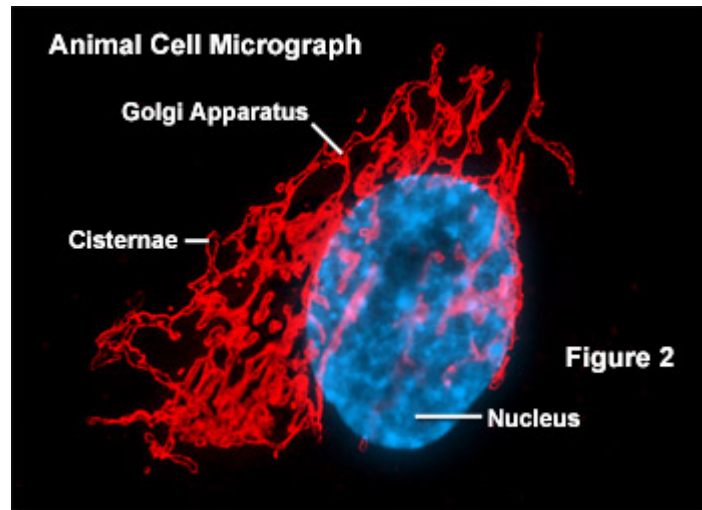
### **The Golgi Apparatus**

The Golgi apparatus (**GA**), also called Golgi body or Golgi complex and found universally in both plant and animal cells, is typically comprised of a series of five to eight cup-shaped, membrane-covered sacs called **cisternae** that look something like a stack of deflated balloons. In some unicellular flagellates, however, as many as 60 cisternae may combine to make up the Golgi apparatus. Similarly, the number of Golgi bodies in a cell varies according to its function. Animal cells generally contain between ten and twenty Golgi stacks per cell, which are linked into a single complex by tubular connections between cisternae. This complex is usually located close to the cell nucleus.



Due to its relatively large size, the Golgi apparatus was one of the first organelles ever observed. In 1897, an Italian physician named Camillo Golgi, who was investigating the nervous system by using a new staining technique he developed (and which is still sometimes used today; known as Golgi staining or Golgi impregnation), observed in a sample under his light microscope a cellular structure that he termed the internal reticular apparatus. Soon after he publicly announced his discovery in 1898, the structure was named after him, becoming universally known as the Golgi apparatus. Yet, many scientists did not believe that what Golgi observed was a real organelle present in the cell and instead argued that the apparent body was a visual distortion caused by staining. The invention of the electron microscope in the twentieth century finally confirmed that the Golgi apparatus is a cellular organelle.

The Golgi apparatus is often considered the distribution and shipping department for the cell's chemical products. It modifies proteins and lipids (fats) that have been built in the endoplasmic reticulum and prepares them for export outside of the cell or for transport to other locations in the cell. Proteins and lipids built in the smooth and rough endoplasmic reticulum bud off in tiny bubble-like vesicles that move through the cytoplasm until they reach the Golgi complex. The vesicles fuse with the Golgi membranes and release their internally stored molecules into the organelle. Once inside, the compounds are further processed by the Golgi apparatus, which adds molecules or chops tiny pieces off the ends. When completed, the product is extruded from the GA in a vesicle and directed to its final destination inside or outside the cell. The exported products are secretions of proteins or glycoproteins that are part of the cell's function in the organism. Other products are returned to the endoplasmic reticulum or may undergo maturation to become lysosomes.



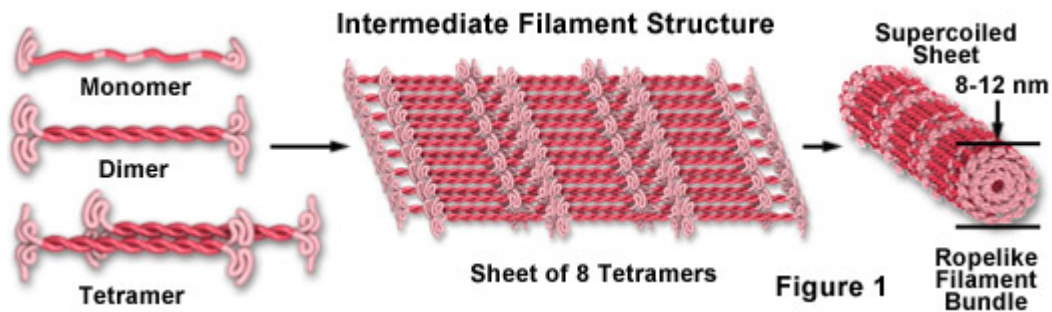
The modifications to molecules that take place in the Golgi apparatus occur in an orderly fashion. Each Golgi stack has two distinct ends, or faces. The **cis** face of a Golgi stack is the end of the organelle where substances enter from the endoplasmic reticulum for processing, while the **trans** face is where they exit in the form of smaller detached vesicles. Consequently, the cis face is found near the endoplasmic reticulum, from whence most of the material it receives comes, and the trans face is positioned near the plasma membrane of the cell, to where many of the substances it modifies are shipped. The chemical make-up of each face is different and the enzymes contained in the lumens (inner open spaces) of the cisternae between the faces are distinctive. Illustrated in Figure 2 is a fluorescence digital image taken through a microscope of the Golgi apparatus (pseudocolored red) in a typical animal cell. Note the close proximity of the Golgi membranes to the cell nucleus.

Proteins, carbohydrates, phospholipids, and other molecules formed in the endoplasmic reticulum are transported to the Golgi apparatus to be biochemically modified during their transition from the cis to the trans poles of the complex. Enzymes present in the Golgi lumen modify the carbohydrate (or sugar) portion of glycoproteins by adding or subtracting individual sugar monomers. In addition, the Golgi apparatus manufactures a variety of macromolecules on its own, including a variety of polysaccharides. The Golgi complex in plant cells produces pectins and other polysaccharides specifically needed by for plant structure and metabolism. The products exported by the Golgi apparatus through the trans face eventually fuse with the plasma membrane of the cell. Among the most important duties of the Golgi apparatus is to sort the wide variety of macromolecules produced by the cell and target them for distribution to their proper location. Specialized molecular identification labels or tags, such as phosphate groups, are added by the Golgi enzymes to aid in this sorting effort.

### Intermediate Filaments

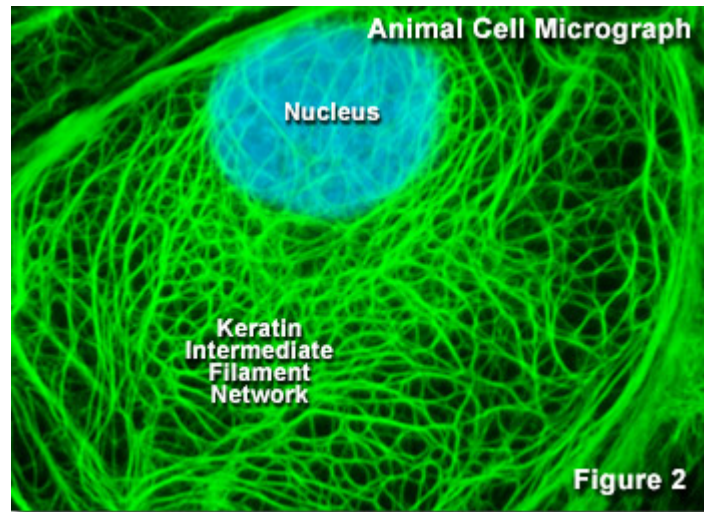
Intermediate filaments are a very broad class of fibrous proteins that play an important role as both structural and functional elements of the cytoskeleton. Ranging in size from 8 to 12 nanometers (in diameter; see Figure 1), intermediate filaments function as tension-bearing elements to help maintain cell shape and rigidity, and serve to anchor in place several organelles,

including the nucleus and desmosomes. Intermediate filaments are also involved in formation of the **nuclear lamina**, a net-like meshwork array that lines the inner nuclear membrane and governs the shape of the nucleus.



Although all eukaryotes contain the common cytoskeletal elements actin and tubulin (both free in the cytoplasm and polymerized in the form of microfilaments and microtubules), intermediate filaments are found only in some metazoan species, including vertebrates, nematodes, and molluscs. In vertebrates, intermediate filament presence and composition is not only species-dependent, it also varies with the tissue type. For example, most animal epithelial cells contain **keratins**, a diverse family of intermediate filaments consisting of more than 50 members, while mesenchymal and muscle cells are rich in the fibrous proteins, **vimentin** and **desmin**, respectively. Intermediate filaments found in neurons and glial cells include **peripherin**, **neurofilaments**, and **glial fibrillary acidic protein (GFAP)**. A variety of associated proteins bind to intermediate filaments, either to improve stability (through crosslinking) or to provide attachment sites for other protein assemblies, such as actin filaments and microtubules.

Another highly specialized class of intermediate filaments are the nuclear **lamins**, which constitute the fibrous protein network that lines the inside of the nuclear membrane, as discussed above. Due to their close proximity to the membrane, nuclear lamins help attach the chromosomes to the nuclear membrane and provide anchorage points for the nuclear pores. Many scientists believe that nuclear lamins are the evolutionary ancestor of cytoplasmic intermediate filaments, which evolved through duplication and translocation of the gene product to the cytoplasm. The rigidity bestowed on cells by intermediate filament networks is especially useful to soft-bodied animals that do not possess an exoskeleton. Because intermediate filaments are very abundant in cells that are often subjected to high mechanical stress *in vivo*, it appears that their primary role is to provide physical strength to cells and tissues.



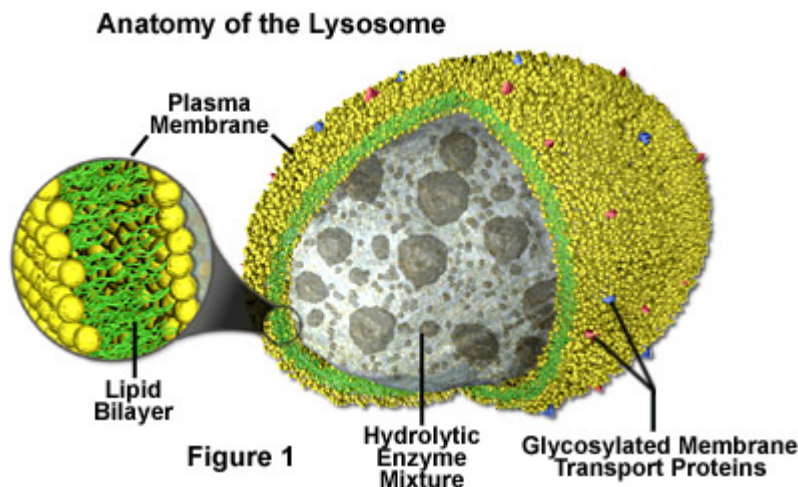
Presented in Figure 2 is a digital image of the keratin intermediate network found in a rat kangaroo (**PtK2** line) epithelial cell as seen through a fluorescence optical microscope. The extensive intertwined network was labeled with primary antibodies to several cytokeratin proteins, which were then stained with secondary antibodies containing a green fluorescent dye. The nucleus was counterstained with a blue dye to note its location in relation to the intermediate filament network. Fluorescence microscopy is an important tool that scientists use to examine the structure and function of internal cellular organelles and the cytoskeleton.

As illustrated in Figure 1, intermediate filament monomer peptides are an elongated fibrous class of proteins with a central *alpha*-helical region capped with globular ends at both the amino and carboxylic acid termini. Two of the monomer units form a coiled-coil dimer that self-associates in an anti-parallel arrangement to form a staggered tetramer, which is the analogous soluble subunit for the globular actin monomer and the tubulin heterodimer (existing free in the cytoplasm). Tetramer units pack together laterally to form a sheet of eight parallel protofilaments that are supercoiled into a tight bundle. Each tightly coiled intermediate filament cross section reveals 32 individual *alpha*-helical peptides, which renders the filament easy to bend but quite difficult to break, thus accounting for the extreme structural rigidity. Although less is understood about the mechanism of intermediate filament assembly and disassembly, it is clear that some classes are highly dynamic structures with a significant rate of turnover in many cell types.

Mutations in intermediate filament genes lead to a host of rather uncommon diseases. For example, defective keratins in skin tissue lead to a disorder known as **epidermolysis bullosa simplex**, manifested by skin blisters produced with even a slight mechanical stress. Similar blistering diseases due to keratin mutations in other tissues affect the esophagus, eyes, and mouth. Several neurodegenerative diseases, such as **amyotrophic lateral sclerosis (ALS or Lou Gehrig's Disease)**, are associated with malfunctions in the intermediate filament (neurofilament) network, and defects in desmin intermediate filaments produce muscle disorders.

## Lysosomes

The main function of these microscopic organelles is to serve as digestion compartments for cellular materials that have exceeded their lifetime or are otherwise no longer useful. In this regard, the lysosomes recycle the cell's organic material in a process known as **autophagy**. Lysosomes break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds, which are then transferred back into the cytoplasm as new cell-building materials. To accomplish the tasks associated with digestion, the lysosomes utilize about 40 different types of hydrolytic enzymes, all of which are manufactured in the endoplasmic reticulum and modified in the Golgi apparatus. Lysosomes are often budded from the membrane of the Golgi apparatus, but in some cases they develop gradually from late endosomes, which are vesicles that carry materials brought into the cell by a process known as **endocytosis**.



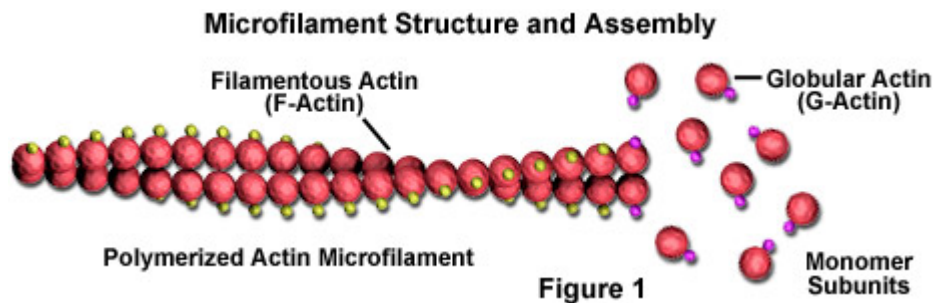
Like other microbodies, lysosomes are spherical organelles contained by a single layer membrane, though their size and shape varies to some extent. This membrane protects the rest of the cell from the harsh digestive enzymes contained in the lysosomes, which would otherwise cause significant damage. The cell is further safeguarded from exposure to the biochemical catalysts present in lysosomes by their dependency on an acidic environment. With an average pH of about 4.8, the lysosomal matrix is favorable for enzymatic activity, but the neutral environment of the cytosol renders most of the digestive enzymes inoperative, so even if a lysosome is ruptured, the cell as a whole may remain uninjured. The acidity of the lysosome is maintained with the help of hydrogen ion pumps, and the organelle avoids self-digestion by glucosylation of inner membrane proteins to prevent their degradation.

The discovery of lysosomes involved the use of a centrifuge to separate the various components of cells. In the mid-twentieth century, the Belgian scientist Christian René de Duve was investigating carbohydrate metabolism of liver cells and observed that the cells released an enzyme called **acid phosphatase** in larger amounts when they received proportionally greater damage in the centrifuge. To explain this phenomenon, de Duve suggested that the digestive enzyme was encased in some sort of membrane-bound organelle within the cell, which he dubbed the lysosome. After estimating the probable size of the lysosome, he was able to identify the organelle in images produced with an electron microscope.

Lysosomes are found in all animal cells, but are most numerous in disease-fighting cells, such as white blood cells. This is because white blood cells must digest more material than most other types of cells in their quest to battle bacteria, viruses, and other foreign intruders. Several human diseases are caused by lysosome enzyme disorders that interfere with cellular digestion. Tay-Sachs disease, for example, is caused by a genetic defect that prevents the formation of an essential enzyme that breaks down complex lipids called **gangliosides**. An accumulation of these lipids damages the nervous system, causes mental retardation, and death in early childhood. Also, arthritis inflammation and pain are related to the escape of lysosome enzymes.

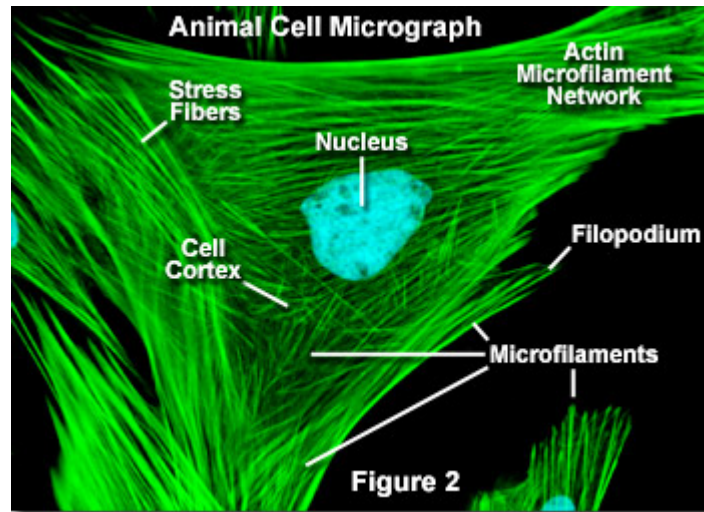
## Microfilaments

Common to all eukaryotic cells, these filaments are primarily structural in function and are an important component of the cytoskeleton, along with microtubules and often the intermediate filaments. Microfilaments range from 5 to 9 nanometers in diameter and are designed to bear large amounts of tension. In association with **myosin**, microfilaments help to generate the forces used in cellular contraction and basic cell movements. The filaments also enable a dividing cell to pinch off into two cells and are involved in amoeboid movements of certain types of cells.



Microfilaments are solid rods made of a protein known as **actin**. When it is first produced by the cell, actin appears in a globular form (**G-actin**; see Figure 1). In microfilaments, however, which are also often referred to as actin filaments, long polymerized chains of the molecules are intertwined in a helix, creating a filamentous form of the protein (**F-actin**). All of the subunits that compose a microfilament are connected in such a way that they have the same orientation. Due to this fact, each microfilament exhibits **polarity**, the two ends of the filament being distinctly different. This polarity affects the growth rate of microfilaments, one end (termed the plus end) typically assembling and disassembling faster than the other (the minus end).

Unlike microtubules, which typically extend out from the centrosome of a cell, microfilaments are typically nucleated at the plasma membrane. Therefore, the periphery (edges) of a cell generally contains the highest concentration of microfilaments. A number of external factors and a group of special proteins influence microfilament characteristics, however, and enable them to make rapid changes if needed, even if the filaments must be completely disassembled in one region of the cell and reassembled somewhere else. When found directly beneath the plasma membrane, microfilaments are considered part of the cell **cortex**, which regulates the shape and movement of the cell's surface. Consequently, microfilaments play a key role in development of various cell surface projections (as illustrated in Figure 2), including filopodia, lamellipodia, and stereocilia.



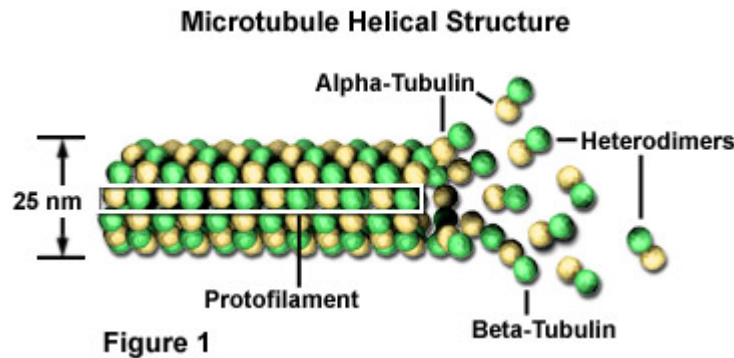
Illustrated in Figure 2 is a fluorescence digital image of an Indian Muntjac deer skin fibroblast cell stained with fluorescent probes targeting the nucleus (blue) and the actin cytoskeletal network (green). Individually, microfilaments are relatively flexible. In the cells of living organisms, however, the actin filaments are usually organized into larger, much stronger structures by various accessory proteins. The exact structural form that a group of microfilaments assumes depends on their primary function and the particular proteins that bind them together. For instance, in the core of surface protrusions called **microspikes**, microfilaments are organized into tight parallel bundles by the bundling protein **fimbrin**. Bundles of the filaments are less tightly packed together, however, when they are bound by **alpha-actinin** or are associated with fibroblast stress fibers (the parallel green fibers in Figure 2). Notably, the microfilament connections created by some cross-linking proteins result in a web-like network or gel form rather than filament bundles.

Over the course of evolutionary history of the cell, actin has remained relatively unchanged. This, along with the fact that all eukaryotic cells heavily depend upon the integrity of their actin filaments in order to be able to survive the many stresses they are faced with in their environment, makes actin an excellent target for organisms seeking to injure cells. Accordingly, many plants, which are unable to physically avoid predators that might want to eat them or harm them in some other way, produce toxins that affect cellular actin and microfilaments as a defensive mechanism. The death cap mushroom, for example, produces a substance called **phalloidin** that binds to and stabilizes actin filaments, which can be fatal to cells.

### Microtubules

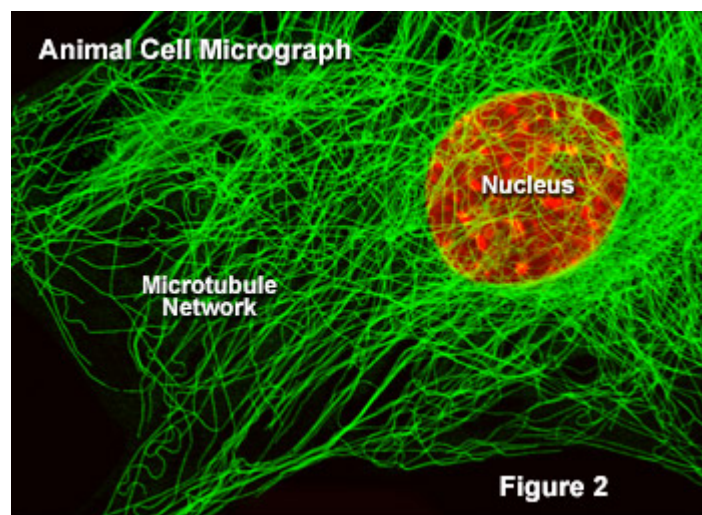
These straight, hollow cylinders are found throughout the cytoplasm of all eukaryotic cells (prokaryotes don't have them) and carry out a variety of functions, ranging from transport to structural support. Microtubules, which are about 25 nanometers in diameter, form part of the **cytoskeleton** that gives structure and shape to a cell, and also serve as conveyor belts moving other organelles throughout the cytoplasm. In addition, microtubules are the major components of cilia and flagella, and participate in the formation of spindle fibers during cell division (mitosis). The length of microtubules in the cell varies between 200 nanometers and 25

micrometers, depending upon the task of a particular microtubule and the state of the cell's life cycle.



Microtubules are biopolymers that are composed of subunits made from an abundant globular cytoplasmic protein known as **tubulin**, as illustrated in Figure 1. Each subunit of the microtubule is made of two slightly different but closely related simpler units called *alpha*-tubulin and *beta*-tubulin that are bound very tightly together to form **heterodimers**. In a microtubule, the subunits are organized in such a way that they all point the same direction to form 13 parallel **protofilaments**. This organization gives the structure **polarity**, with only the *alpha*-tubulin proteins exposed at one end and only *beta*-tubulin proteins at the other.

By adding or removing globular tubulin proteins, the length of polymeric microtubules can be increased or decreased. Because the two ends of a microtubule are not the same, however, the rate at which growth or depolymerization occurs at each pole is different. The end of a polarized filament that grows and shrinks the fastest is known as the plus end and the opposing end is called the minus end. For all microtubules, the minus end is the one with exposed *alpha*-tubulins. In an animal cell, it is this end that is located at the centriole-containing centrosome found near the nucleus, while the plus end, comprised of exposed *beta*-units, is projected out toward the cell's surface. Microtubules are continuously being assembled and disassembled so that tubulin monomers can be transported elsewhere to build microtubules when needed.



Presented in Figure 2 is a digital image of the microtubule network found in an embryonic mouse cell as seen through a fluorescence optical microscope. The extensive intertwined network is labeled with primary antibodies to *alpha*-tubulin, which are then stained with secondary antibodies containing a green fluorescent dye. The nucleus was counterstained with a red dye to note its location in relation to the microtubule network. Fluorescence microscopy is an important tool that scientists use to examine the structure and function of internal cellular organelles.

In addition to their structural support role, microtubules also serve as a highway system along which organelles can be transported with the aid of motor proteins. For instance, the microtubule network interconnects the Golgi apparatus with the plasma membrane to guide secretory vesicles for export, and also transports mitochondria back and forth in the cytoplasm. Another example is the translocation of vesicles containing neurotransmitters by microtubules to the tips of nerve cell axons. The motor proteins involved in organelle transport operate by altering their three-dimensional conformation using adenosine triphosphate (**ATP**) as fuel to move back and forth along a microtubule. With each step, the motor molecule releases one portion of the microtubule and grips a second site farther long the filament. Motor proteins, which are grouped into several distinct classes, attach to organelles through specialized receptors.

Since eukaryotic cells greatly depend upon the integrity of microtubules and other cytoskeletal filaments to maintain their structure and essentially to survive, many plants produce natural toxins aimed at disrupting the microtubule network as a means of self-defense. **Taxol**, for example, is a toxic substance produced by a species of yew trees that increases microtubule polymerization (building a macromolecule) by binding to the filament and stabilizing it. Other natural toxins, such as the **colchicine** produced by the meadow saffron, destabilize microtubules and hinder their polymerization. Both kinds of events can be fatal to the affected cell, though in some circumstances, this can be beneficial to animals, as demonstrated by taxol, which is commonly used as a cancer medication

### **Mitochondria**

Mitochondria are rod-shaped organelles that can be considered the power generators of the cell, converting oxygen and nutrients into adenosine triphosphate (**ATP**). ATP is the chemical energy "currency" of the cell that powers the cell's metabolic activities. This process is called **aerobic respiration** and is the reason animals breathe oxygen. Without mitochondria (singular, mitochondrion), higher animals would likely not exist because their cells would only be able to obtain energy from anaerobic respiration (in the absence of oxygen), a process much less efficient than aerobic respiration. In fact, mitochondria enable cells to produce 15 times more ATP than they could otherwise, and complex animals, like humans, need large amounts of energy in order to survive.

### Mitochondria Structural Features

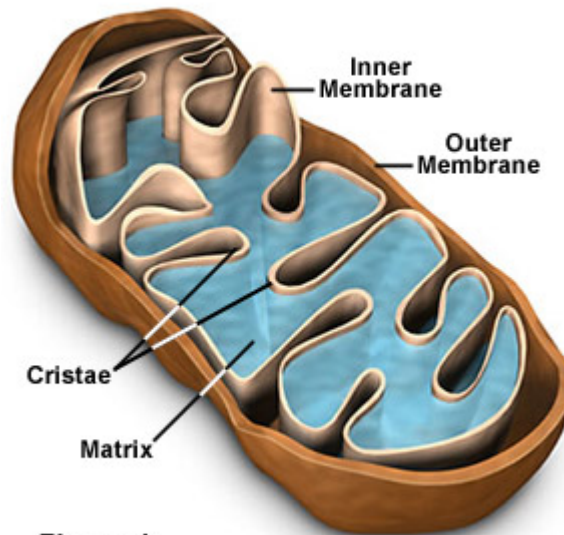


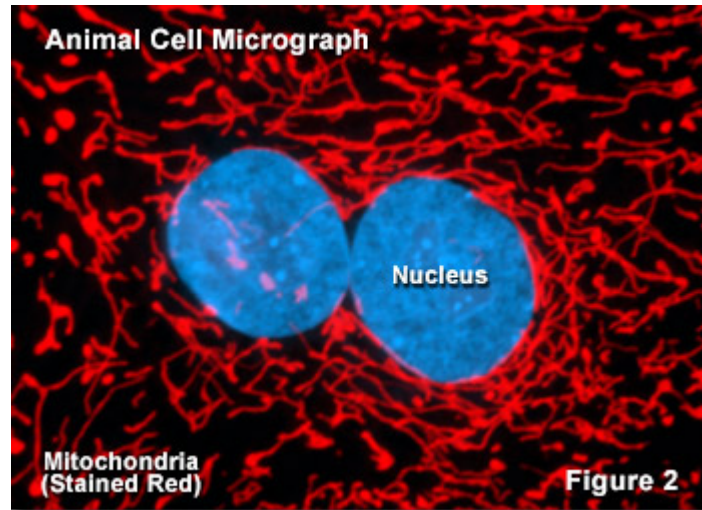
Figure 1

The number of mitochondria present in a cell depends upon the metabolic requirements of that cell, and may range from a single large mitochondrion to thousands of the organelles. Mitochondria, which are found in nearly all eukaryotes, including plants, animals, fungi, and protists, are large enough to be observed with a light microscope and were first discovered in the 1800s. The name of the organelles was coined to reflect the way they looked to the first scientists to observe them, stemming from the Greek words for "thread" and "granule." For many years after their discovery, mitochondria were commonly believed to transmit hereditary information. It was not until the mid-1950s when a method for isolating the organelles intact was developed that the modern understanding of mitochondrial function was worked out.

The elaborate structure of a mitochondrion is very important to the functioning of the organelle (see Figure 1). Two specialized membranes encircle each mitochondrion present in a cell, dividing the organelle into a narrow **intermembrane space** and a much larger internal **matrix**, each of which contains highly specialized proteins. The outer membrane of a mitochondrion contains many channels formed by the protein **porin** and acts like a sieve, filtering out molecules that are too big. Similarly, the inner membrane, which is highly convoluted so that a large number of infoldings called **cristae** are formed, also allows only certain molecules to pass through it and is much more selective than the outer membrane. To make certain that only those materials essential to the matrix are allowed into it, the inner membrane utilizes a group of transport proteins that will only transport the correct molecules. Together, the various compartments of a mitochondrion are able to work in harmony to generate ATP in a complex multi-step process.

Mitochondria are generally oblong organelles, which range in size between 1 and 10 micrometers in length, and occur in numbers that directly correlate with the cell's level of metabolic activity. The organelles are quite flexible, however, and time-lapse studies of living cells have demonstrated that mitochondria change shape rapidly and move about in the cell almost constantly. Movements of the organelles appear to be linked in some way to the

microtubules present in the cell, and are probably transported along the network with motor proteins. Consequently, mitochondria may be organized into lengthy traveling chains, packed tightly into relatively stable groups, or appear in many other formations based upon the particular needs of the cell and the characteristics of its microtubular network.



Presented in Figure 2 is a digital image of the mitochondrial network found in the ovarian tissue from a mountain goat relative, known as the Himalayan Tahr, as seen through a fluorescence optical microscope. The extensive intertwined network is labeled with a synthetic dye named MitoTracker Red (red fluorescence) that localizes in the respiring mitochondria of living cells in culture. The rare twin nuclei in this cell were counterstained with a blue dye (cyan fluorescence) to denote their centralized location in relation to the mitochondrial network. Fluorescence microscopy is an important tool that scientists use to examine the structure and function of internal cellular organelles.

The mitochondrion is different from most other organelles because it has its own circular DNA (similar to the DNA of prokaryotes) and reproduces independently of the cell in which it is found; an apparent case of **endosymbiosis**. Scientists hypothesize that millions of years ago small, free-living prokaryotes were engulfed, but not consumed, by larger prokaryotes, perhaps because they were able to resist the digestive enzymes of the host organism. The two organisms developed a symbiotic relationship over time, the larger organism providing the smaller with ample nutrients and the smaller organism providing ATP molecules to the larger one. Eventually, according to this view, the larger organism developed into the eukaryotic cell and the smaller organism into the mitochondrion.

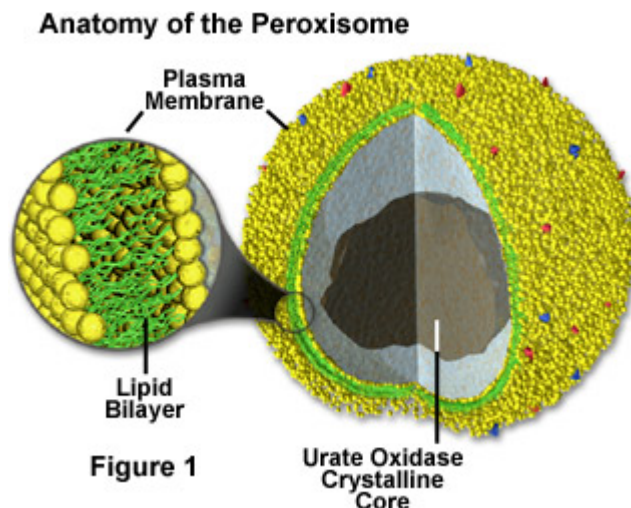
Mitochondrial DNA is localized to the matrix, which also contains a host of enzymes, as well as ribosomes for protein synthesis. Many of the critical metabolic steps of cellular respiration are catalyzed by enzymes that are able to diffuse through the mitochondrial matrix. The other proteins involved in respiration, including the enzyme that generates ATP, are embedded within the mitochondrial inner membrane. Infolding of the cristae dramatically increases the surface area available for hosting the enzymes responsible for cellular respiration.

Mitochondria are similar to plant chloroplasts in that both organelles are able to produce energy and metabolites that are required by the host cell. As discussed above, mitochondria are the sites of respiration, and generate chemical energy in the form of ATP by metabolizing sugars, fats, and other chemical fuels with the assistance of molecular oxygen. Chloroplasts, in contrast, are found only in plants and algae, and are the primary sites of photosynthesis. These organelles work in a different manner to convert energy from the sun into the biosynthesis of required organic nutrients using carbon dioxide and water. Like mitochondria, chloroplasts also contain their own DNA and are able to grow and reproduce independently within the cell.

In most animal species, mitochondria appear to be primarily inherited through the maternal lineage, though some recent evidence suggests that in rare instances mitochondria may also be inherited via a paternal route. Typically, a sperm carries mitochondria in its tail as an energy source for its long journey to the egg. When the sperm attaches to the egg during fertilization, the tail falls off. Consequently, the only mitochondria the new organism usually gets are from the egg its mother provided. Therefore, unlike nuclear DNA, mitochondrial DNA doesn't get shuffled every generation, so it is presumed to change at a slower rate, which is useful for the study of human evolution. Mitochondrial DNA is also used in forensic science as a tool for identifying corpses or body parts, and has been implicated in a number of genetic diseases, such as Alzheimer's disease and diabetes.

### **Peroxisomes**

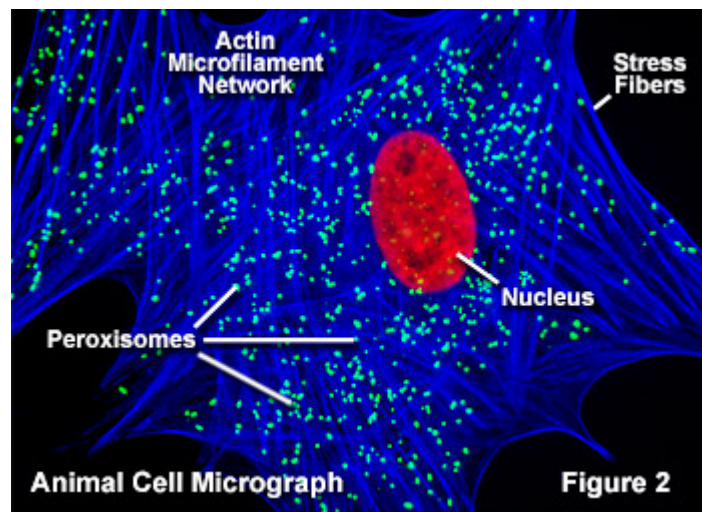
Microbodies are a diverse group of organelles that are found in the cytoplasm of almost all cells, roughly spherical, and bound by a single membrane. There are several types of microbodies, including lysosomes, but peroxisomes are the most common. All eukaryotes are comprised of one or more cells that contain peroxisomes. The organelles were first discovered by the Belgian scientist Christian de Duve, who also discovered lysosomes.



Peroxisomes contain a variety of enzymes, which primarily function together to rid the cell of toxic substances, and in particular, hydrogen peroxide (a common byproduct of cellular metabolism). These organelles contain enzymes that convert the hydrogen peroxide to water, rendering the potentially toxic substance safe for release back into the cell. Some types of

peroxisomes, such as those in liver cells, detoxify alcohol and other harmful compounds by transferring hydrogen from the poisons to molecules of oxygen (a process termed **oxidation**). Others are more important for their ability to initiate the production of phospholipids, which are typically used in the formation of membranes.

In order to carry out their activities, peroxisomes use significant amounts of oxygen. This characteristic of the organelles would have been extremely important millions of years ago, before cells contained mitochondria, when the Earth's atmosphere first began to amass large amounts of oxygen due to the actions of photosynthetic bacteria. Peroxisomes would have been primarily responsible at that time for detoxifying cells by decreasing their levels of oxygen, which was then poisonous to most forms of life. The organelles would have provided the cellular benefit of carrying out a number of advantageous reactions as well. Later, when mitochondria eventually evolved, peroxisomes became less important (in some ways) to the cell since mitochondria also utilize oxygen to carry out many of the same reactions, but with the additional benefit of generating energy in the form of adenosine triphosphate (ATP) at the same time.

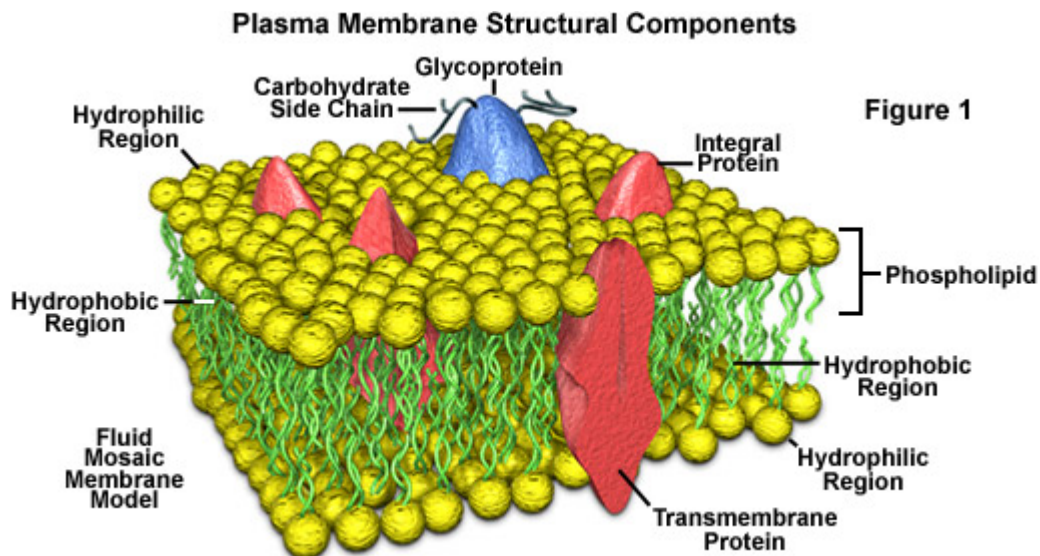


Peroxisomes are similar in appearance to lysosomes, another type of microbody, but the two have very different origins. Lysosomes are generally formed in the Golgi complex, whereas peroxisomes self-replicate. Unlike self-replicating mitochondria, however, peroxisomes do not have their own internal DNA molecules. Consequently, the organelles must import the proteins they need to make copies of themselves from the surrounding cytosol. The importation process of peroxisomes is not yet well understood, but it appears to be heavily dependent upon **peroxisomal targeting signals** composed of specific amino acid sequences. These signals are thought to interact with receptor proteins present in the cytosol and docking proteins present in the peroxisomal membrane. As more and more proteins are imported into lumen of a peroxisome or are inserted into its membrane, the organelle gets larger and eventually reaches a point where fission takes place, resulting in two daughter peroxisomes. Illustrated in Figure 2 is a fluorescence digital image of an African water mongoose skin fibroblast cell stained with fluorescent probes targeting the nucleus (red), actin cytoskeletal network (blue), and peroxisomes (green).

Since the early 1980s, a number of metabolic disorders have been discovered to be caused by molecular defects in peroxisomes. Two major categories have been described so far. The first category consists of disorders of peroxisome biogenesis in which the organelle fails to develop normally, causing defects in numerous peroxisomal proteins. The second category involves defects of single peroxisomal enzymes. Studies indicate that approximately one in every 20,000 people has some type of a peroxisomal disorder. The most serious of these disorders is Zellweger syndrome, which is characterized by an absence or reduced number of peroxisomes in the cells. Present in patients at birth (congenital), Zellweger syndrome has no cure or effective treatment and usually causes death within the first year of life.

## Plasma Membrane

All living cells, prokaryotic and eukaryotic, have a plasma membrane that encloses their contents and serves as a semi-porous barrier to the outside environment. The membrane acts as a boundary, holding the cell constituents together and keeping other substances from entering. The plasma membrane is permeable to specific molecules, however, and allows nutrients and other essential elements to enter the cell and waste materials to leave the cell. Small molecules, such as oxygen, carbon dioxide, and water, are able to pass freely across the membrane, but the passage of larger molecules, such as amino acids and sugars, is carefully regulated.



According to the accepted current theory, known as the **fluid mosaic model**, the plasma membrane is composed of a double layer (**bilayer**) of lipids, oily substances found in all cells (see Figure 1). Most of the lipids in the bilayer can be more precisely described as **phospholipids**, that is, lipids that feature a phosphate group at one end of each molecule. Phospholipids are characteristically **hydrophilic** ("water-loving") at their phosphate ends and **hydrophobic** ("water-fearing") along their lipid tail regions. In each layer of a plasma membrane, the hydrophobic lipid tails are oriented inwards and the hydrophilic phosphate groups are aligned so they face outwards, either toward the aqueous cytosol of the cell or the outside environment. Phospholipids tend to spontaneously aggregate by this mechanism whenever they are exposed to water.

Within the phospholipid bilayer of the plasma membrane, many diverse proteins are embedded, while other proteins simply adhere to the surfaces of the bilayer. Some of these proteins, primarily those that are at least partially exposed on the external side of the membrane, have carbohydrates attached to their outer surfaces and are, therefore, referred to as **glycoproteins**. The positioning of proteins along the plasma membrane is related in part to the organization of the filaments that comprise the cytoskeleton, which help anchor them in place. The arrangement of proteins also involves the hydrophobic and hydrophilic regions found on the surfaces of the proteins: hydrophobic regions associate with the hydrophobic interior of the plasma membrane and hydrophilic regions extend past the surface of the membrane into either the inside of the cell or the outer environment.

Plasma membrane proteins function in several different ways. Many of the proteins play a role in the selective transport of certain substances across the phospholipid bilayer, either acting as channels or active transport molecules. Others function as receptors, which bind information-providing molecules, such as hormones, and transmit corresponding signals based on the obtained information to the interior of the cell. Membrane proteins may also exhibit enzymatic activity, catalyzing various reactions related to the plasma membrane.

Since the 1970s, the plasma membrane has been frequently described as a **fluid mosaic**, which is reflective of the discovery that oftentimes the lipid molecules in the bilayer can move about in the plane of the membrane. However, depending upon a number of factors, including the exact composition of the bilayer and temperature, plasma membranes can undergo **phase transitions** which render their molecules less dynamic and produce a more gel-like or nearly solid state. Cells are able to regulate the fluidity of their plasma membranes to meet their particular needs by synthesizing more of certain types of molecules, such as those with specific kinds of bonds that keep them fluid at lower temperatures. The presence of cholesterol and glycolipids, which are found in most cell membranes, can also affect molecular dynamics and inhibit phase transitions.

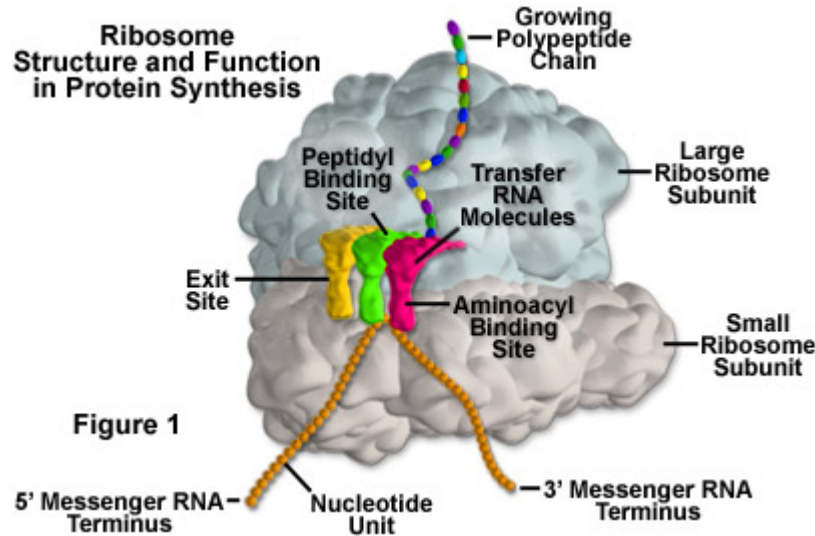
In prokaryotes and plants, the plasma membrane is an inner layer of protection since a rigid cell wall forms the outside boundary for their cells. The cell wall has pores that allow materials to enter and leave the cell, but they are not very selective about what passes through. The plasma membrane, which lines the cell wall, provides the final filter between the cell interior and the environment.

Eukaryotic animal cells are generally thought to have descended from prokaryotes that lost their cell walls. With only the flexible plasma membrane left to enclose them, these primordial creatures would have been able to expand in size and complexity. Eukaryotic cells are generally ten times larger than prokaryotic cells and have membranes enclosing interior components, the organelles. Like the exterior plasma membrane, these membranes also regulate the flow of materials, allowing the cell to segregate its chemical functions into discrete internal compartments.

### **Ribosomes**

All living cells contain ribosomes, tiny organelles composed of approximately 60 percent ribosomal RNA (**rRNA**) and 40 percent protein. However, though they are generally described

as organelles, it is important to note that ribosomes are not bound by a membrane and are much smaller than other organelles. Some cell types may hold a few million ribosomes, but several thousand is more typical. The organelles require the use of an electron microscope to be visually detected.



Ribosomes are mainly found bound to the endoplasmic reticulum and the nuclear envelope, as well as freely scattered throughout the cytoplasm, depending upon whether the cell is plant, animal, or bacteria. The organelles serve as the protein production machinery for the cell and are consequently most abundant in cells that are active in protein synthesis, such as pancreas and brain cells. Some of the proteins synthesized by ribosomes are for the cell's own internal use, especially those that are produced by free ribosomes. Many of the proteins produced by bound ribosomes, however, are transported outside of the cell.

In eukaryotes, the rRNA in ribosomes is organized into four strands, and in prokaryotes, three strands. Eukaryote ribosomes are produced and assembled in the nucleolus. Ribosomal proteins enter the nucleolus and combine with the four rRNA strands to create the two ribosomal subunits (one small and one large) that will make up the completed ribosome (see Figure 1). The ribosome units leave the nucleus through the nuclear pores and unite once in the cytoplasm for the purpose of protein synthesis. When protein production is not being carried out, the two subunits of a ribosome are separated.

In 2000, the complete three-dimensional structure of the large and small subunits of a ribosome was established. Evidence based on this structure suggests, as had long been assumed, that it is the rRNA that provides the ribosome with its basic formation and functionality, not proteins. Apparently the proteins in a ribosome help fill in structural gaps and enhance protein synthesis, although the process can take place in their absence, albeit at a much slower rate.

The units of a ribosome are often described by their Svedberg (s) values, which are based upon their rate of sedimentation in a centrifuge. The ribosomes in a eukaryotic cell generally have a Svedberg value of 80S and are comprised of 40s and 60s subunits. Prokaryotic cells, on the other hand, contain 70S ribosomes, each of which consists of a 30s and a 50s subunit. As demonstrated

by these values, Svedberg units are not additive, so the values of the two subunits of a ribosome do not add up to the Svedberg value of the entire organelle. This is because the rate of sedimentation of a molecule depends upon its size and shape, rather than simply its molecular weight.

Protein synthesis requires the assistance of two other kinds of RNA molecules in addition to rRNA. Messenger RNA (**mRNA**) provides the template of instructions from the cellular DNA for building a specific protein. Transfer RNA (**tRNA**) brings the protein building blocks, amino acids, to the ribosome. There are three adjacent tRNA binding sites on a ribosome: the **aminoacyl** binding site for a tRNA molecule attached to the next amino acid in the protein (as illustrated in Figure 1), the **peptidyl** binding site for the central tRNA molecule containing the growing peptide chain, and an **exit** binding site to discharge used tRNA molecules from the ribosome.

Once the protein backbone amino acids are polymerized, the ribosome releases the protein and it is transported to the cytoplasm in prokaryotes or to the Golgi apparatus in eukaryotes. There, the proteins are completed and released inside or outside the cell. Ribosomes are very efficient organelles. A single ribosome in a eukaryotic cell can add 2 amino acids to a protein chain every second. In prokaryotes, ribosomes can work even faster, adding about 20 amino acids to a polypeptide every second.

In addition to the most familiar cellular locations of ribosomes, the organelles can also be found inside mitochondria and the chloroplasts of plants. These ribosomes notably differ in size and makeup than other ribosomes found in eukaryotic cells, and are more akin to those present in bacteria and blue-green algae cells. The similarity of mitochondrial and chloroplast ribosomes to prokaryotic ribosomes is generally considered strong supportive evidence that mitochondria and chloroplasts evolved from ancestral prokaryotes.