Organelle in Cell Biology

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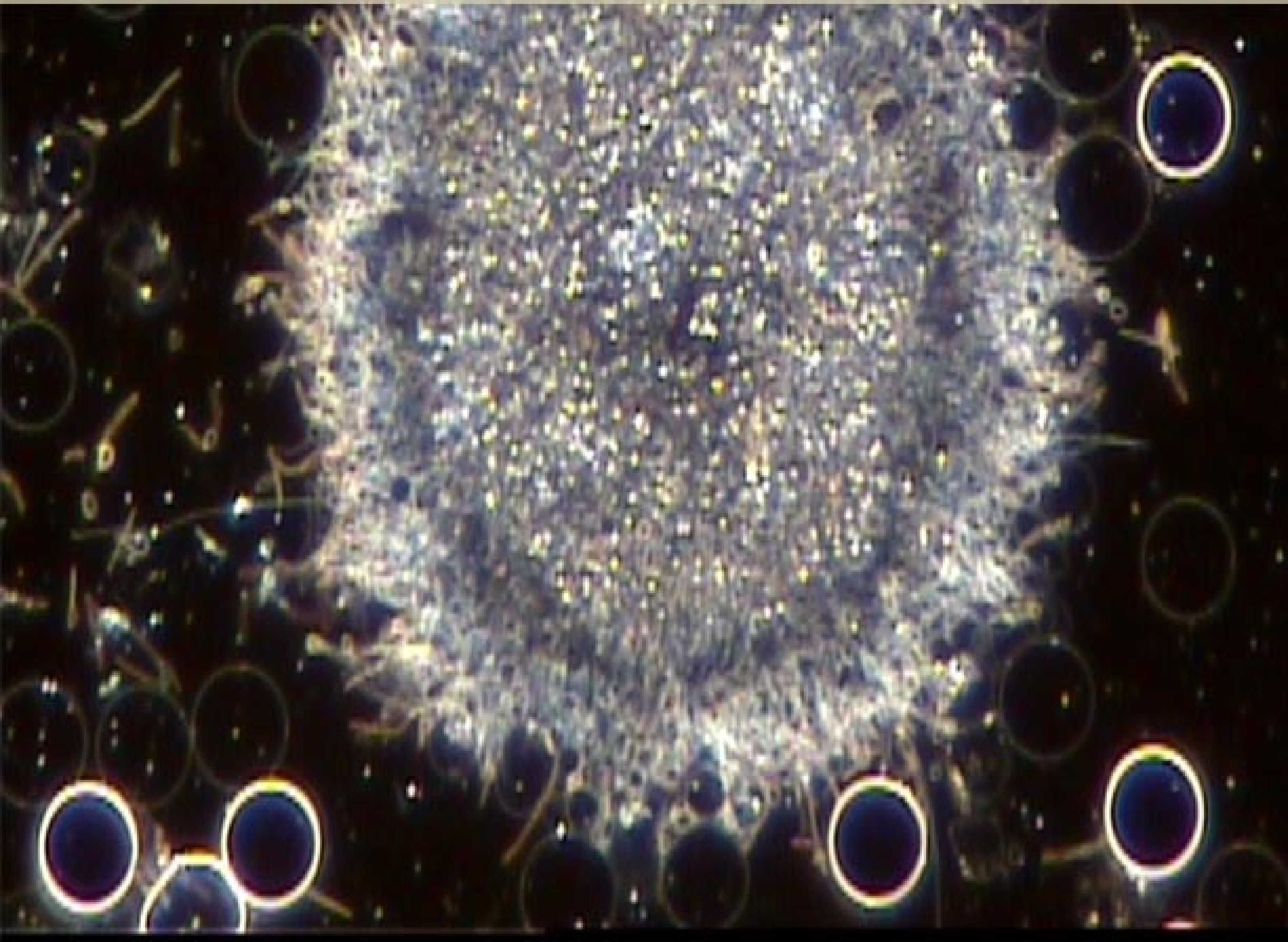

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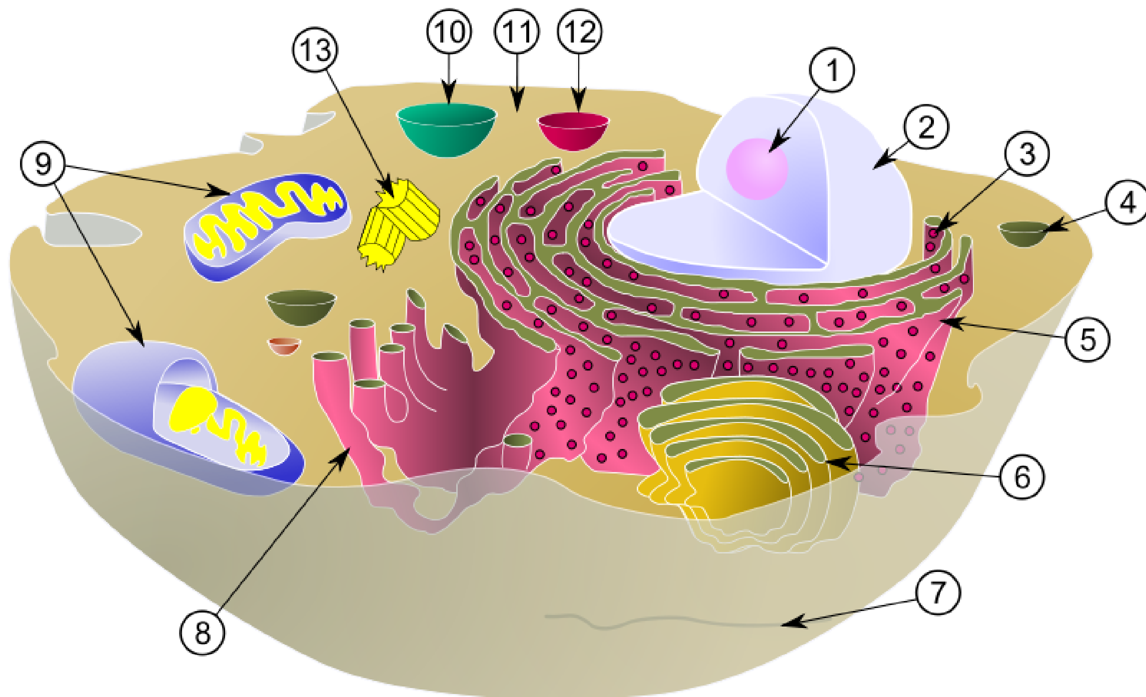
Chapter 1

Organelle



A typical animal cell. Within the cytoplasm, the major organelles and cellular structures include: (1) nucleolus (2) nucleus (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (6) Golgi apparatus (7) cytoskeleton (8) smooth endoplasmic reticulum (9) mitochondria (10) vacuole (11) cytosol (12) lysosome (13) centriole.

In cell biology, an **organelle** is a specialized subunit within a cell that has a specific function, and is usually separately enclosed within its own lipid bilayer.

The name *organelle* comes from the idea that these structures are to cells what an organ is to the body (hence the name *organelle,* the suffix *-elle* being a diminutive). Organelles are identified by microscopy, and can also be purified by cell fractionation. There are many types of organelles, particularly in eukaryotic cells. Prokaryotes were once thought not to have organelles, but some examples have now been identified.

History and terminology

In biology, *organs* are defined as confined functional units within an organism. The analogy of bodily organs to microscopic cellular substructures is obvious, as from even early works, authors of respective textbooks rarely elaborate on the distinction between the two.

Credited as the first to use a diminutive of *organ* (*i.e.* little organ) for cellular structures was German zoologist Karl August Möbius (1884), who used the term "organula" (plural form of *organulum*, the diminutive of latin *organum*). From the context, it is clear that he referred to reproduction related structures of protists. In a footnote, which was published as a correction in the next issue of the journal, he justified his suggestion to call organs of unicellular organisms "organella" since they are only differently formed parts of one cell, in contrast to multicellular organs of multicellular organisms. Thus, the original definition was limited to structures of unicellular organisms.

It would take several years before *organulum*, or the later term *organelle*, became accepted and expanded in meaning to include subcellular structures in multicellular organisms. Books around 1900 from Valentin Häcker, Edmund Wilson and Oscar Hertwig still referred to cellular *organs*. Later, both terms came to be used side by side: Bengt Lidforss wrote 1915 (in German) about "Organs or Organells".

Around 1920, the term organelle was used to describe propulsion structures ("motor organelle complex", i.e., flagella and their anchoring) and other protist structures, such as ciliates. Alfred Kühn wrote about centrioles as division organelles, although he stated that, for Vahlkampfias, the alternative 'organelle' or 'product of structural build-up' had not yet been decided, without explaining the difference between the alternatives.

In his 1953 textbook, Max Hartmann used the term for extracellular (pellicula, shells, cell walls) and intracellular skeletons of protists.

Later, the now-widely-used definition of organelle emerged, after which only cellular structures with surrounding membrane had been considered organelles. However, the more original definition of subcellular functional unit in general still coexists.

In 1978, Albert Frey-Wyssling suggested that the term organelle should refer only to structures that convert energy, such as centrosomes, ribosomes, and nucleoli. This new definition, however, did not win wide recognition.

Examples

While most cell biologists consider the term **organelle** to be synonymous with "cell compartment", other cell biologists choose to limit the term organelle to include only those that are DNA-containing, having originated from formerly-autonomous microscopic organisms acquired via endosymbiosis.

The most notable of these organelles having originated from endosymbiont bacteria are:

- mitochondria (in almost all eukaryotes)
- chloroplasts (in plants, algae and protists).

Other organelles are also suggested to have endosymbiotic origins, (notably the flagellum).

Under the more restricted definition of membrane-bound structures, some parts of the cell do not qualify as organelles. Nevertheless, the use of organelle to refer to non-membrane bound structures such as ribosomes is common. This has led some texts to delineate between membrane-bound and non-membrane bound organelles. These structures are large assemblies of macromolecules that carry out particular and specialized functions, but they lack membrane boundaries. Such cell structures include:

- ribosome
- cytoskeleton
- flagellum
- centriole and microtubule-organizing center (MTOC).

Eukaryotic organelles

Eukaryotes are one of the structurally complex cell type, and by definition are in part organized by smaller interior compartments, that are themselves enclosed by lipid membranes that resemble the outermost cell membrane. The larger organelles, such as the nucleus and vacuoles, are easily visible with the light microscope. They were among the first biological discoveries made after the invention of the microscope.

Not all eukaryotic cells have each of the organelles listed below. Exceptional organisms have cells which do not include some organelles that might otherwise be considered universal to eukaryotes (such as mitochondria). There are also occasional exceptions to the number of membranes surrounding organelles, listed in the tables below (e.g., some that are listed as double-membrane are sometimes found with single or triple membranes). In addition, the number of individual organelles of each type found in a given cell varies depending upon the function of that cell.

Major eukaryotic organelles

Organelle	Main function	Structure	Organisms	Notes
chloroplast (plastid)	photosynthesis, traps energy from sunlight	double-membrane compartment	plants, protists (rare kleptoplastic organisms)	has some genes; theorized to be engulfed by the ancestral eukaryotic cell (endosymbiosis)
endoplasmic reticulum	translation and folding of new	single-membrane	all eukaryotes	rough endoplasmic reticulum is covered

	proteins (rough endoplasmic reticulum), expression of lipids (smooth endoplasmic reticulum)	compartment		with ribosomes, has folds that are flat sacs; smooth endoplasmic reticulum has folds that are tubular
Golgi apparatus	sorting and modification of proteins	single-membrane compartment	all eukaryotes	cis-face (convex) nearest to rough endoplasmic reticulum; trans-face (concave) farthest from rough endoplasmic reticulum
mitochondria	energy production from the oxidation of food substances and the release of adenosine triphosphate	double-membrane compartment	most eukaryotes	has some DNA; theorized to be engulfed by an ancestral eukaryotic cell (endosymbiosis)
vacuole	storage, helps maintain homeostasis	single-membrane compartment	eukaryotes	
nucleus	DNA maintenance, controls all activities of the cell, RNA transcription	double-membrane compartment	all eukaryotes	contains bulk of genome

Mitochondria and chloroplasts, which have double-membranes and their own DNA, are believed to have originated from incompletely consumed or invading prokaryotic organisms, which were adopted as a part of the invaded cell. This idea is supported in the Endosymbiotic theory.

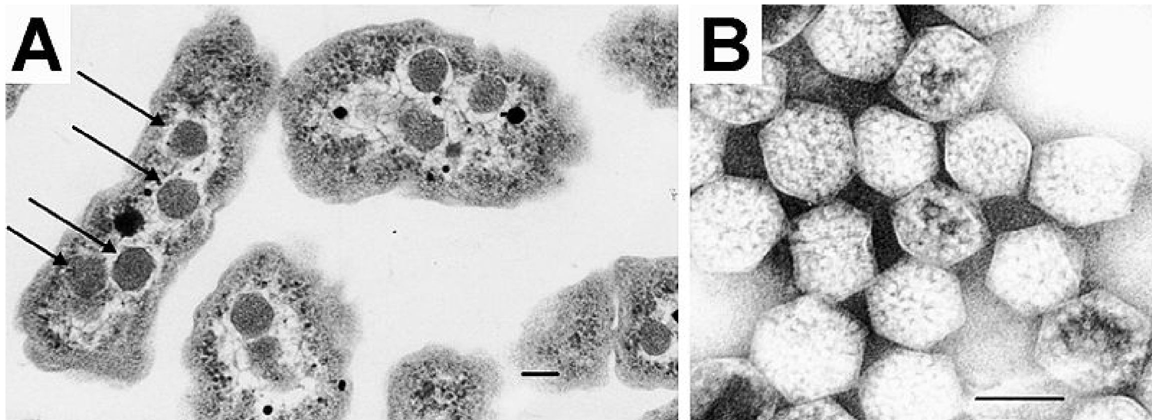
Minor eukaryotic organelles and cell components

Organelle/Macromolecule	Main function	Structure	Organisms
acrosome	helps spermatoza fuse with ovum	single-membrane compartment	many animals
autophagosome	vesicle which sequesters cytoplasmic material and organelles for degradation	double-membrane compartment	all eukaryotic cells
centriole	anchor for cytoskeleton, helps in cell division by forming spindle fibres	Microtubule protein	animals

cilium	movement in or of external medium; "critical developmental signaling pathway".	Microtubule protein	animals, protists, few plants
eyespot apparatus	detects light, allowing phototaxis to take place		green algae and other unicellular photosynthetic organisms such as euglenids
glycosome	carries out glycolysis	single-membrane compartment	Some protozoa, such as *Trypanosomes*.
glyoxysome	conversion of fat into sugars	single-membrane compartment	plants
hydrogenosome	energy & hydrogen production	double-membrane compartment	a few unicellular eukaryotes
lysosome	breakdown of large molecules (e.g., proteins + polysaccharides)	single-membrane compartment	most eukaryotes
melanosome	pigment storage	single-membrane compartment	animals
mitosome	not characterized	double-membrane compartment	a few unicellular eukaryotes
myofibril	muscular contraction	bundled filaments	animals
nucleolus	ribosome production	protein-DNA-RNA	most eukaryotes
parenthesome	not characterized	not characterized	fungi
peroxisome	breakdown of metabolic hydrogen peroxide	single-membrane compartment	all eukaryotes
ribosome	translation of RNA into proteins	RNA-protein	eukaryotes, prokaryotes
vesicle	material transport	single-membrane compartment	all eukaryotes

Other related structures:

- cytosol
- endomembrane system
- nucleosome
- microtubule
- cell membrane



(A) Electron micrograph of *Halothiobacillus neapolitanus* cells, arrows highlight carboxysomes. (B) Image of intact carboxysomes isolated from *H. neapolitanus*. Scale bars are 100 nm.

Prokaryotic organelles

Prokaryotes are not as structurally complex as eukaryotes, and were once thought not to have any internal structures enclosed by lipid membranes. In the past, they were often viewed as having little internal organization; but, slowly, details are emerging about prokaryotic internal structures. An early false turn was the idea developed in the 1970s that bacteria might contain membrane folds termed mesosomes, but these were later shown to be artifacts produced by the chemicals used to prepare the cells for electron microscopy.

However, more recent research has revealed that at least some prokaryotes have microcompartments such as carboxysomes. These subcellular compartments are 100 - 200 nm in diameter and are enclosed by a shell of proteins. Even more striking is the description of membrane-bound magnetosomes in bacteria, as well as the nucleus-like structures of the *Planctomycetes* that are surrounded by lipid membranes.

Prokaryotic organelles and cell components

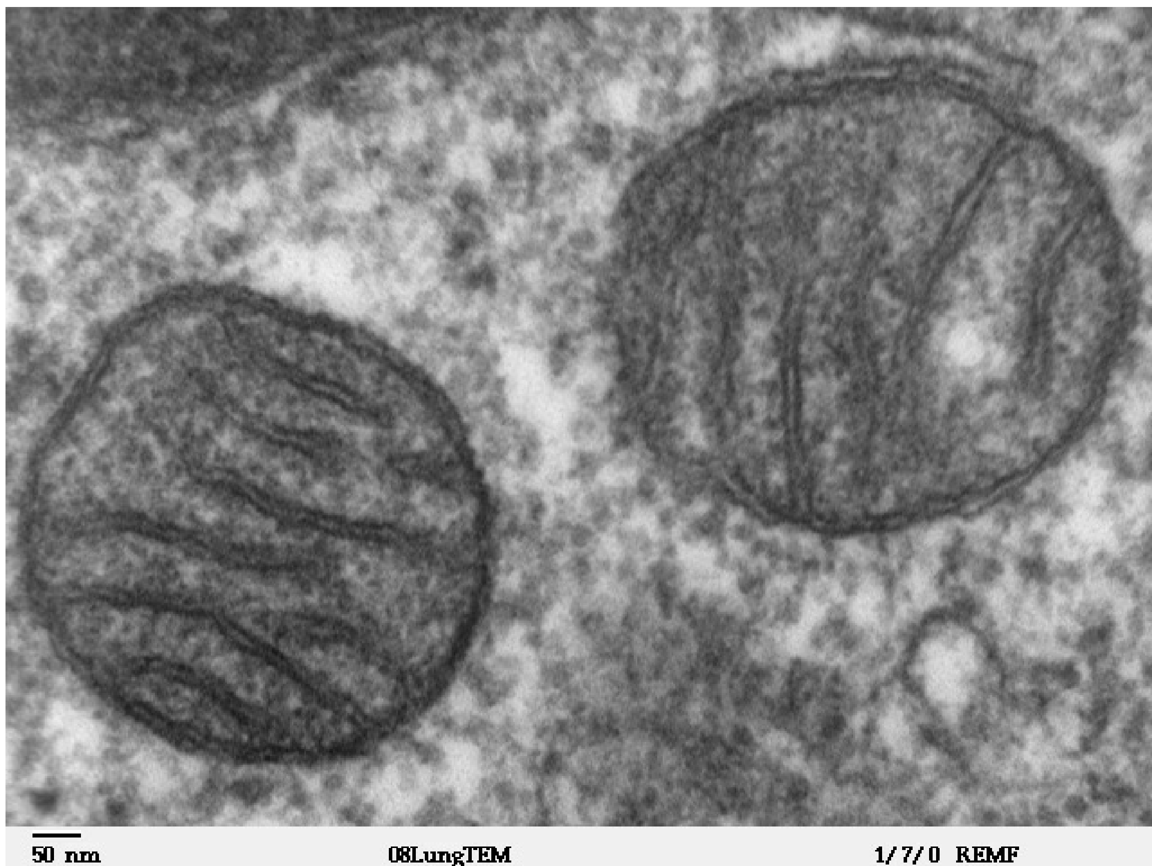
Organelle/Macromolecule	Main function	Structure	Organisms
carboxysome	carbon fixation	protein-shell compartment	some bacteria
chlorosome	photosynthesis	light harvesting complex	green sulfur bacteria
flagellum	movement in external medium	protein filament	some prokaryotes and eukaryotes
magnetosome	magnetic orientation	inorganic crystal, lipid membrane	magnetotactic bacteria
nucleoid	DNA maintenance, transcription to RNA	DNA-protein	prokaryotes
plasmid	DNA exchange	circular DNA	some bacteria
ribosome	translation of RNA into proteins	RNA-protein	eukaryotes, prokaryotes
thylakoid	photosynthesis	photosystem proteins and pigments	mostly cyanobacteria

Proteins and organelles

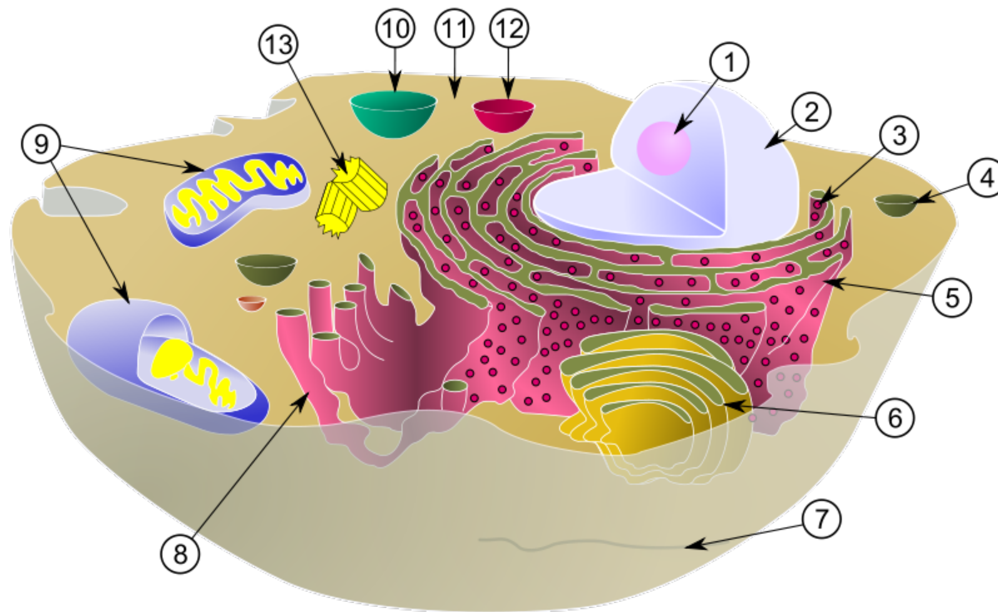
The function of a protein is closely correlated with the organelle in which it resides. Some methods were proposed for predicting the organelle in which an uncharacterized protein is located according to its amino acid composition and some methods were based on pseudo amino acid composition.

Chapter 2

Mitochondrion



Two mitochondria from mammalian lung tissue displaying their matrix and membranes as shown by electron microscopy.



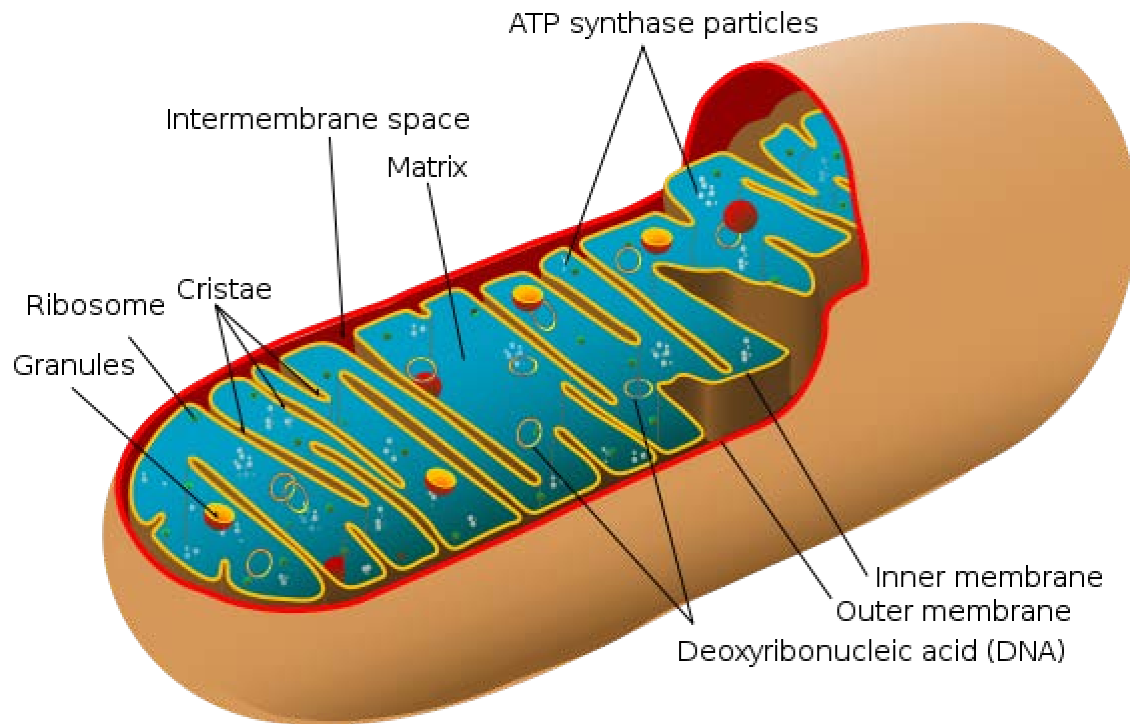
Schematic of typical animal cell, showing subcellular components. Organelles:
(1) nucleolus
(2) nuclear membrane
(3) Ribosomes
(4) Vesicle
(5) Rough endoplasmic reticulum (ER)
(6) Golgi body
(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(13) Centrioles within centrosome

In cell biology, a **mitochondrion** (plural **mitochondria**) is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from 0.5 to 10 micrometers (μm) in diameter. Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth. Mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process. The word mitochondrion comes from the Greek *μίτος* or *mitos*, thread + *χονδρίον* or *chondrion*, granule.

Several characteristics make mitochondria unique. The number of mitochondria in a cell varies widely by organism and tissue type. Many cells have only a single mitochondrion, whereas others can contain several thousand mitochondria. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the

cristae and matrix. Mitochondrial proteins vary depending on the tissue and the species. In humans, 615 distinct types of proteins have been identified from cardiac mitochondria, whereas in Murinae (rats), 940 proteins encoded by distinct genes have been reported. The mitochondrial proteome is thought to be dynamically regulated. Although most of a cell's DNA is contained in the cell nucleus, the mitochondrion has its own independent genome. Further, its DNA shows substantial similarity to bacterial genomes.

Structure



A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins. The two membranes, however, have different properties. Because of this double-membraned organization, there are five distinct compartments within the mitochondrion. There is the outer mitochondrial membrane, the intermembrane space (the space between the outer and inner membranes), the inner mitochondrial membrane, the cristae space (formed by infoldings of the inner membrane), and the matrix (space within the inner membrane).

Outer membrane

The outer mitochondrial membrane, which encloses the entire organelle, has a protein-to-phospholipid ratio similar to that of the eukaryotic plasma membrane (about 1:1 by weight). It contains large numbers of integral proteins called *porins*. These porins form channels that allow molecules 5000 Daltons or less in molecular weight to freely diffuse from one side of the membrane to the other. Larger proteins can enter the mitochondrion if a signaling sequence at their N-terminus binds to a large multisubunit protein called

translocase of the outer membrane, which then actively moves them across the membrane. Disruption of the outer membrane permits proteins in the intermembrane space to leak into the cytosol, leading to certain cell death. The mitochondrial outer membrane can associate with the endoplasmic reticulum (ER) membrane, in a structure called MAM (mitochondria-associated ER-membrane). This is important in ER-mitochondria calcium signaling and involved in the transfer of lipids between the ER and mitochondria.

Intermembrane space

The intermembrane space is the space between the outer membrane and the inner membrane. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules such as ions and sugars in the intermembrane space is the same as the cytosol. However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol. One protein that is localized to the intermembrane space in this way is cytochrome c.

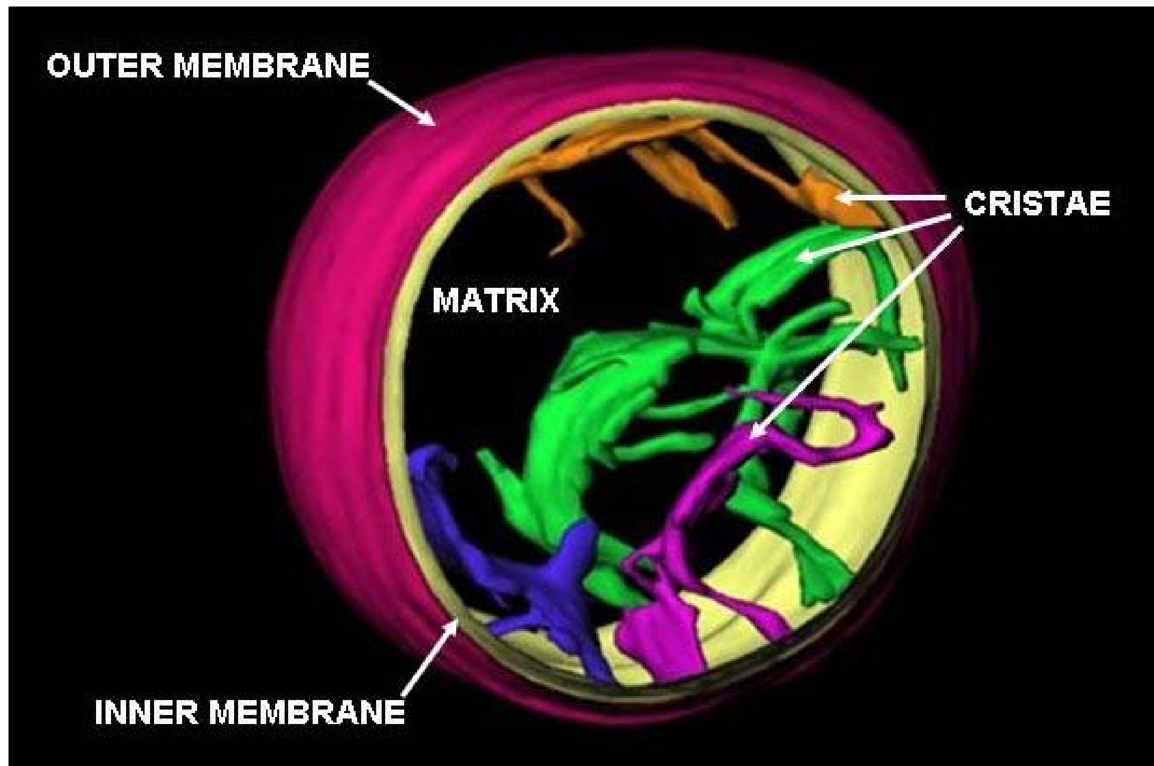
Inner membrane

The inner mitochondrial membrane contains proteins with five types of functions:

1. Those that perform the redox reactions of oxidative phosphorylation
2. ATP synthase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery.
5. Mitochondria fusion and fission protein

It contains more than 151 different polypeptides, and has a very high protein-to-phospholipid ratio (more than 3:1 by weight, which is about 1 protein for 15 phospholipids). The inner membrane is home to around 1/5 of the total protein in a mitochondrion. In addition, the inner membrane is rich in an unusual phospholipid, cardiolipin. This phospholipid was originally discovered in cow hearts in 1942, and is usually characteristic of mitochondrial and bacterial plasma membranes. Cardiolipin contains four fatty acids rather than two and may help to make the inner membrane impermeable. Unlike the outer membrane, the inner membrane doesn't contain porins and is highly impermeable to all molecules. Almost all ions and molecules require special membrane transporters to enter or exit the matrix. Proteins are ferried into the matrix via the translocase of the inner membrane (TIM) complex or via Oxa1. In addition, there is a membrane potential across the inner membrane formed by the action of the enzymes of the electron transport chain.

Cristae



Cross-sectional image of cristae in rat liver mitochondrion to demonstrate the likely 3D structure and relationship to the inner membrane.

The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria the area of the inner membrane is about five times greater than the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies known as F_1 particles or oxysomes. These are not simple random folds but rather invaginations of the inner membrane, which can affect overall chemiosmotic function.

One recent mathematical modeling study has suggested that the optical properties of the christae in filamentous mitochondria may affect the generation and propogation of light within the tissue.

Matrix

The matrix is the space enclosed by the inner membrane. It contains about 2/3 of the total protein in a mitochondrion. The matrix is important in the production of ATP with the aid of the ATP synthase contained in the inner membrane. The matrix contains a highly-concentrated mixture of hundreds of enzymes, special mitochondrial ribosomes, tRNA,

and several copies of the mitochondrial DNA genome. Of the enzymes, the major functions include oxidation of pyruvate and fatty acids, and the citric acid cycle.

Mitochondria have their own genetic material, and the machinery to manufacture their own RNAs and proteins. A published human mitochondrial DNA sequence revealed 16,569 base pairs encoding 37 total genes: 22 tRNA, 2 rRNA, and 13 peptide genes. The 13 mitochondrial peptides in humans are integrated into the inner mitochondrial membrane, along with proteins encoded by genes that reside in the host cell's nucleus.

Organization and distribution

Mitochondria are found in nearly all eukaryotes. They vary in number and location according to cell type. A single mitochondrion is often found in unicellular organisms. Conversely, numerous mitochondria are found in human liver cells, with about 1000–2000 mitochondria per cell making up 1/5th of the cell volume. The mitochondria can be found nestled between myofibrils of muscle or wrapped around the sperm flagellum. Often they form a complex 3D branching network inside the cell with the cytoskeleton. The association with the cytoskeleton determines mitochondrial shape, which can affect the function as well. Recent evidence suggests vimentin, one of the components of the cytoskeleton, is critical to the association with the cytoskeleton.

Function

The most prominent roles of mitochondria are to produce ATP (i.e., phosphorylation of ADP) through respiration, and to regulate cellular metabolism. The central set of reactions involved in ATP production are collectively known as the citric acid cycle, or the Krebs Cycle. However, the mitochondrion has many other functions in addition to the production of ATP.

Energy conversion

A dominant role for the mitochondria is the production of ATP, as reflected by the large number of proteins in the inner membrane for this task. This is done by oxidizing the major products of glucose, pyruvate, and NADH, which are produced in the cytosol. This process of cellular respiration, also known as aerobic respiration, is dependent on the presence of oxygen. When oxygen is limited, the glycolytic products will be metabolized by anaerobic respiration, a process that is independent of the mitochondria. The production of ATP from glucose has an approximately 13-fold higher yield during aerobic respiration compared to anaerobic respiration. Recently it has been shown that plant mitochondria can produce a limited amount of ATP without oxygen by using the alternate substrate nitrite.

Pyruvate and the citric acid cycle

Each pyruvate molecule produced by glycolysis is actively transported across the inner mitochondrial membrane, and into the matrix where it is oxidized and combined with coenzyme A to form CO_2, acetyl-CoA, and NADH.

The acetyl-CoA is the primary substrate to enter the *citric acid cycle*, also known as the *tricarboxylic acid (TCA) cycle* or *Krebs cycle*. The enzymes of the citric acid cycle are located in the mitochondrial matrix, with the exception of succinate dehydrogenase, which is bound to the inner mitochondrial membrane as part of Complex II. The citric acid cycle oxidizes the acetyl-CoA to carbon dioxide, and, in the process, produces reduced cofactors (three molecules of NADH and one molecule of $FADH_2$) that are a source of electrons for the *electron transport chain*, and a molecule of GTP (that is readily converted to an ATP).

NADH and $FADH_2$: the electron transport chain

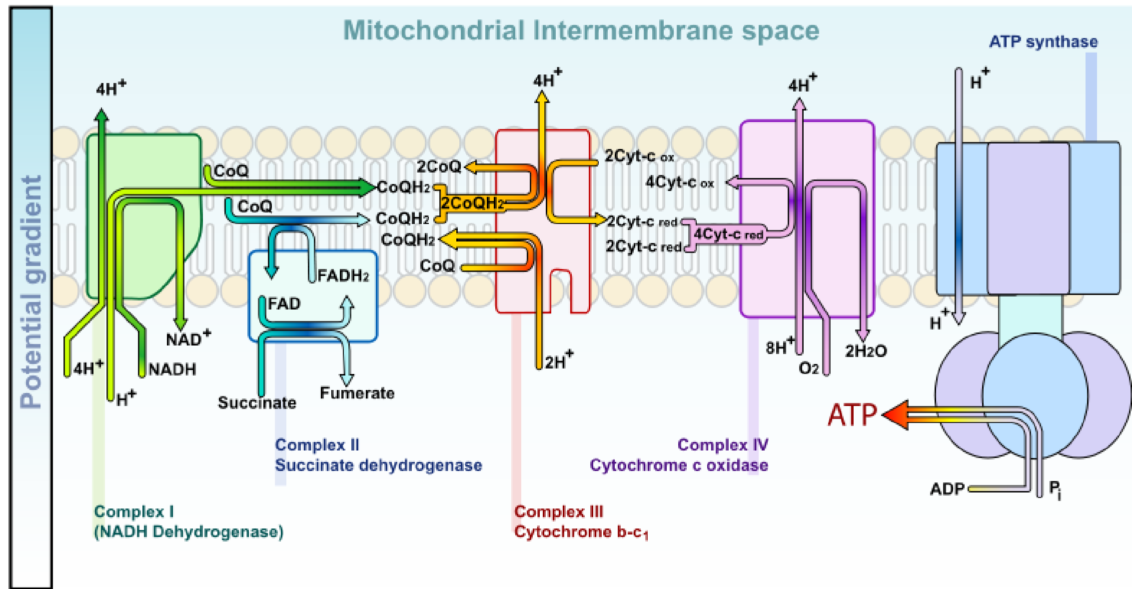


Diagram of the electron transport chain in the mitonchondrial intermembrane space

The redox energy from NADH and $FADH_2$ is transferred to oxygen (O_2) in several steps via the electron transport chain. These energy-rich molecules are produced within the matrix via the citric acid cycle but are also produced in the cytoplasm by glycolysis. Reducing equivalents from the cytoplasm can be imported via the malate-aspartate shuttle system of antiporter proteins or feed into the electron transport chain using a glycerol phosphate shuttle. Protein complexes in the inner membrane (NADH dehydrogenase, cytochrome c reductase, and cytochrome c oxidase) perform the transfer and the incremental release of energy is used to pump protons (H^+) into the intermembrane space. This process is efficient, but a small percentage of electrons may prematurely reduce oxygen, forming reactive oxygen species such as superoxide. This

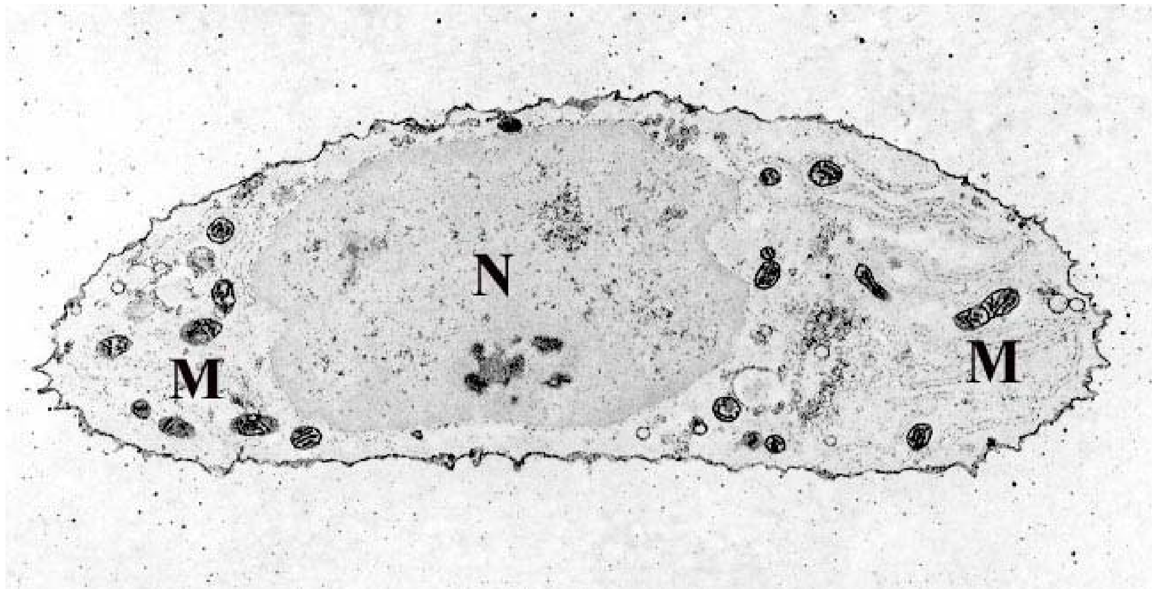
can cause oxidative stress in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.

As the proton concentration increases in the intermembrane space, a strong electrochemical gradient is established across the inner membrane. The protons can return to the matrix through the ATP synthase complex, and their potential energy is used to synthesize ATP from ADP and inorganic phosphate (P_i). This process is called chemiosmosis, and was first described by Peter Mitchell who was awarded the 1978 Nobel Prize in Chemistry for his work. Later, part of the 1997 Nobel Prize in Chemistry was awarded to Paul D. Boyer and John E. Walker for their clarification of the working mechanism of ATP synthase.

Heat production

Under certain conditions, protons can re-enter the mitochondrial matrix without contributing to ATP synthesis. This process is known as *proton leak* or *mitochondrial uncoupling* and is due to the facilitated diffusion of protons into the matrix. The process results in the unharnessed potential energy of the proton electrochemical gradient being released as heat. The process is mediated by a proton channel called thermogenin, or UCP1. Thermogenin is a 33kDa protein first discovered in 1973. Thermogenin is primarily found in brown adipose tissue, or brown fat, and is responsible for non-shivering thermogenesis. Brown adipose tissue is found in mammals, and is at its highest levels in early life and in hibernating animals. In humans, brown adipose tissue is present at birth and decreases with age.

Storage of calcium ions



Mitochondria (M) within a chondrocyte stained for calcium as shown by electron microscopy.

The concentrations of free calcium in the cell can regulate an array of reactions and is important for signal transduction in the cell. Mitochondria can transiently store calcium, a contributing process for the cell's homeostasis of calcium. In fact, their ability to rapidly take in calcium for later release makes them very good "cytosolic buffers" for calcium. The endoplasmic reticulum (ER) is the most significant storage site of calcium, and there is a significant interplay between the mitochondrion and ER with regard to calcium. The calcium is taken up into the matrix by a calcium uniporter on the inner mitochondrial membrane. It is primarily driven by the mitochondrial membrane potential. Release of this calcium back into the cell's interior can occur via a sodium-calcium exchange protein or via "calcium-induced-calcium-release" pathways. This can initiate calcium spikes or calcium waves with large changes in the membrane potential. These can activate a series of second messenger system proteins that can coordinate processes such as neurotransmitter release in nerve cells and release of hormones in endocrine cells.

Additional functions

Mitochondria play a central role in many other metabolic tasks, such as:

- Regulation of the membrane potential
- Apoptosis-programmed cell death
- Calcium signaling (including calcium-evoked apoptosis)
- Cellular proliferation regulation
- Regulation of cellular metabolism
- Certain heme synthesis reactions
- Steroid synthesis.

Some mitochondrial functions are performed only in specific types of cells. For example, mitochondria in liver cells contain enzymes that allow them to detoxify ammonia, a waste product of protein metabolism. A mutation in the genes regulating any of these functions can result in mitochondrial diseases.

Origin

Mitochondria have many features in common with prokaryotes. As a result, they are believed to be originally derived from endosymbiotic prokaryotes.

A mitochondrion contains DNA, which is organized as several copies of a single, circular chromosome. This mitochondrial chromosome contains genes for redox proteins such as those of the respiratory chain. The CoRR hypothesis proposes that this **co**-location is required for **r**edox **r**egulation. The mitochondrial genome codes for some RNAs of ribosomes, and the twenty-two tRNAs necessary for the translation of messenger RNAs into protein. The circular structure is also found in prokaryotes, and the similarity is extended by the fact that mitochondrial DNA is organized with a variant genetic code similar to that of Proteobacteria. This suggests that their ancestor, the so-called proto-mitochondrion, was a member of the Proteobacteria. In particular, the proto-mitochondrion was probably closely related to the rickettsia. However, the exact

relationship of the ancestor of mitochondria to the alpha-proteobacteria and whether the mitochondria was formed at the same time or after the nucleus, remains controversial.

The ribosomes coded for by the mitochondrial DNA are similar to those from bacteria in size and structure. They closely resemble the bacterial 70S ribosome and not the 80S cytoplasmic ribosomes, which are coded for by nuclear DNA.

The endosymbiotic relationship of mitochondria with their host cells was popularized by Lynn Margulis. The endosymbiotic hypothesis suggests that mitochondria descended from bacteria that somehow survived endocytosis by another cell, and became incorporated into the cytoplasm. The ability of these bacteria to conduct respiration in host cells that had relied on glycolysis and fermentation would have provided a considerable evolutionary advantage. In a similar manner, host cells with symbiotic bacteria capable of photosynthesis would have had an advantage. The incorporation of symbiotes would have increased the number of environments in which the cells could survive. This symbiotic relationship probably developed 1.7-2 billion years ago.

A few groups of unicellular eukaryotes lack mitochondria: the microsporidians, metamonads, and archamoebae. These groups appear as the most primitive eukaryotes on phylogenetic trees constructed using rRNA information, which once suggested that they appeared before the origin of mitochondria. However, this is now known to be an artifact of long-branch attraction—they are derived groups and retain genes or organelles derived from mitochondria (e.g., mitosomes and hydrogenosomes).

Genome

The human mitochondrial genome is a circular DNA molecule of about 16 kilobases. It encodes 37 genes: 13 for subunits of respiratory complexes I, III, IV and V, 22 for mitochondrial tRNA (for the 20 standard amino acids, plus an extra gene for leucine and serine), and 2 for rRNA. One mitochondrion can contain two to ten copies of its DNA.

As in prokaryotes, there is a very high proportion of coding DNA and an absence of repeats. Mitochondrial genes are transcribed as multigenic transcripts, which are cleaved and polyadenylated to yield mature mRNAs. Not all proteins necessary for mitochondrial function are encoded by the mitochondrial genome; most are coded by genes in the cell nucleus and the corresponding proteins are imported into the mitochondrion. The exact number of genes encoded by the nucleus and the mitochondrial genome differs between species. In general, mitochondrial genomes are circular, although exceptions have been reported. In general, mitochondrial DNA lacks introns, as is the case in the human mitochondrial genome; however, introns have been observed in some eukaryotic mitochondrial DNA, such as that of yeast and protists, including *Dictyostelium discoideum*.

In animals the mitochondrial genome is typically a single circular chromosome that is approximately 16-kb long and has 37 genes. The genes while highly conserved may vary in location. Curiously this pattern is not found in the human body louse (*Pediculus*

humanus). Instead this mitochondrial genome is arranged in 18 minicircular chromosomes each of which is 3–4 kb long and has one to three genes. This pattern is also found in other sucking lice but not in chewing lice. Recombination has been shown to occur between the minichromosomes. The reason for this difference is not known.

While slight variations on the standard code had been predicted earlier, none was discovered until 1979, when researchers studying human mitochondrial genes determined that they used an alternative code. Many slight variants have been discovered since, including various alternative mitochondrial codes. Further, the AUA, AUC, and AUU codons are all allowable start codons.

Exceptions to the universal genetic code (UGC) in mitochondria

Organism	Codon	Standard	Novel
	AGA, AGG	Arginine	Stop codon
Mammalian	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
	AGA, AGG	Arginine	Serine
Invertebrates	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
	AUA	Isoleucine	Methionine
Yeast	UGA	Stop codon	Tryptophan
	CUA	Leucine	Threonine

Some of these differences should be regarded as pseudo-changes in the genetic code due to the phenomenon of RNA editing, which is common in mitochondria. In higher plants, it was thought that CGG encoded for tryptophan and not arginine; however, the codon in the processed RNA was discovered to be the UGG codon, consistent with the universal genetic code for tryptophan. Of note, the arthropod mitochondrial genetic code has undergone parallel evolution within a phylum, with some organisms uniquely translating AGG to lysine.

Mitochondrial genomes have far fewer genes than the bacteria from which they are thought to be descended. Although some have been lost altogether, many have been transferred to the nucleus, such as the respiratory complex II protein subunits. This is thought to be relatively common over evolutionary time. A few organisms, such as the *Cryptosporidium*, actually have mitochondria that lack any DNA, presumably because all their genes have been lost or transferred. In *Cryptosporidium*, the mitochondria have an altered ATP generation system that renders the parasite resistant to many classical mitochondrial inhibitors such as cyanide, azide, and atovaquone.

Replication and inheritance

Mitochondria divide by binary fission similar to bacterial cell division; unlike bacteria, however, mitochondria can also fuse with other mitochondria. The regulation of this division differs between eukaryotes. In many single-celled eukaryotes, their growth and division is linked to the cell cycle. For example, a single mitochondrion may divide synchronously with the nucleus. This division and segregation process must be tightly controlled so that each daughter cell receives at least one mitochondrion. In other eukaryotes (in mammals for example), mitochondria may replicate their DNA and divide mainly in response to the energy needs of the cell, rather than in phase with the cell cycle. When the energy needs of a cell are high, mitochondria grow and divide. When the energy use is low, mitochondria are destroyed or become inactive. In such examples, and in contrast to the situation in many single celled eukaryotes, mitochondria are apparently randomly distributed to the daughter cells during the division of the cytoplasm.

An individual's mitochondrial genes are not inherited by the same mechanism as nuclear genes. At fertilization of an egg cell by a sperm, the egg nucleus and sperm nucleus each contribute equally to the genetic makeup of the zygote nucleus. In contrast, the mitochondria, and therefore the mitochondrial DNA, usually comes from the egg only. The sperm's mitochondria enter the egg but do not contribute genetic information to the embryo. Instead, paternal mitochondria are marked with ubiquitin to select them for later destruction inside the embryo. The egg cell contains relatively few mitochondria, but it is these mitochondria that survive and divide to populate the cells of the adult organism. Mitochondria are, therefore, in most cases inherited down the female line, known as maternal inheritance. This mode is seen in most organisms including all animals. However, mitochondria in some species can sometimes be inherited paternally. This is the norm among certain coniferous plants, although not in pine trees and yew trees. It has been suggested that it occurs at a very low level in humans.

Uniparental inheritance leads to little opportunity for genetic recombination between different lineages of mitochondria, although a single mitochondrion can contain 2–10 copies of its DNA. For this reason, mitochondrial DNA usually is thought to reproduce by binary fission. What recombination does take place maintains genetic integrity rather than maintaining diversity. However, there are studies showing evidence of recombination in mitochondrial DNA. It is clear that the enzymes necessary for recombination are present in mammalian cells. Further, evidence suggests that animal mitochondria can undergo recombination. The data are a bit more controversial in humans, although indirect evidence of recombination exists. If recombination does not occur, the whole mitochondrial DNA sequence represents a single haplotype, which makes it useful for studying the evolutionary history of populations.

Population genetic studies

The near-absence of genetic recombination in mitochondrial DNA makes it a useful source of information for scientists involved in population genetics and evolutionary biology. Because all the mitochondrial DNA is inherited as a single unit, or haplotype,

the relationships between mitochondrial DNA from different individuals can be represented as a gene tree. Patterns in these gene trees can be used to infer the evolutionary history of populations. The classic example of this is in human evolutionary genetics, where the molecular clock can be used to provide a recent date for mitochondrial Eve. This is often interpreted as strong support for a recent modern human expansion out of Africa. Another human example is the sequencing of mitochondrial DNA from Neanderthal bones. The relatively large evolutionary distance between the mitochondrial DNA sequences of Neanderthals and living humans has been interpreted as evidence for lack of interbreeding between Neanderthals and anatomically-modern humans.

However, mitochondrial DNA reflects the history of only females in a population and so may not represent the history of the population as a whole. This can be partially overcome by the use of paternal genetic sequences, such as the non-recombining region of the Y-chromosome. In a broader sense, only studies that also include nuclear DNA can provide a comprehensive evolutionary history of a population.

Dysfunction and disease

Mitochondrial diseases

With their central place in cell metabolism, damage — and subsequent dysfunction — in mitochondria is an important factor in a wide range of human diseases. Mitochondrial disorders often present as neurological disorders, but can manifest as myopathy, diabetes, multiple endocrinopathy, or a variety of other systemic manifestations. Diseases caused by mutation in the mtDNA include Kearns-Sayre syndrome, MELAS syndrome and Leber's hereditary optic neuropathy. In the vast majority of cases, these diseases are transmitted by a female to her children, as the zygote derives its mitochondria and hence its mtDNA from the ovum. Diseases such as Kearns-Sayre syndrome, Pearson's syndrome, and progressive external ophthalmoplegia are thought to be due to large-scale mtDNA rearrangements, whereas other diseases such as MELAS syndrome, Leber's hereditary optic neuropathy, myoclonic epilepsy with ragged red fibers (MERRF), and others are due to point mutations in mtDNA.

In other diseases, defects in nuclear genes lead to dysfunction of mitochondrial proteins. This is the case in Friedreich's ataxia, hereditary spastic paraplegia, and Wilson's disease. These diseases are inherited in a dominance relationship, as applies to most other genetic diseases. A variety of disorders can be caused by nuclear mutations of oxidative phosphorylation enzymes, such as coenzyme Q10 deficiency and Barth syndrome. Environmental influences may interact with hereditary predispositions and cause mitochondrial disease. For example, there may be a link between pesticide exposure and the later onset of Parkinson's disease.

Other pathologies with etiology involving mitochondrial dysfunction include schizophrenia, bipolar disorder, dementia, Alzheimer's disease, Parkinson's disease, epilepsy, stroke, cardiovascular disease, retinitis pigmentosa, and diabetes mellitus. A

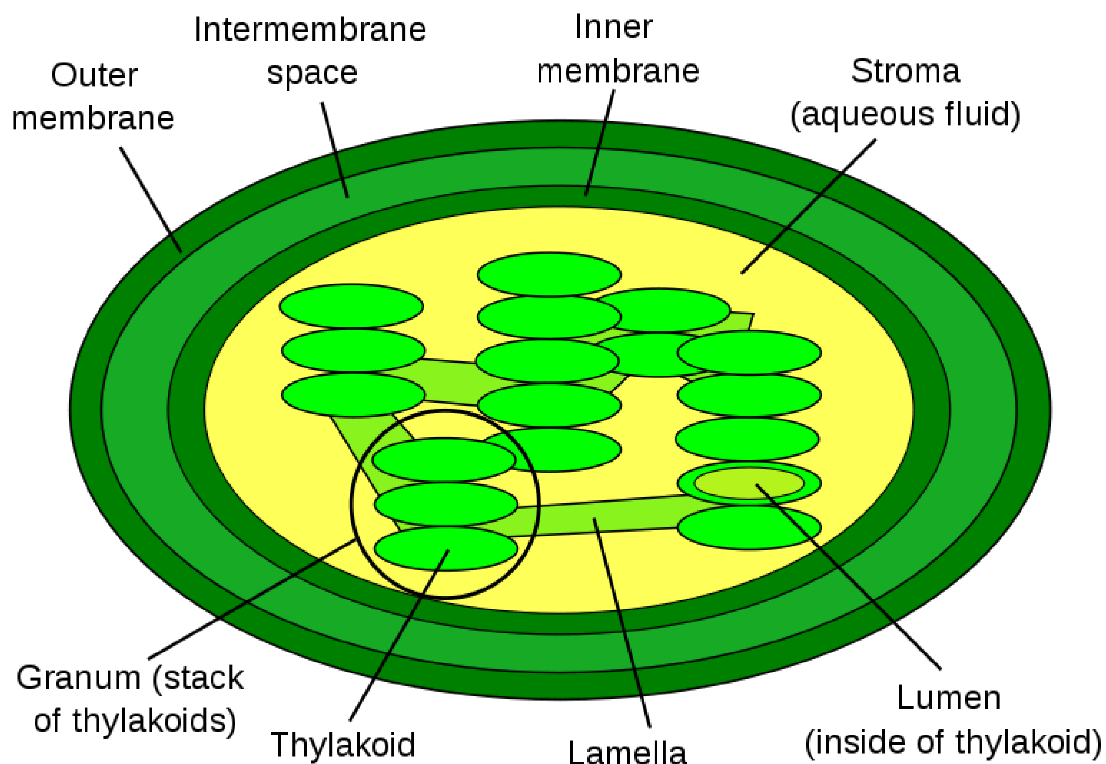
common thread thought to link these seemingly-unrelated conditions is cellular damage causing oxidative stress. How exactly mitochondrial dysfunction fits into the etiology of these pathologies is yet to be elucidated.

Possible relationships to aging

Given the role of mitochondria as the cell's powerhouse, there may be some leakage of the high-energy electrons in the respiratory chain to form reactive oxygen species. This can result in significant oxidative stress in the mitochondria with high mutation rates of mitochondrial DNA. A vicious cycle is thought to occur, as oxidative stress leads to mitochondrial DNA mutations, which can lead to enzymatic abnormalities and further oxidative stress. A number of changes occur to mitochondria during the aging process. Tissues from elderly patients show a decrease in enzymatic activity of the proteins of the respiratory chain. Large deletions in the mitochondrial genome can lead to high levels of oxidative stress and neuronal death in Parkinson's disease. Hypothesized links between aging and oxidative stress are not new and were proposed over 50 years ago; however, there is much debate over whether mitochondrial changes are causes of aging or merely characteristics of aging. One notable study in mice demonstrated shortened lifespan but no increase in reactive oxygen species despite increasing mitochondrial DNA mutations, suggesting that mitochondrial DNA mutations can cause lifespan shortening by other mechanisms. As a result, the exact relationships between mitochondria, oxidative stress, and aging have not yet been settled.

Chapter 3

Chloroplast

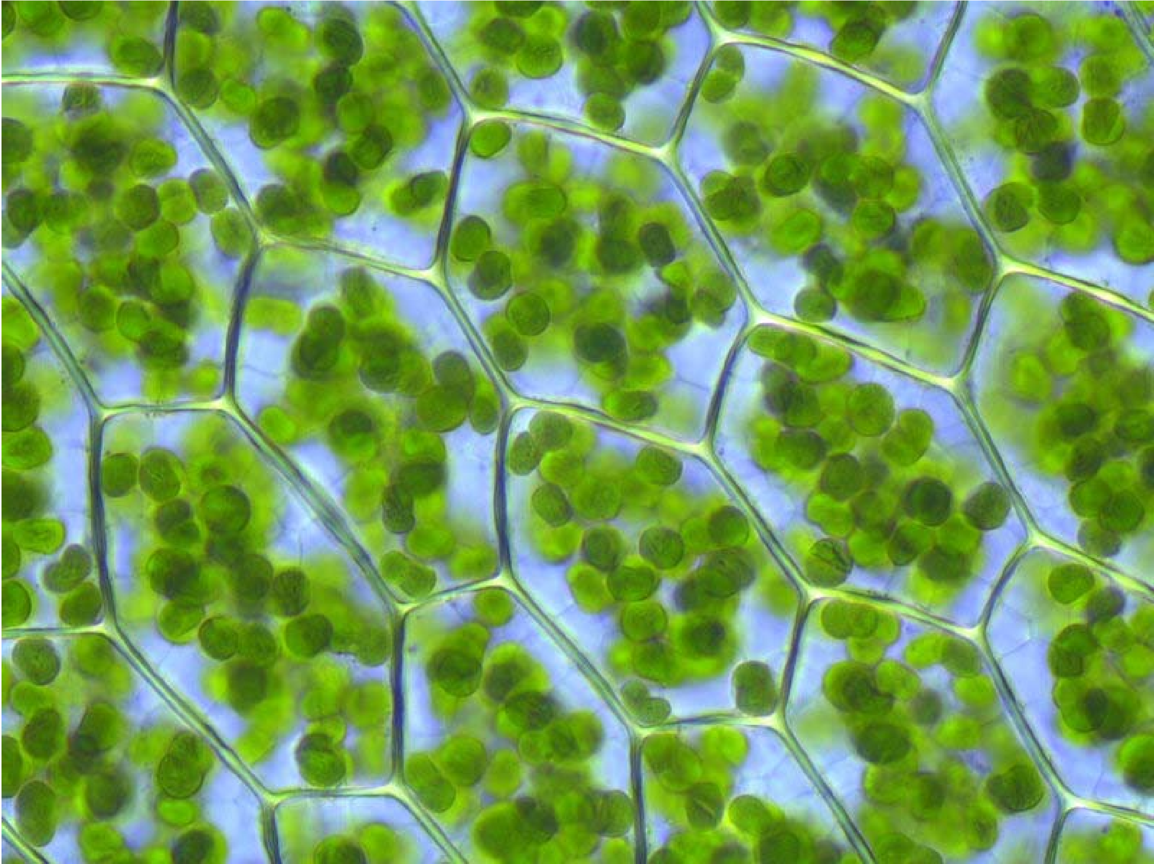


The simplified internal structure of a chloroplast

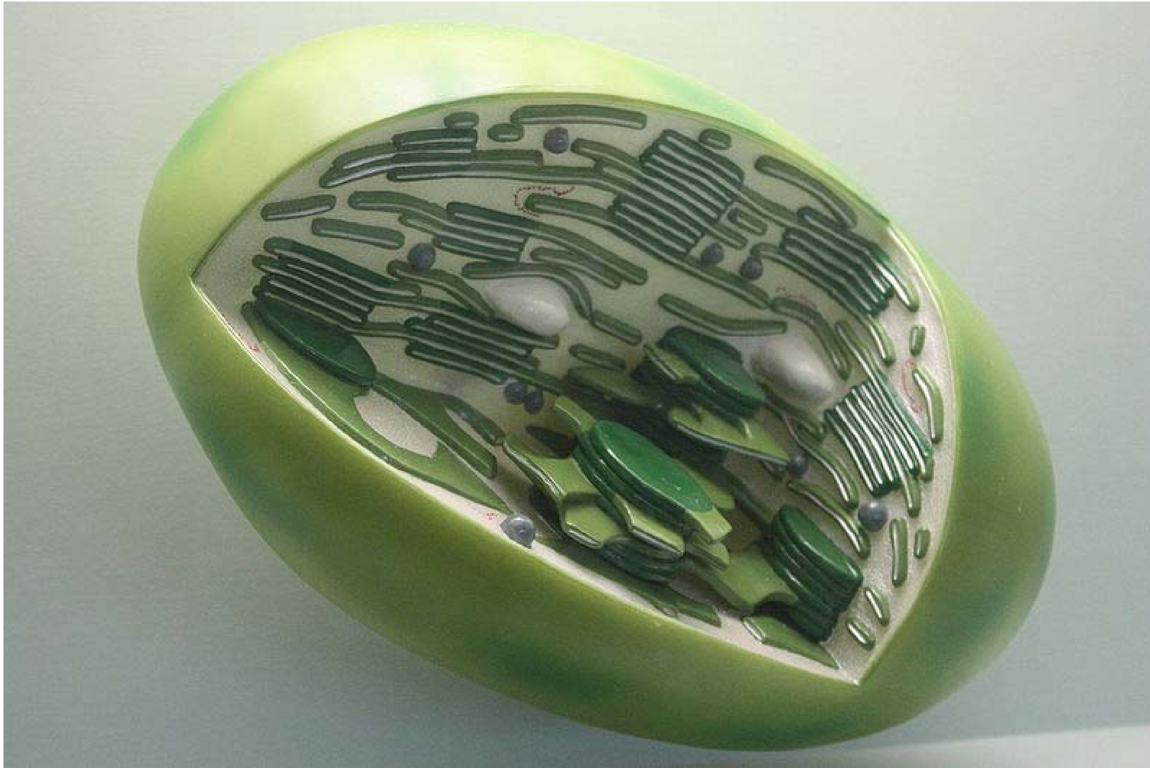
Chloroplasts are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

The word chloroplast (χλωροπλάστης) is derived from the Greek words *chloros* (χλωρός), which means green, and *plastis* (πλάστης), which means "the one who forms". Chloroplasts are members of a class of organelles known as plastids.

Evolutionary origin



Chloroplasts visible in the cells of *Plagiomnium affine* — Many-fruited Thyme-moss



A model chloroplast

Chloroplasts are one of the many different types of organelles in the plant cell. In general, they are considered to have originated from cyanobacteria through endosymbiosis. This was first suggested by Mereschkowsky in 1905 after an observation by Schimper in 1883 that chloroplasts closely resemble cyanobacteria. All chloroplasts are thought to derive directly or indirectly from a single endosymbiotic event (in the Archaeplastida), except for *Paulinella chromatophora*, which has recently acquired a photosynthetic cyanobacterial endosymbiont which is not closely related to chloroplasts of other eukaryotes. In that they derive from an endosymbiotic event, chloroplasts are similar to mitochondria, but chloroplasts are found only in plants and protista. The chloroplast is surrounded by a double-layered composite membrane with an intermembrane space; further, it has reticulations, or many infoldings, filling the inner spaces. The chloroplast has its own DNA, which codes for redox proteins involved in electron transport in photosynthesis; this is termed the plastome.

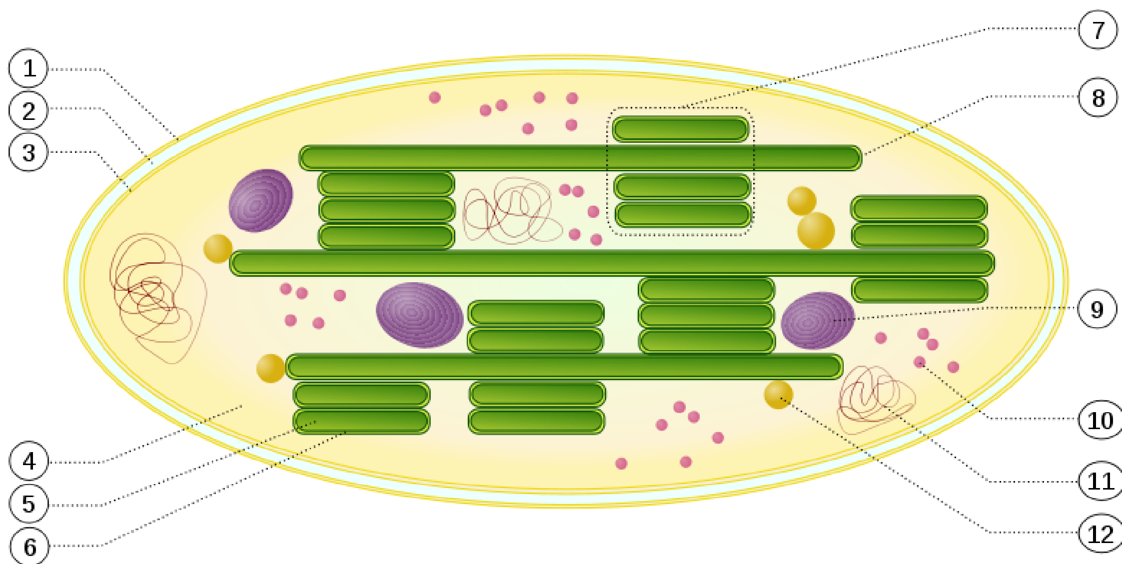
In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. They are believed to correspond to the outer and inner membranes of the ancestral cyanobacterium. Chloroplasts have their own genome, which is considerably reduced compared to that of free-living cyanobacteria, but the parts that are still present show clear similarities with the cyanobacterial genome. Plastids may contain 60-100 genes whereas cyanobacteria often contain more than 1500 genes. Many of the missing genes are encoded in the nuclear genome of the host. The transfer of nuclear information has been estimated in tobacco plants at one gene for every 16000 pollen grains.

In some algae (such as the heterokonts and other protists such as Euglenozoa and Cercozoa), chloroplasts seem to have evolved through a secondary event of endosymbiosis, in which a eukaryotic cell engulfed a second eukaryotic cell containing chloroplasts, forming chloroplasts with three or four membrane layers. In some cases, such secondary endosymbionts may have themselves been engulfed by still other eukaryotes, thus forming tertiary endosymbionts. In the alga *Chlorella*, there is only one chloroplast, which is bell-shaped.

In some groups of mixotrophic protists such as the dinoflagellates, chloroplasts are separated from a captured alga or diatom and used temporarily. These klepto chloroplasts may only have a lifetime of a few days and are then replaced.

Structure

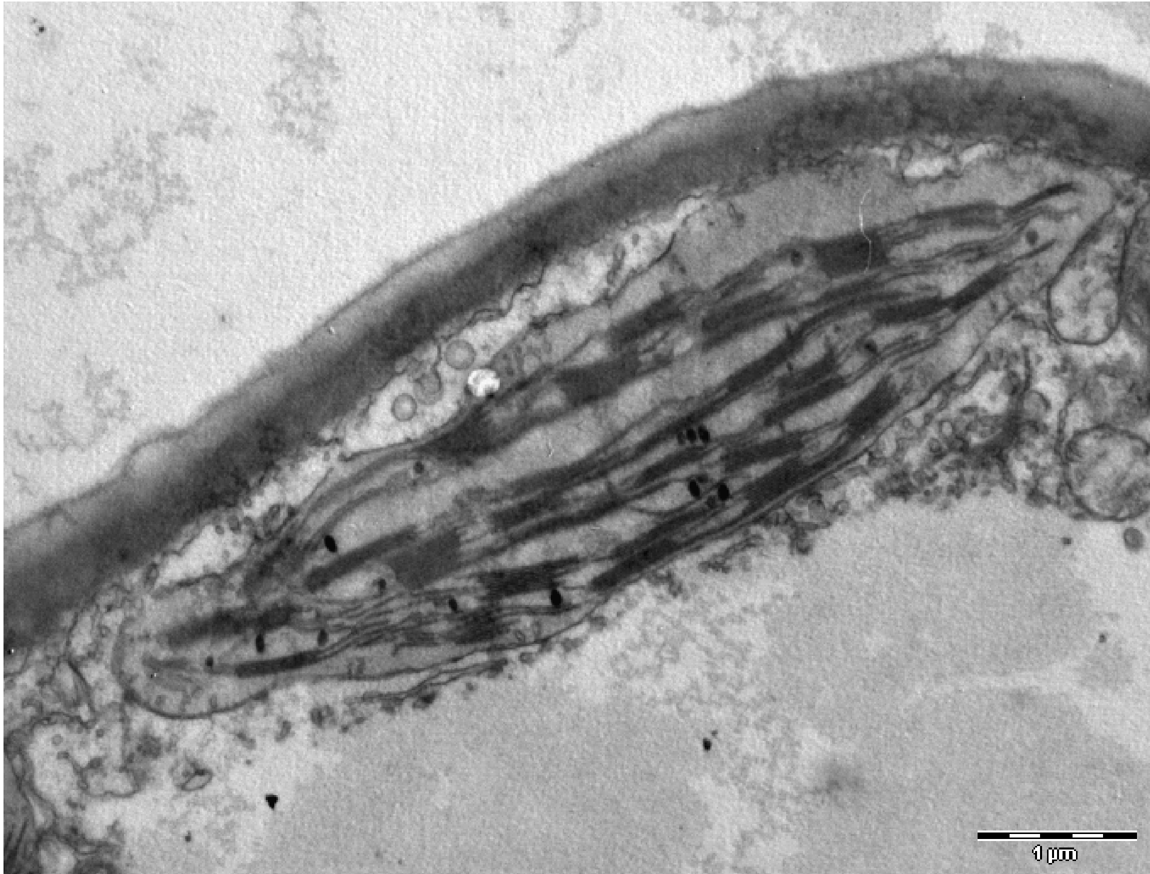
Chloroplasts are observable as flat discs usually 2 to 10 micrometers in diameter and 1 micrometer thick. In land plants, they are, in general, 5 µm in diameter and 2.3 µm thick. The chloroplast is contained by an envelope that consists of an inner and an outer phospholipid membrane. Between these two layers is the intermembrane space. A typical parenchyma cell contains about 10 to 100 chloroplasts.



Chloroplast ultrastructure:
1. outer membrane
2. intermembrane space
3. inner membrane (1+2+3: envelope)
4. stroma (aqueous fluid)
5. thylakoid lumen (inside of thylakoid)
6. thylakoid membrane
7. granum (stack of thylakoids)
8. thylakoid (lamella)

9. starch
10. ribosome
11. plastidial DNA
12. plastoglobule (drop of lipids)

The material within the chloroplast is called the stroma, corresponding to the cytosol of the original bacterium, and contains one or more molecules of small circular DNA. It also contains ribosomes; however most of its proteins are encoded by genes contained in the host cell nucleus, with the protein products transported to the chloroplast.



TEM image of a chloroplast

Within the stroma are stacks of thylakoids, the sub-organelles, which are the site of photosynthesis. The thylakoids are arranged in stacks called grana (singular: granum). A thylakoid has a flattened disk shape. Inside it is an empty area called the thylakoid space or lumen. Photosynthesis takes place on the thylakoid membrane; as in mitochondrial oxidative phosphorylation, it involves the coupling of cross-membrane fluxes with biosynthesis via the dissipation of a proton electrochemical gradient.

In the electron microscope, thylakoid membranes appear as alternating light-and-dark bands, each 0.01 μm thick. Embedded in the thylakoid membrane are antenna complexes, each of which consists of the light-absorbing pigments, including chlorophyll and

carotenoids, as well as proteins that bind the pigments. This complex both increases the surface area for light capture, and allows capture of photons with a wider range of wavelengths. The energy of the incident photons is absorbed by the pigments and funneled to the reaction centre of this complex through resonance energy transfer. Two chlorophyll molecules are then ionised, producing an excited electron, which then passes onto the photochemical reaction centre.

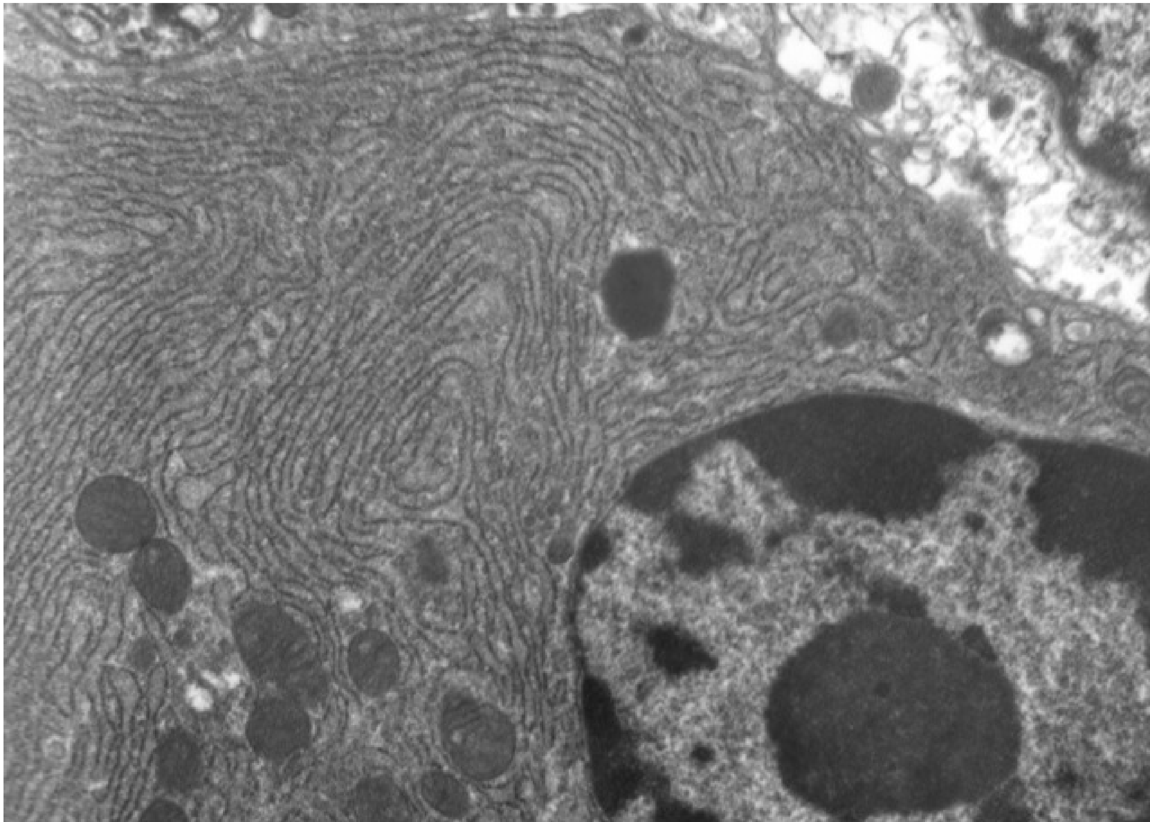
Recent studies have shown that chloroplasts can be interconnected by tubular bridges called stromules, formed as extensions of their outer membranes. Chloroplasts appear to be able to exchange proteins via stromules, and thus function as a network.

Transplastomic plants

Recently, chloroplasts have caught attention by developers of genetically modified plants. In most flowering plants, chloroplasts are not inherited from the male parent, although in plants such as pines, chloroplasts are inherited from males. Where chloroplasts are inherited only from the female, transgenes in these plastids cannot be disseminated by pollen. This makes plastid transformation a valuable tool for the creation and cultivation of genetically modified plants that are biologically contained, thus posing significantly lower environmental risks. This biological containment strategy is therefore suitable for establishing the coexistence of conventional and organic agriculture. While the reliability of this mechanism has not yet been studied for all relevant crop species, recent results in tobacco plants are promising, showing a failed containment rate of transplastomic plants at 3 in 1,000,000.

Chapter 4

Endoplasmic Reticulum

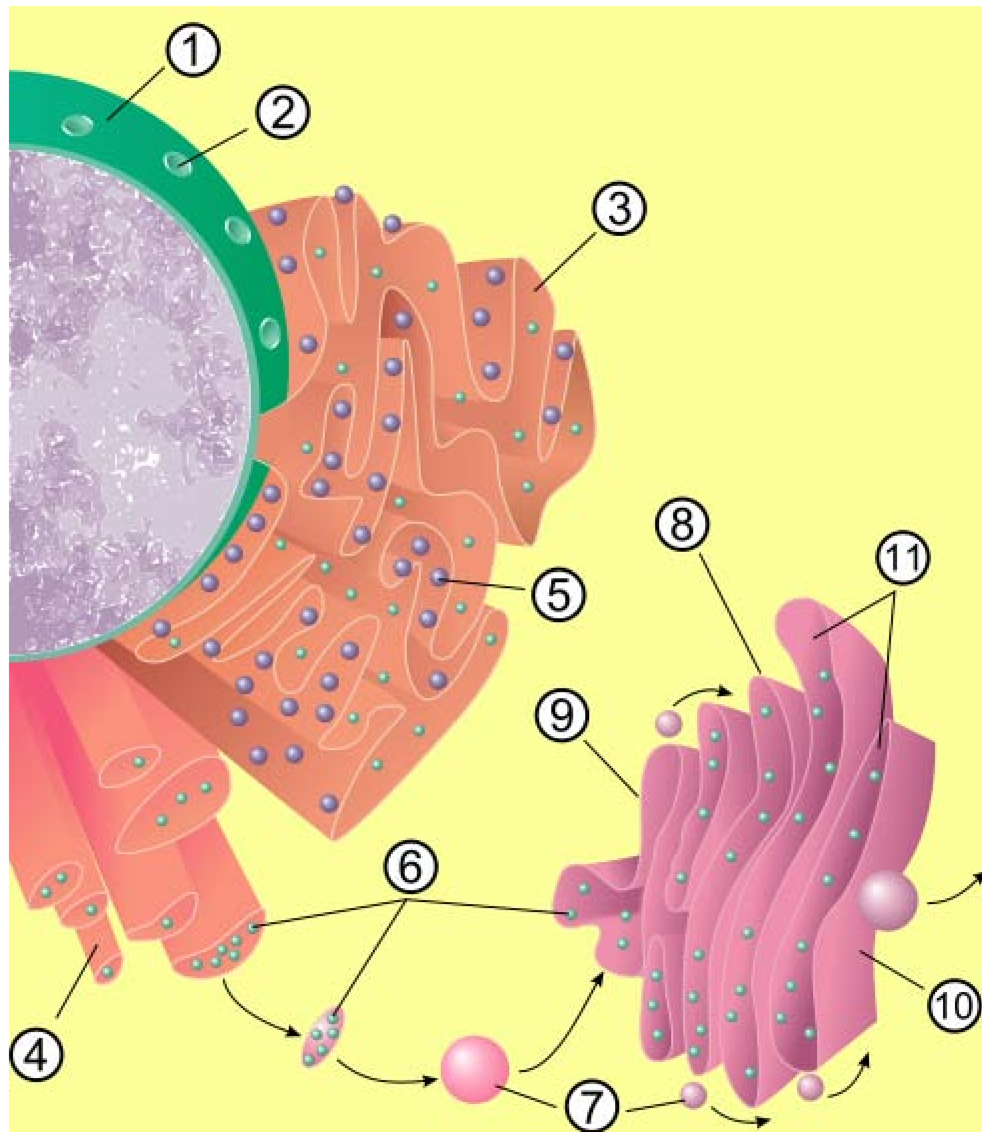


Micrograph of rough endoplasmic reticulum network around the nucleus (shown in lower right-hand side of the picture). Dark small circles in the network are mitochondria.

The **endoplasmic reticulum** (**ER**) is a eukaryotic organelle that forms an interconnected network of tubules, vesicles, and cisternae within cells. **Rough endoplasmic reticula** synthesize proteins, while **smooth endoplasmic reticula** synthesize lipids and steroids, metabolize carbohydrates and steroids, and regulate calcium concentration, drug detoxification, and attachment of receptors on cell membrane proteins. **Sarcoplasmic reticula** solely regulate calcium levels.

The lacey membranes of the endoplasmic reticulum were first seen by Keith R. Porter, Albert Claude, and Ernest F. Fullam in 1945.

Structure



1 Nucleus **2** Nuclear pore **3** Rough endoplasmic reticulum (RER) **4** Smooth endoplasmic reticulum (SER) **5** Ribosome on the rough ER **6** Proteins that are transported **7** Transport vesicle **8** Golgi apparatus **9** Cis face of the Golgi apparatus **10** Trans face of the Golgi apparatus **11** Cisternae of the Golgi apparatus

The general structure of the endoplasmic reticulum is an extensive membrane network of cisternae (sac-like structures) held together by the cytoskeleton. The phospholipid membrane encloses a space, the cisternal space (or lumen), which is continuous with the perinuclear space but separate from the cytosol. The functions of the endoplasmic reticulum vary greatly depending on the exact type of endoplasmic reticulum and the type

of cell in which it resides. The three varieties are called *rough endoplasmic reticulum*, *smooth endoplasmic reticulum* and *sarcoplasmic reticulum*.

The quantity of RER and SER in a cell can quickly interchange from one type to the other, depending on changing metabolic needs: one type will undergo numerous changes including new proteins embedded in the membranes in order to transform. Also, massive changes in the protein content can occur without any noticeable structural changes, depending on the enzymatic needs of the cell (as per the functions listed below).

Rough endoplasmic reticulum

The surface of the rough endoplasmic reticulum (RER) is studded with protein-manufacturing ribosomes giving it a "rough" appearance (hence its name). However, the ribosomes bound to the RER at any one time are not a stable part of this organelle's structure as ribosomes are constantly being bound and released from the membrane. A ribosome only binds to the ER once it begins to synthesize a protein destined for the secretory pathway. Here, a ribosome in the cytosol begins synthesizing a protein until a signal recognition particle recognizes the pre-piece of 5-15 hydrophobic amino acids preceded by a positively charged amino acid. This signal sequence allows the recognition particle to bind to the ribosome, causing the ribosome to bind to the RER and pass the new protein through the ER membrane. The pre-piece is then cleaved off within the lumen of the ER and the ribosome released back into the cytosol.

The membrane of the RER is continuous with the outer layer of the nuclear envelope. Although there is no continuous membrane between the RER and the Golgi apparatus, membrane-bound vesicles shuttle proteins between these two compartments. Vesicles are surrounded by coating proteins called COPI and COPII. COPII targets vesicles to the golgi and COPI marks them to be brought back to the RER. The RER works in concert with the Golgi complex to target new proteins to their proper destinations. A second method of transport out of the ER are areas called membrane contact sites, where the membranes of the ER and other organelles are held closely together, allowing the transfer of lipids and other small molecules.

The RER is key in multiple functions:

- lysosomal enzymes with a mannose-6-phosphate marker added in the *cis*-Golgi network
- Secreted proteins, either secreted constitutively with no tag, or regulated secretion involving clathrin and paired basic amino acids in the signal peptide.
- integral membrane proteins that stay imbedded in the membrane as vesicles exit and bind to new membranes. Rab proteins are key in targeting the membrane, SNAP and SNARE proteins are key in the fusion event.
- initial glycosylation as assembly continues. This is either N-linked (O-linking occur in the golgi).
    - N-linked glycosylation: if the protein is properly folded, glycosyltransferase recognizes the AA sequence NXS or NXT (with the

S/T residue phosphorylated) and adds a 14 sugar backbone (2 *N*-acetylglucosamine, 9 branching mannose, and 3 glucose at the end) to the side chain nitrogen of Asn.

Smooth endoplasmic reticulum

The smooth endoplasmic reticulum (SER) has functions in several metabolic processes, including synthesis of lipids and steroids, metabolism of carbohydrates, regulation of calcium concentration, drug detoxification, attachment of receptors on cell membrane proteins, and steroid metabolism. It is connected to the nuclear envelope. Smooth endoplasmic reticulum is found in a variety of cell types (both animal and plant) and it serves different functions in each. The Smooth ER also contains the enzyme glucose-6-phosphatase which converts glucose-6-phosphate to glucose, a step in gluconeogenesis. The SER consists of tubules and vesicles that branch forming a network. In some cells there are dilated areas like the sacs of RER. The network of SER allows increased surface area for the action or storage of key enzymes and the products of these enzymes.

Sarcoplasmic reticulum

The sarcoplasmic reticulum (SR), from the Greek *sarx*, ("flesh"), is a special type of smooth ER found in smooth and striated muscle. The only structural difference between this organelle and the SER is the medley of proteins they have, both bound to their membranes and drifting within the confines of their lumens. This fundamental difference is indicative of their functions: the SER synthesizes molecules while the SR stores and pumps calcium ions. The SR contains large stores of calcium, which it sequesters and then releases when the muscle cell is stimulated. The SR's release of calcium upon electrical stimulation of the cell plays a major role in excitation-contraction coupling.

Functions

The endoplasmic reticulum serves many general functions, including the facilitation of protein folding and the transport of synthesized proteins in sacs called cisternae.

Correct folding of newly-made proteins is made possible by several endoplasmic reticulum chaperone proteins, including protein disulfide isomerase (PDI), ERp29, the Hsp70 family member Grp78, calnexin, calreticulin, and the peptidylpropyl isomerase family. Only properly-folded proteins are transported from the rough ER to the Golgi complex.

Transport of proteins

Secretory proteins, mostly glycoproteins, are moved across the endoplasmic reticulum membrane. Proteins that are transported by the endoplasmic reticulum and from there throughout the cell are marked with an address tag called a signal sequence. The N-terminus (one end) of a polypeptide chain (i.e., a protein) contains a few amino acids that work as an address tag, which are removed when the polypeptide reaches its destination.

Proteins that are destined for places outside the endoplasmic reticulum are packed into transport vesicles and moved along the cytoskeleton toward their destination.

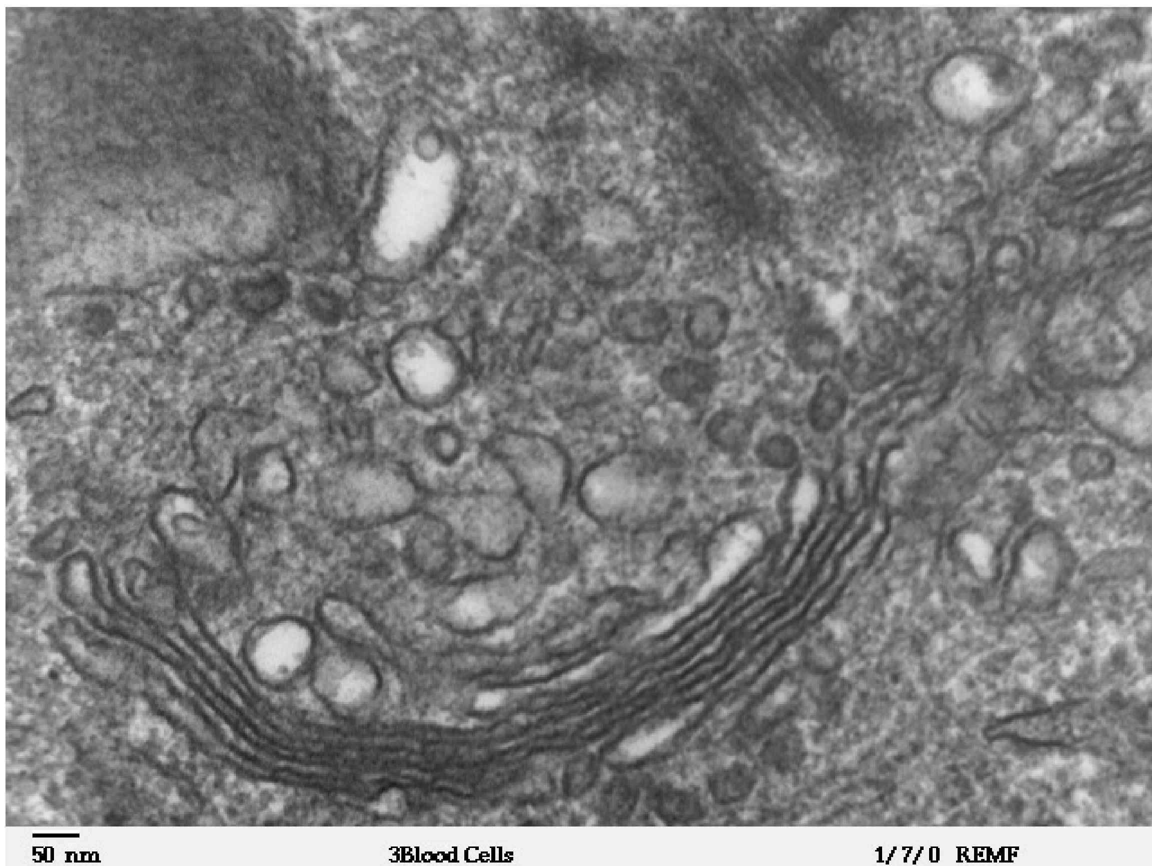
The endoplasmic reticulum is also part of a protein sorting pathway. It is, in essence, the transportation system of the eukaryotic cell. The majority of endoplasmic reticulum resident proteins are retained in the endoplasmic reticulum through a retention motif. This motif is composed of four amino acids at the end of the protein sequence. The most common retention sequence is KDEL (*lys-asp-glu-leu*). However, variation on KDEL does occur and other sequences can also give rise to endoplasmic reticulum retention. It is not known if such variation can lead to sub-endoplasmic reticulum localizations. There are three KDEL receptors in mammalian cells, and they have a very high degree of sequence identity. The functional differences between these receptors remain to be established.

Other functions

- **Insertion of proteins into the endoplasmic reticulum membrane:** Integral membrane proteins are inserted into the endoplasmic reticulum membrane as they are being synthesized (co-translational translocation). Insertion into the endoplasmic reticulum membrane requires the correct topogenic signal sequences in the protein.
- **Glycosylation**: Glycosylation involves the attachment of oligosaccharides.
- **Disulfide bond formation and rearrangement**: Disulfide bonds stabilize the tertiary and quaternary structure of many proteins.
- **Drug metabolism**: The smooth ER is the site at which some drugs are modified by microsomal enzymes which include the cytochrome P450 enzymes.

Chapter 5

Golgi Apparatus



Micrograph of Golgi apparatus, visible as a stack of semicircular black rings near the bottom. Numerous circular vesicles can be seen in proximity to the organelle

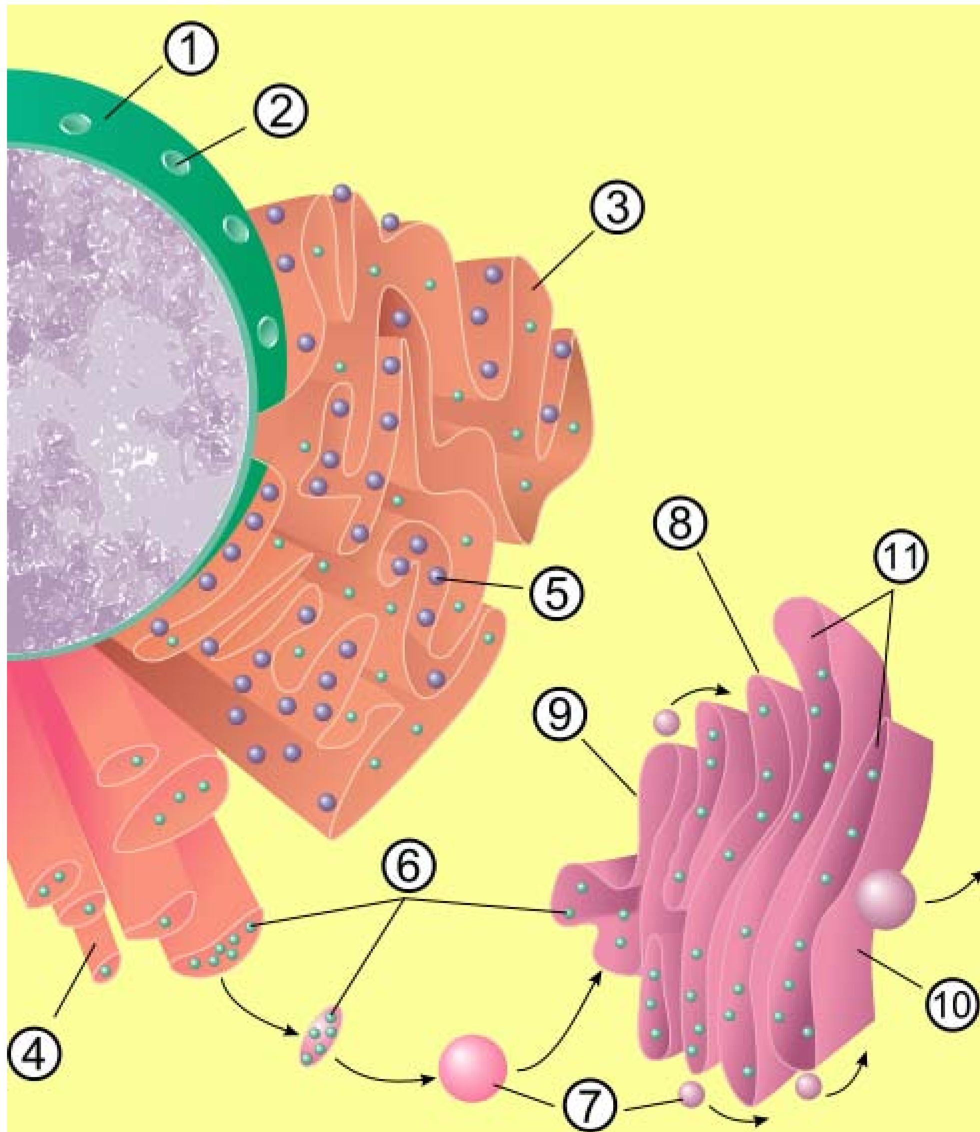


Diagram of secretory process from endoplasmic reticulum (orange) to Golgi apparatus (pink). 1. Nuclear membrane; 2. Nuclear pore; 3. Rough endoplasmic reticulum (RER); 4. Smooth endoplasmic reticulum (SER); 5. Ribosome attached to RER; 6. Macromolecules; 7. Transport vesicles; 8. Golgi apparatus; 9. *Cis* face of Golgi apparatus; 10. *Trans* face of Golgi apparatus; 11. Cisternae of lipids

The Golgi Apparatus (also **Golgi body** or the **Golgi complex**) is an organelle found in most eukaryotic cells. It was identified in 1897 by the Italian physician Camillo Golgi, after whom the Golgi apparatus is named.

It processes and packages macromolecules, such as proteins and lipids, after their synthesis and before they make their way to their destination; it is particularly important in the processing of proteins for secretion. The Golgi apparatus forms a part of the cellular endomembrane system.

Evolution

The Golgi apparatus appears to have existed even in the "ancestral eukaryote" from which all modern eukaryotes evolved, even though some no longer have it.

Discovery

Due to its fairly large size, the Golgi apparatus was one of the first organelles to be discovered and observed in detail. The apparatus was discovered in 1897 by Italian physician Camillo Golgi during an investigation of the nervous system. After first observing it under his microscope, he termed the structure the *internal reticular apparatus*. The structure was then renamed after Golgi not long after the announcement of his discovery in 1898. However, some doubted the discovery at first, arguing that the appearance of the structure was merely an optical illusion created by the observation technique used by Golgi. With the development of modern microscopes in the 20th century, the discovery was confirmed.

Structure

Found in both plant and animal cells, the Golgi is composed of stacks of membrane-bound structures known as cisternae (singular: *cisterna*). An individual stack is sometimes called a dictyosome (from Greek *dictyon*, net + *soma*, body), especially in plant cells. A mammalian cell typically contains 40 to 100 stacks. Between four and eight cisternae are usually present in a stack; however, in some protists as many as sixty have been observed. Each cisterna comprises a flat, membrane enclosed disc that includes special Golgi enzymes which modify or help to modify cargo proteins that travel through it.

The cisternae stack has four functional regions: the cis-Golgi network, medial-Golgi, endo-Golgi, and trans-Golgi network. Vesicles from the endoplasmic reticulum (via the vesicular-tubular clusters) fuse with the network and subsequently progress through the stack to the trans Golgi network, where they are packaged and sent to the required destination. Each region contains different enzymes which selectively modify the contents depending on where they reside. The cisternae also carry structural proteins important for their maintenance as flattened membranes which stack upon each other.

Function

Cells synthesize a large number of different macromolecules. The Golgi apparatus is integral in modifying, sorting, and packaging these macromolecules for cell secretion (exocytosis) or use within the cell. It primarily modifies proteins delivered from the rough endoplasmic reticulum but is also involved in the transport of lipids around the cell, and the creation of lysosomes. In this respect it can be thought of as similar to a post office; it packages and labels items which it then sends to different parts of the cell.

Enzymes within the cisternae are able to modify the proteins by addition of carbohydrates (glycosylation) and phosphates (phosphorylation). In order to do so, the Golgi imports substances such as nucleotide sugars from the cytosol. These modifications may also form a signal sequence which determines the final destination of the protein. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes.

The Golgi plays an important role in the synthesis of proteoglycans, which are molecules present in the extracellular matrix of animals. It is also a major site of carbohydrate synthesis. This includes the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans. Enzymes in the Golgi polymerize several of these GAGs via a xylose link onto the core protein. Another task of the Golgi involves the sulfation of certain molecules passing through its lumen via sulphotranferases that gain their sulphur molecule from a donor called PAPs. This process occurs on the GAGs of proteoglycans as well as on the core protein. The level of sulfation is very important to the proteoglycans' signalling abilities as well as giving the proteoglycan its overall negative charge.

The phosphorylation of molecules requires that ATP is imported into the lumen of the Golgi and then utilised by resident kinases such as casein kinase 1 and casein kinase 2. One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constitute of blood serum. It is thought that the phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

The Golgi has a putative role in apoptosis, with several Bcl-2 family members localised there, as well as to the mitochondria. A newly characterized protein, GAAP (Golgi anti-apoptotic protein), almost exclusively resides in the Golgi and protects cells from apoptosis by an as-yet undefined mechanism.

Vesicular transport

The vesicles that leave the rough endoplasmic reticulum are transported to the *cis* face of the Golgi apparatus, where they fuse with the Golgi membrane and empty their contents into the lumen. Once inside the lumen, the molecules are modified, sorted and shipped towards their final destination. The Golgi apparatus tends to be larger and more numerous in cells that synthesise and secrete large amounts of substances, for example, the plasma B cells and the antibody-secreting cells of the immune system have prominent Golgi complexes.

Those proteins destined for areas of the cell other than either the endoplasmic reticulum or Golgi apparatus are moved towards the *trans* face, to a complex network of membranes and associated vesicles known as the *trans-Golgi network* (TGN). This area of the Golgi is the point at which proteins are sorted and shipped to their intended

destinations by their placement into one of at least three different types of vesicles, depending upon the molecular marker they carry:

Type	Description	Example
Exocytotic vesicles *(continuous)*	Vesicle contains proteins destined for extracellular release. After packaging the vesicles bud off and immediately move towards the plasma membrane, where they fuse and release the contents into the extracellular space in a process known as *constitutive secretion*.	Antibody release by activated plasma B cells
Secretory vesicles *(regulated)*	Vesicle contains proteins destined for extracellular release. After packaging, the vesicles bud off and are stored in the cell until a signal is given for their release. When the appropriate signal is received they move towards the membrane and fuse to release their contents. This process is known as *regulated secretion*.	Neurotransmitter release from neurons
Lysosomal vesicles	Vesicle contains proteins destined for the lysosome, an organelle of degradation containing many acid hydrolases, or to lysosome-like storage organelles. These proteins include both digestive enzymes and membrane proteins. The vesicle first fuses with the late endosome, and the contents are then transferred to the lysosome via unknown mechanisms.	Digestive proteases destined for the lysosome

Transport mechanism

The transport mechanism which proteins use to progress through the Golgi apparatus is not yet clear; however a number of hypotheses currently exist. Until recently, the vesicular transport mechanism was favoured but now more evidence is coming to light to support cisternal maturation. The two proposed models may actually work in conjunction with each other, rather than being mutually exclusive. This is sometimes referred to as the *combined* model.

- ***Cisternal maturation model***: the cisternae of the Golgi apparatus move by being built at the *cis* face and destroyed at the *trans* face. Vesicles from the endoplasmic reticulum fuse with each other to form a cisterna at the *cis* face, consequently this cisterna would appear to move through the Golgi stack when a new cisterna is formed at the *cis* face. This model is supported by the fact that structures larger than the transport vesicles, such as collagen rods, were observed microscopically to progress through the Golgi apparatus. This was initially a popular hypothesis, but lost favour in the 1980s. Recently it has made a comeback, as laboratories at the University of Chicago and the University of Tokyo have been able to use new technology to directly observe Golgi compartments maturing. Additional evidence comes from the fact that COPI vesicles move in the retrograde direction,

transporting Endoplasmic Reticulum proteins back to where they belong by recognizing a signal peptide.

- ***Vesicular transport model***: Vesicular transport views the Golgi as a very stable organelle, divided into compartments in the cis to trans direction. Membrane bound carriers transport material between the ER and the different compartments of the Golgi. Experimental evidence includes the abundance of small vesicles (known technically as shuttle vesicles) in proximity to the Golgi apparatus. To direct the vesicles, actin filaments connect packaging proteins to the membrane to ensure that they fuse with the correct compartment.

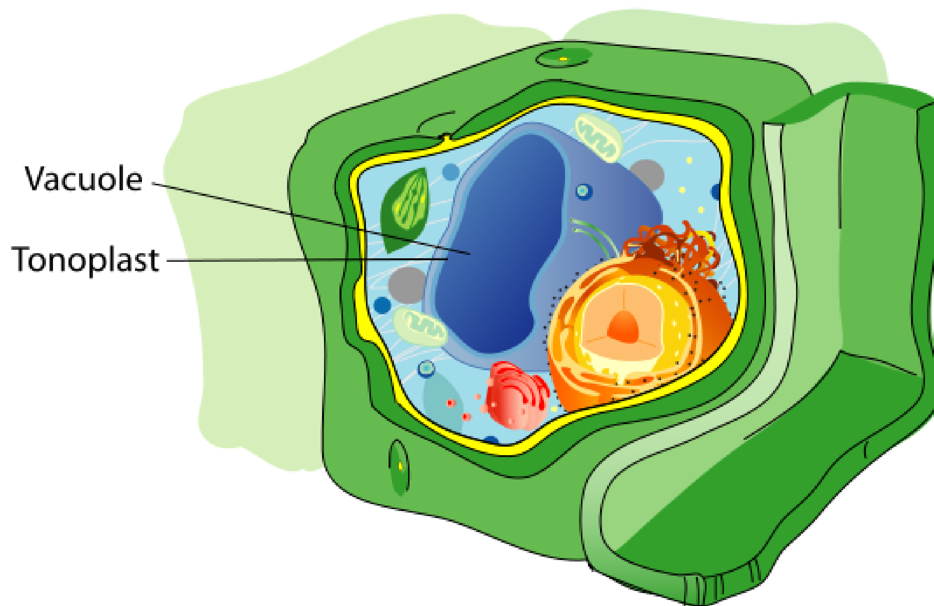
Golgi apparatus during mitosis

In animal cells, the Golgi apparatus will break up and disappear following the onset of mitosis, or cellular division. During the telophase of mitosis, the Golgi apparatus reappears; however, it is still uncertain how this occurs.

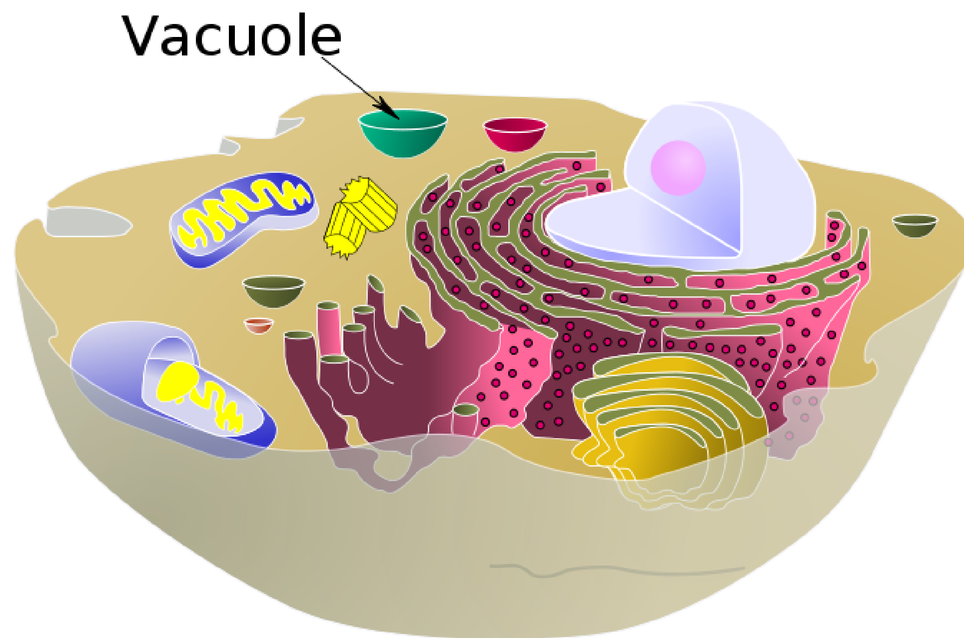
Intriguingly, the same is not true of plant or yeast Golgi stacks, which have been observed to remain intact throughout the cell cycle. The reason for this difference is not yet known, but it may, in part, be a consequence of golgin proteins.

Chapter 6

Vacuole



Plant cell structure



Animal cell structure

A **vacuole** is a membrane-bound organelle which is present in all plant and fungal cells and some protist, animal and bacterial cells. Vacuoles are essentially enclosed compartments which are filled with water containing inorganic and organic molecules including enzymes in solution, though in certain cases they may contain solids which have been engulfed. Vacuoles are formed by the fusion of multiple membrane vesicles and are effectively just larger forms of these. The organelle has no basic shape or size, its structure varies according to the needs of the cell.

The function and importance of vacuoles varies greatly according to the type of cell in which they are present, having much greater prominence in the cells of plants, fungi and certain protists than those of animals and bacteria. In general, the functions of the vacuole include:

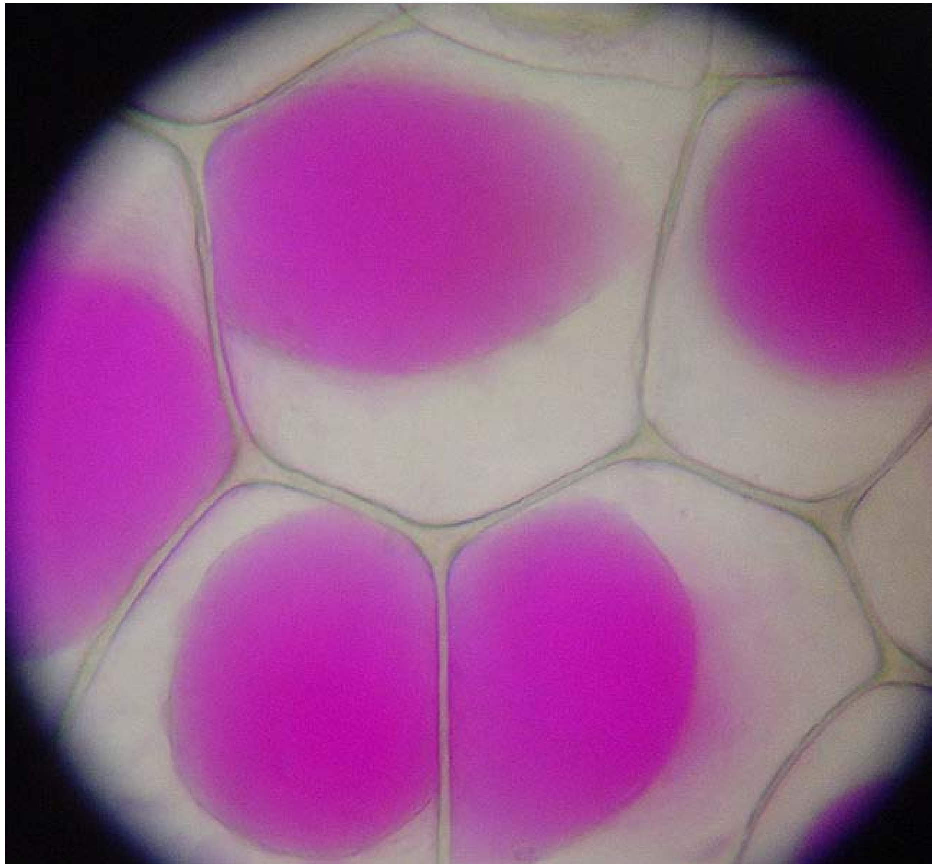
- Isolating materials that might be harmful or a threat to the cell
- Containing waste products
- Containing water in plant cells
- Maintaining internal hydrostatic pressure or turgor within the cell
- Maintaining an acidic internal pH
- Containing small molecules
- Exporting unwanted substances from the cell
- Allows plants to support structures such as leaves and flowers due to the pressure of the central vacuole
- In seeds, stored proteins needed for germination are kept in 'protein bodies', which are modified vacuoles.

Vacuoles also play a major role in autophagy, maintaining a balance between biogenesis (production) and degradation (or turnover), of many substances and cell structures in certain organisms. They also aid in the lysis and recycling of misfolded proteins that have begun to build up within the cell. Thomas Boller and others proposed that the vacuole participates in the destruction of invading bacteria and Robert B Mellor proposed organ-specific forms have a role in 'housing' symbiotic bacteria. In protists, vacuoles have the additional function of storing food which has been absorbed by the organism and assisting in the digestive and waste management process for the cell.

Bacteria

Large vacuoles are found in three genera of filamentous sulfur bacteria, the *Thioploca*, *Beggiatoa* and *Thiomargarita*. The cytosol is extremely reduced in these genera and the vacuole can occupy between 40-98% of the cell. The vacuole contains high concentrations of nitrate ions and is therefore thought to be a storage organelle.

Plants



The anthocyanin-storing vacuoles of *Rhoeo spathacea*, a spiderwort, in cells that have plasmolyzed

Most mature plant cells have one large central vacuole that typically occupies more than 30% of the cell's volume, and that can occupy as much as 80% of the volume for certain cell types and conditions. Strands of cytoplasm often run through the vacuole.

A vacuole is surrounded by a membrane called the **tonoplast** (word origin: Gk tón(os) + -o-, meaning “stretching”, “tension”, “tone” + comb. form repr. Gk plastós formed, molded). Also called the **vacuolar membrane**, the tonoplast is the cytoplasmic membrane surrounding a vacuole, separating the vacuolar contents from the cell's cytoplasm. As a membrane, it is mainly involved in regulating the movements of ions around the cell, and isolating materials that might be harmful or a threat to the cell.

Transport of protons from the cytosol to the vacuole stabilises cytoplasmic pH, while making the vacuolar interior more acidic creating a proton motive force which the cell can use to transport nutrients into or out of the vacuole. The low pH of the vacuole also allows degradative enzymes to act. Although single large central vacuoles are most common, the size and number of vacuoles may vary in different tissues and stages of development. For example, developing cells in the meristems contain small provacuoles and cells of the vascular cambium have many small vacuoles in the winter and one large one in the summer.

Aside from storage, the main role of the central vacuole is to maintain turgor pressure against the cell wall. Proteins found in the tonoplast (aquaporins) control the flow of water into and out of the vacuole through active transport, pumping potassium (K^+) ions into and out of the vacuolar interior. Due to osmosis, water will diffuse into the vacuole, placing pressure on the cell wall. If water loss leads to a significant decline in turgor pressure, the cell will plasmolyse. Turgor pressure exerted by vacuoles is also required for cellular elongation: as the cell wall is partially degraded by the action of expansins, the less rigid wall is expanded by the pressure coming from within the vacuole. Turgor pressure exerted by the vacuole is also essential in supporting plants in an upright position. Another function of a central vacuole is that it pushes all contents of the cell's cytoplasm against the cellular membrane, and thus keeps the chloroplasts closer to light.

Most plants store chemicals in the vacuole that react with chemicals in the cytosol. If the cell is broken, for example by a herbivore, then the two chemicals can react forming toxic chemicals. In garlic, alliin and the enzyme alliinase are normally separated but form allicin if the vacuole is broken. A similar reaction is responsible for the production of syn-propanethial-S-oxide when onions are cut.

Fungi

Vacuoles in fungal cells perform similar functions to those in plants and there can be more than one vacuole per cell. In yeast cells the vacuole is a dynamic structure that can rapidly modify its morphology. They are involved in many processes including the homeostasis of cell pH and the concentration of ions, osmoregulation, storing amino acids and polyphosphate and degradative processes. Toxic ions, such as strontium (Sr^{2+}),

cobalt(II) (Co^{2+}), and lead(II) (Pb^{2+}) are transported into the vacuole to isolate them from the rest of the cell.

Animals

In animal cells, vacuoles perform mostly subordinate roles, assisting in larger processes of exocytosis and endocytosis.

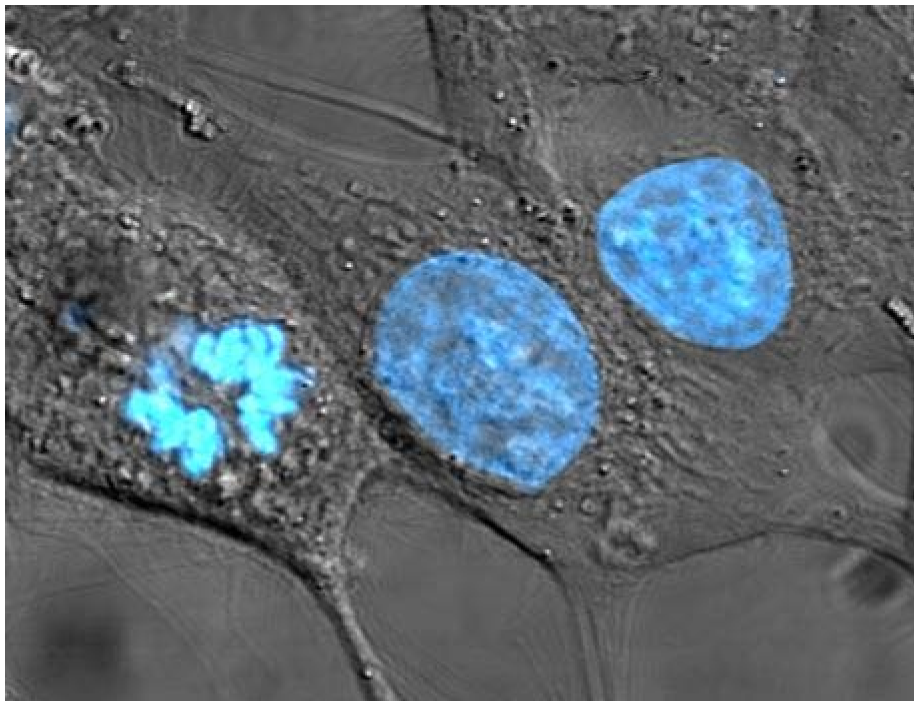
Animal vacuoles are relatively smaller that their vegetable counterparts but also usually greater in number. There are also animal cells that do not have any vacuoles.

Exocytosis is the extrusion process of proteins and lipids from the cell. These materials are absorbed into secretory granules within the Golgi apparatus before being transported to the cell membrane and secreted into the extracellular environment. In this capacity, vacuoles are simply storage vesicles which allow for the containment, transport and disposal of selected proteins and lipids to the extracellular environment.

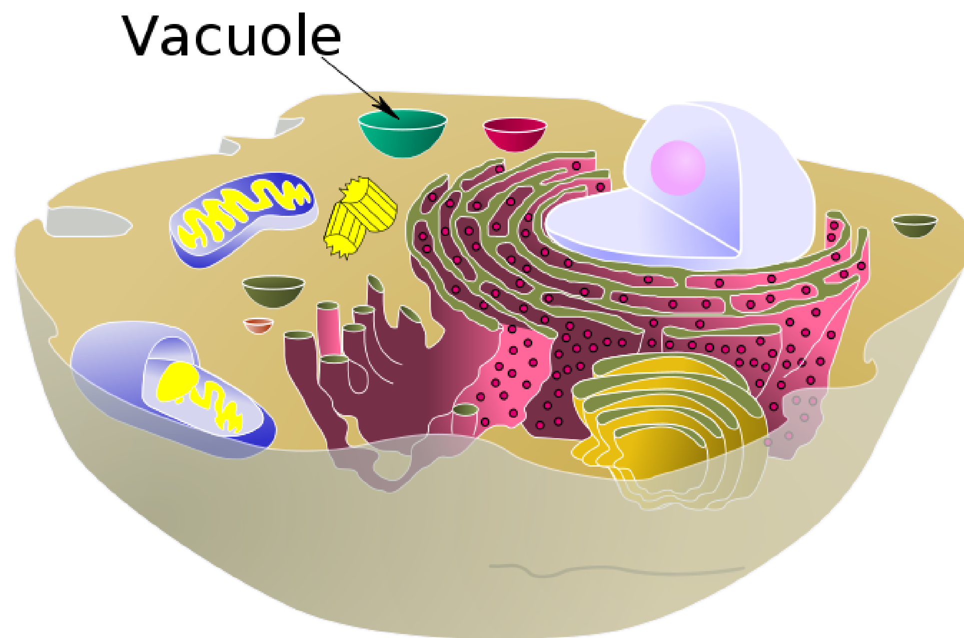
Endocytosis is the reverse of exocytosis and can occur in a variety of forms. Phagocytosis ("cell eating") is the process by which bacteria, dead tissue, or other bits of material visible under the microscope are engulfed by cells. The material makes contact with the cell membrane, which then invaginates. The invagination is pinched off, leaving the engulfed material in the membrane-enclosed vacuole and the cell membrane intact. Pinocytosis ("cell drinking") is essentially the same process, the difference being that the substances ingested are in solution and not visible under the microscope. Phagocytosis and Pinocytosis are both undertaken in association with lysosomes which complete the breakdown of the material which has been engulfed.

Chapter 7

Cell Nucleus



HeLa cells stained for DNA with the Blue Hoechst dye. The central and rightmost cell are in interphase, thus their entire nuclei are labeled. On the left, a cell is going through mitosis and its DNA has condensed ready for division.



Schematic of typical animal cell, showing subcellular components. Organelles: (1) nucleolus (2) **nucleus** (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

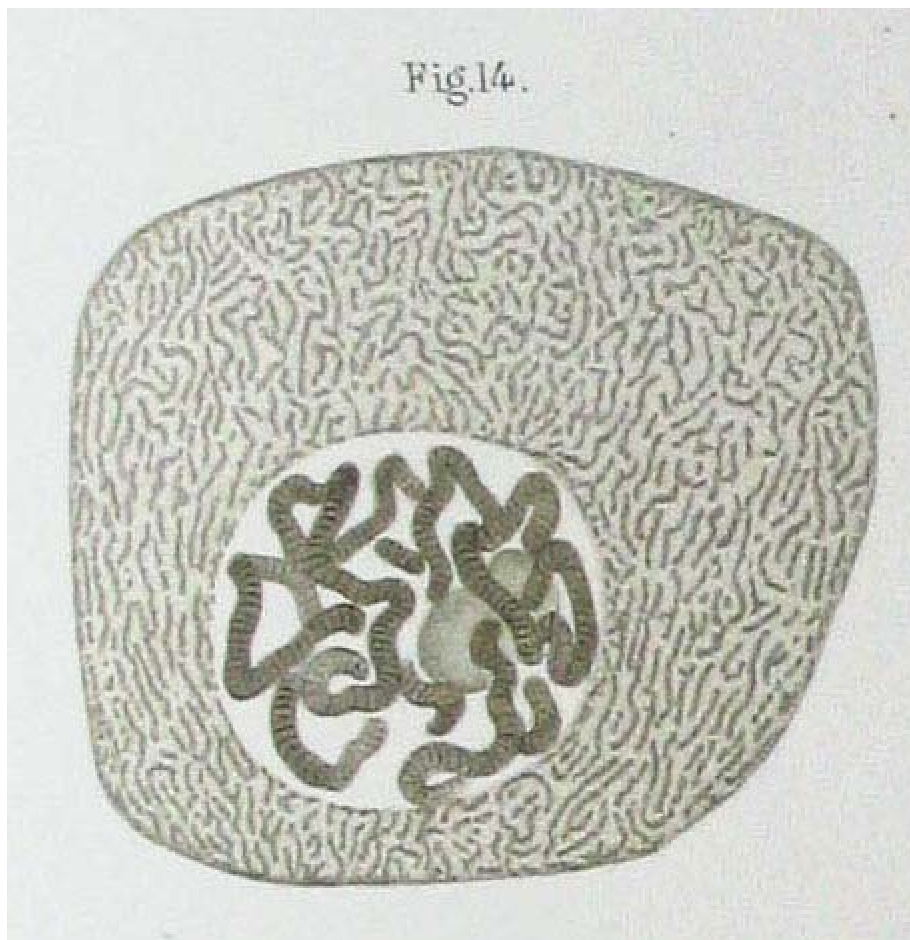
In cell biology, the **nucleus** (pl. *nuclei*; from Latin *nucleus* or *nuculeus*, meaning kernel) is a membrane-enclosed organelle found in eukaryotic cells. It contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression — the nucleus is, therefore, the control center of the cell. The main structures making up the nucleus are the nuclear envelope, a double membrane that encloses the entire organelle and separates its contents from the cellular cytoplasm, and the nuclear lamina, a meshwork within the nucleus that adds mechanical support, much like the cytoskeleton, which supports the cell as a whole. Because the nuclear membrane is impermeable to most molecules, nuclear pores are required to allow movement of molecules across the envelope. These pores cross both of the membranes, providing a channel that allows free movement of small molecules and ions. The movement of larger molecules such as proteins is carefully controlled, and requires active transport regulated by carrier proteins. Nuclear transport is crucial to cell function, as movement through the pores is required for both gene expression and chromosomal maintenance.

Although the interior of the nucleus does not contain any membrane-bound subcompartments, its contents are not uniform, and a number of *subnuclear bodies* exist, made up of unique proteins, RNA molecules, and particular parts of the chromosomes. The best-known of these is the nucleolus, which is mainly involved in the assembly of

ribosomes. After being produced in the nucleolus, ribosomes are exported to the cytoplasm where they translate mRNA.

History

Oldest known depiction of cells and their nuclei by Antonie van Leeuwenhoek, 1719.



Drawing of a *Chironomus* salivary gland cell published by Walther Flemming in 1882. The nucleus contains Polytene chromosomes.

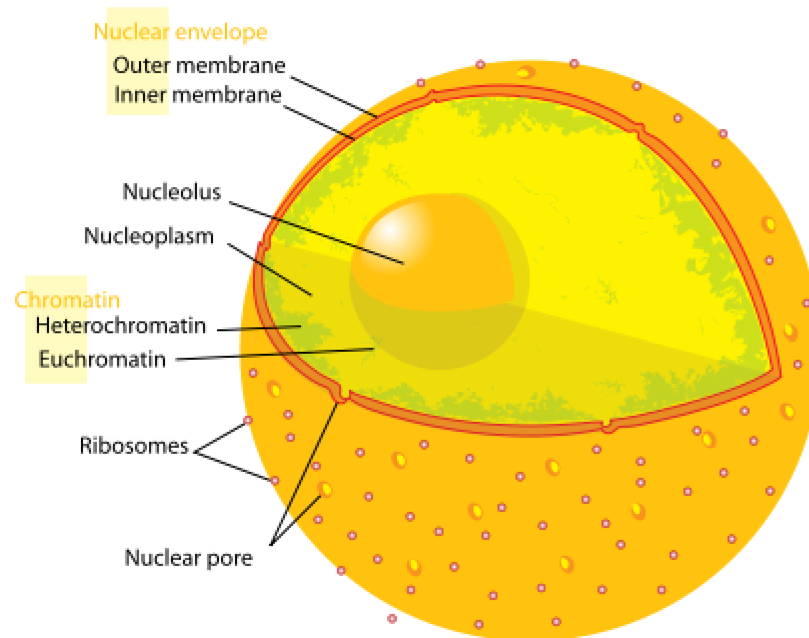
The nucleus was the first organelle to be discovered. The probably oldest preserved drawing dates back to the early microscopist Antonie van Leeuwenhoek (1632 – 1723). He observed a "Lumen", the nucleus, in the red blood cells of salmon. Unlike mammalian red blood cells, those of other vertebrates still possess nuclei. The nucleus was also described by Franz Bauer in 1804 and in more detail in 1831 by Scottish botanist Robert Brown in a talk at the Linnean Society of London. Brown was studying orchids under microscope when he observed an opaque area, which he called the areola or nucleus, in the cells of the flower's outer layer. He did not suggest a potential function. In 1838, Matthias Schleiden proposed that the nucleus plays a role in generating cells, thus he introduced the name "Cytoblast" (cell builder). He believed that he had observed new cells assembling around "cytoblasts". Franz Meyen was a strong opponent of this view, having already described cells multiplying by division and believing that many cells would have no nuclei. The idea that cells can be generated de novo, by the "cytoblast" or otherwise, contradicted work by Robert Remak (1852) and Rudolf Virchow (1855) who decisively propagated the new paradigm that cells are generated solely by cells ("Omnis cellula e cellula"). The function of the nucleus remained unclear.

Between 1876 and 1878, Oscar Hertwig published several studies on the fertilization of sea urchin eggs, showing that the nucleus of the sperm enters the oocyte and fuses with its nucleus. This was the first time it was suggested that an individual develops from a (single) nucleated cell. This was in contradiction to Ernst Haeckel's theory that the complete phylogeny of a species would be repeated during embryonic development, including generation of the first nucleated cell from a "Monerula", a structureless mass of primordial mucus ("Urschleim"). Therefore, the necessity of the sperm nucleus for fertilization was discussed for quite some time. However, Hertwig confirmed his observation in other animal groups, e.g., amphibians and molluscs. Eduard Strasburger produced the same results for plants (1884). This paved the way to assign the nucleus an important role in heredity. In 1873, August Weismann postulated the equivalence of the maternal and paternal germ *cells* for heredity. The function of the nucleus as carrier of genetic information became clear only later, after mitosis was discovered and the Mendelian rules were rediscovered at the beginning of the 20th century; the chromosome theory of heredity was developed.

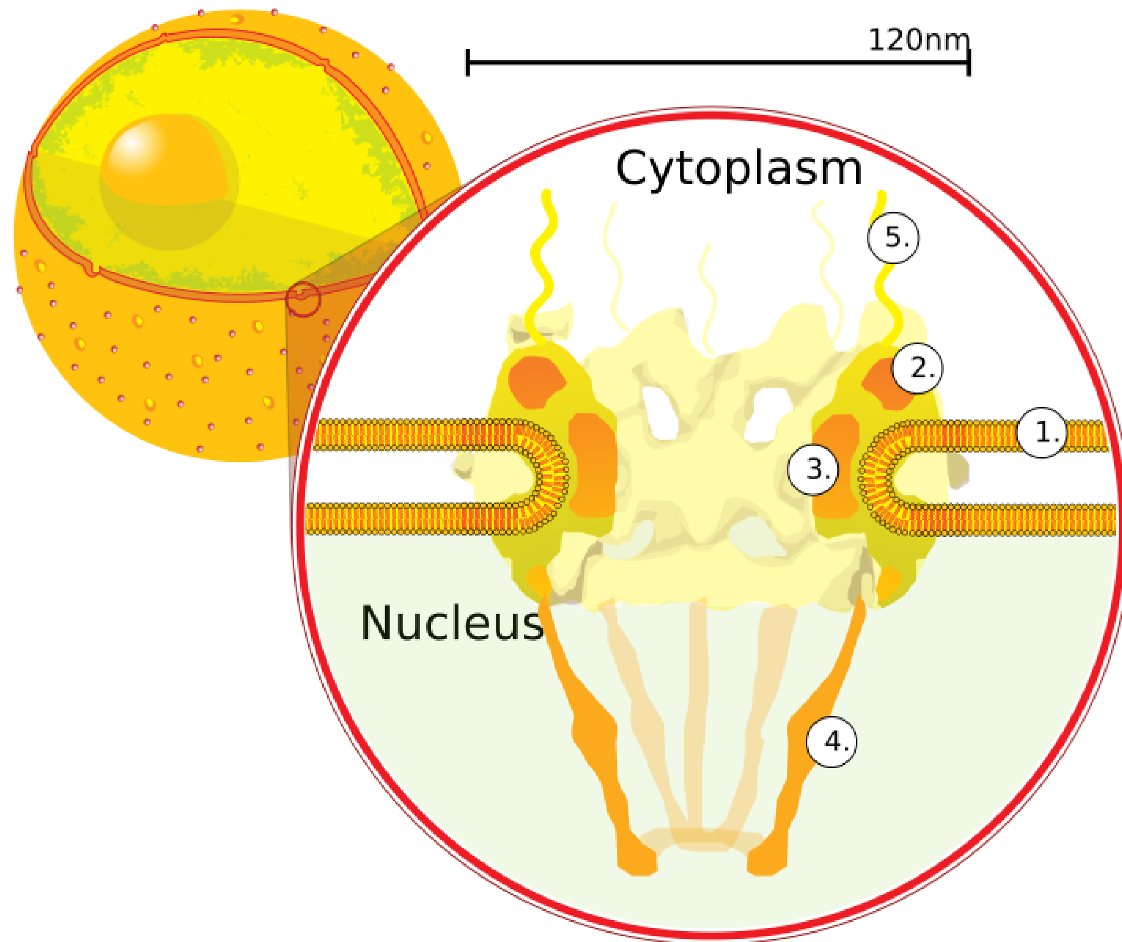
Structures

The nucleus is the largest cellular organelle in animals. In mammalian cells, the average diameter of the nucleus is approximately 6 micrometers (μm), which occupies about 10% of the total cell volume. The viscous liquid within it is called nucleoplasm, and is similar in composition to the cytosol found outside the nucleus. It appears as a dense, roughly spherical organelle.

Nuclear envelope and pores



The eukaryotic cell nucleus. Visible in this diagram are the ribosome-studded double membranes of the nuclear envelope, the DNA (complexed as chromatin), and the nucleolus. Within the cell nucleus is a viscous liquid called nucleoplasm, similar to the cytoplasm found outside the nucleus.



A cross section of a nuclear pore on the surface of the nuclear envelope (1). Other diagram labels show (2) the outer ring, (3) spokes, (4) basket, and (5) filaments.

The nuclear envelope otherwise known as nuclear membrane consists of two cellular membranes, an inner and an outer membrane, arranged parallel to one another and separated by 10 to 50 nanometers (nm). The nuclear envelope completely encloses the nucleus and separates the cell's genetic material from the surrounding cytoplasm, serving as a barrier to prevent macromolecules from diffusing freely between the nucleoplasm and the cytoplasm. The outer nuclear membrane is continuous with the membrane of the rough endoplasmic reticulum (RER), and is similarly studded with ribosomes. The space between the membranes is called the perinuclear space and is continuous with the RER lumen.

Nuclear pores, which provide aqueous channels through the envelope, are composed of multiple proteins, collectively referred to as nucleoporins. The pores are about 125 million daltons in molecular weight and consist of around 50 (in yeast) to 100 proteins (in vertebrates). The pores are 100& nbsp;nm in total diameter; however, the gap through which molecules freely diffuse is only about 9 nm wide, due to the presence of regulatory systems within the center of the pore. This size allows the free passage of small water-soluble molecules while preventing larger molecules, such as nucleic acids and larger

proteins, from inappropriately entering or exiting the nucleus. These large molecules must be actively transported into the nucleus instead. The nucleus of a typical mammalian cell will have about 3000 to 4000 pores throughout its envelope, each of which contains a donut-shaped, eightfold-symmetric ring-shaped structure at a position where the inner and outer membranes fuse. Attached to the ring is a structure called the *nuclear basket* that extends into the nucleoplasm, and a series of filamentous extensions that reach into the cytoplasm. Both structures serve to mediate binding to nuclear transport proteins.

Most proteins, ribosomal subunits, and some RNAs are transported through the pore complexes in a process mediated by a family of transport factors known as karyopherins. Those karyopherins that mediate movement into the nucleus are also called importins, whereas those that mediate movement out of the nucleus are called exportins. Most karyopherins interact directly with their cargo, although some use adaptor proteins. Steroid hormones such as cortisol and aldosterone, as well as other small lipid-soluble molecules involved in intercellular signaling, can diffuse through the cell membrane and into the cytoplasm, where they bind nuclear receptor proteins that are trafficked into the nucleus. There they serve as transcription factors when bound to their ligand; in the absence of ligand, many such receptors function as histone deacetylases that repress gene expression.

Nuclear lamina

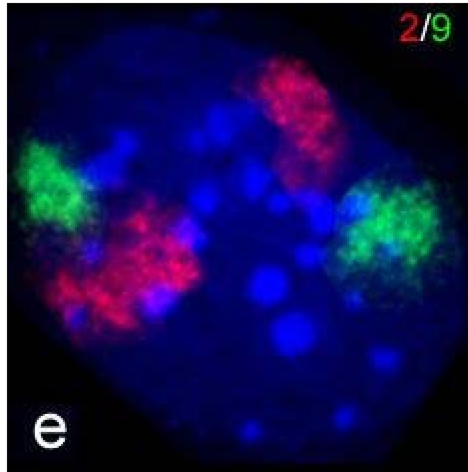
In animal cells, two networks of intermediate filaments provide the nucleus with mechanical support: The nuclear lamina forms an organized meshwork on the internal face of the envelope, while less organized support is provided on the cytosolic face of the envelope. Both systems provide structural support for the nuclear envelope and anchoring sites for chromosomes and nuclear pores.

The nuclear lamina is composed mostly of lamin proteins. Like all proteins, lamins are synthesized in the cytoplasm and later transported into the nucleus interior, where they are assembled before being incorporated into the existing network of nuclear lamina. Lamins are also found inside the nucleoplasm where they form another regular structure, known as the *nucleoplasmic veil*, that is visible using fluorescence microscopy. The actual function of the veil is not clear, although it is excluded from the nucleolus and is present during interphase. The lamin structures that make up the veil bind chromatin and disrupting their structure inhibits transcription of protein-coding genes.

Like the components of other intermediate filaments, the lamin monomer contains an alpha-helical domain used by two monomers to coil around each other, forming a dimer structure called a coiled coil. Two of these dimer structures then join side by side, in an antiparallel arrangement, to form a tetramer called a *protofilament*. Eight of these protofilaments form a lateral arrangement that is twisted to form a ropelike *filament*. These filaments can be assembled or disassembled in a dynamic manner, meaning that changes in the length of the filament depend on the competing rates of filament addition and removal.

Mutations in lamin genes leading to defects in filament assembly are known as *laminopathies*. The most notable laminopathy is the family of diseases known as progeria, which causes the appearance of premature aging in its sufferers. The exact mechanism by which the associated biochemical changes give rise to the aged phenotype is not well understood.

Chromosomes



A mouse fibroblast nucleus in which DNA is stained blue. The distinct chromosome territories of chromosome 2 (red) and chromosome 9 (green) are visible stained with fluorescent in situ hybridization.

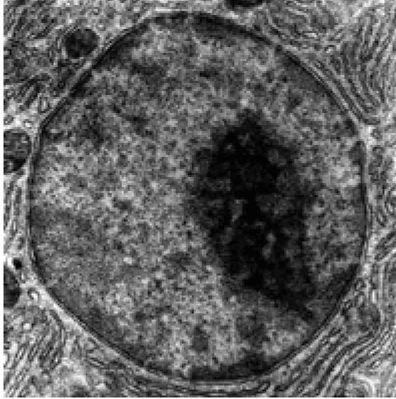
The cell nucleus contains the majority of the cell's genetic material, in the form of multiple linear DNA molecules organized into structures called chromosomes. During most of the cell cycle these are organized in a DNA-protein complex known as chromatin, and during cell division the chromatin can be seen to form the well-defined chromosomes familiar from a karyotype. A small fraction of the cell's genes are located instead in the mitochondria.

There are two types of chromatin. Euchromatin is the less compact DNA form, and contains genes that are frequently expressed by the cell. The other type, heterochromatin, is the more compact form, and contains DNA that are infrequently transcribed. This structure is further categorized into *facultative* heterochromatin, consisting of genes that are organized as heterochromatin only in certain cell types or at certain stages of development, and *constitutive* heterochromatin that consists of chromosome structural components such as telomeres and centromeres. During interphase the chromatin organizes itself into discrete individual patches, called *chromosome territories*. Active genes, which are generally found in the euchromatic region of the chromosome, tend to be located towards the chromosome's territory boundary.

Antibodies to certain types of chromatin organization, in particular, nucleosomes, have been associated with a number of autoimmune diseases, such as systemic lupus erythematosus. These are known as anti-nuclear antibodies (ANA) and have also been

observed in concert with multiple sclerosis as part of general immune system dysfunction. As in the case of progeria, the role played by the antibodies in inducing the symptoms of autoimmune diseases is not obvious.

Nucleolus



An electron micrograph of a cell nucleus, showing the darkly stained nucleolus.

The nucleolus is a discrete densely stained structure found in the nucleus. It is not surrounded by a membrane, and is sometimes called a *suborganelle*. It forms around tandem repeats of rDNA, DNA coding for ribosomal RNA (rRNA). These regions are called nucleolar organizer regions (NOR). The main roles of the nucleolus are to synthesize rRNA and assemble ribosomes. The structural cohesion of the nucleolus depends on its activity, as ribosomal assembly in the nucleolus results in the transient association of nucleolar components, facilitating further ribosomal assembly, and hence further association. This model is supported by observations that inactivation of rDNA results in intermingling of nucleolar structures.

The first step in ribosomal assembly is transcription of the rDNA, by a protein called RNA polymerase I, forming a large pre-rRNA precursor. This is cleaved into the subunits 5.8S, 18S, and 28S rRNA. The transcription, post-transcriptional processing, and assembly of rRNA occurs in the nucleolus, aided by small nucleolar RNA (snoRNA) molecules, some of which are derived from spliced introns from messenger RNAs encoding genes related to ribosomal function. The assembled ribosomal subunits are the largest structures passed through the nuclear pores.

When observed under the electron microscope, the nucleolus can be seen to consist of three distinguishable regions: the innermost *fibrillar centers* (FCs), surrounded by the *dense fibrillar component* (DFC), which in turn is bordered by the *granular component* (GC). Transcription of the rDNA occurs either in the FC or at the FC-DFC boundary, and, therefore, when rDNA transcription in the cell is increased, more FCs are detected. Most of the cleavage and modification of rRNAs occurs in the DFC, while the latter steps involving protein assembly onto the ribosomal subunits occur in the GC.

Other subnuclear bodies

Subnuclear structure sizes	
Structure name	**Structure diameter**
Cajal bodies	0.2–2.0 µm
PIKA	5 µm
PML bodies	0.2–1.0 µm
Paraspeckles	0.2–1.0 µm
Speckles	20–25 nm

Besides the nucleolus, the nucleus contains a number of other non-membrane-delineated bodies. These include Cajal bodies, Gemini of coiled bodies, polymorphic interphase karyosomal association (PIKA), promyelocytic leukaemia (PML) bodies, paraspeckles, and splicing speckles. Although little is known about a number of these domains, they are significant in that they show that the nucleoplasm is not uniform mixture, but rather contains organized functional subdomains.

Other subnuclear structures appear as part of abnormal disease processes. For example, the presence of small intranuclear rods has been reported in some cases of nemaline myopathy. This condition typically results from mutations in actin, and the rods themselves consist of mutant actin as well as other cytoskeletal proteins.

Cajal bodies and gems

A nucleus typically contains between 1 and 10 compact structures called Cajal bodies or coiled bodies (CB), whose diameter measures between 0.2 µm and 2.0 µm depending on the cell type and species. When seen under an electron microscope, they resemble balls of tangled thread and are dense foci of distribution for the protein coilin. CBs are involved in a number of different roles relating to RNA processing, specifically small nucleolar RNA (snoRNA) and small nuclear RNA (snRNA) maturation, and histone mRNA modification.

Similar to Cajal bodies are Gemini of coiled bodies, or gems, whose name is derived from the Gemini constellation in reference to their close "twin" relationship with CBs. Gems are similar in size and shape to CBs, and in fact are virtually indistinguishable under the microscope. Unlike CBs, gems do not contain small nuclear ribonucleoproteins (snRNPs), but do contain a protein called *survivor of motor neurons* (SMN) whose function relates to snRNP biogenesis. Gems are believed to assist CBs in snRNP biogenesis, though it has also been suggested from microscopy evidence that CBs and gems are different manifestations of the same structure.

RAFA and PTF domains

RAFA domains, or polymorphic interphase karyosomal associations, were first described in microscopy studies in 1991. Their function was and remains unclear, though they were not thought to be associated with active DNA replication, transcription, or RNA processing. They have been found to often associate with discrete domains defined by dense localization of the transcription factor PTF, which promotes transcription of snRNA.

PML bodies

Promyelocytic leukaemia bodies (PML bodies) are spherical bodies found scattered throughout the nucleoplasm, measuring around 0.2–1.0 µm. They are known by a number of other names, including nuclear domain 10 (ND10), Kremer bodies, and PML oncogenic domains. They are often seen in the nucleus in association with Cajal bodies and cleavage bodies. It has been suggested that they play a role in regulating transcription.

Paraspeckles

Discovered by Fox et al. in 2002, paraspeckles are irregularly shaped compartments in the nucleus' interchromatin space. First documented in HeLa cells, where there are generally 10–30 per nucleus, paraspeckles are now known to also exist in all human primary cells, transformed cell lines, and tissue sections. Their name is derived from their distribution in the nucleus; the "para" is short for parallel and the "speckles" refers to the splicing speckles to which they are always in close proximity.

Paraspeckles are dynamic structures that are altered in response to changes in cellular metabolic activity. They are transcription dependent and in the absence of RNA Pol II transcription, the paraspeckle disappears and all of its associated protein components (PSP1, p54nrb, PSP2, CFI(m)68, and PSF) form a crescent shaped perinucleolar cap in the nucleolus. This phenomenon is demonstrated during the cell cycle. In the cell cycle, paraspeckles are present during interphase and during all of mitosis except for telophase. During telophase, when the two daughter nuclei are formed, there is no RNA Pol II transcription so the protein components instead form a perinucleolar cap.

Splicing speckles

Speckles are subnuclear structures that are enriched in pre-messenger RNA splicing factors and are located in the interchromatin regions of the nucleoplasm of mammalian cells. At the fluorescence-microscope level they appear as irregular, punctate structures, which vary in size and shape, and when examined by electron microscopy they are seen as clusters of interchromatin granules. Speckles are dynamic structures, and both their protein and RNA-protein components can cycle continuously between speckles and other nuclear locations, including active transcription sites. Studies on the composition, structure and behaviour of speckles have provided a model for understanding the

functional compartmentalization of the nucleus and the organization of the gene-expression machinery.

Sometimes referred to as *interchromatin granule clusters* or as *splicing-factor compartments*, speckles are rich in splicing snRNPs and other splicing proteins necessary for pre-mRNA processing. Because of a cell's changing requirements, the composition and location of these bodies changes according to mRNA transcription and regulation via phosphorylation of specific proteins.

Function

The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle. The nucleus provides a site for genetic transcription that is segregated from the location of translation in the cytoplasm, allowing levels of gene regulation that are not available to prokaryotes.

Cell compartmentalization

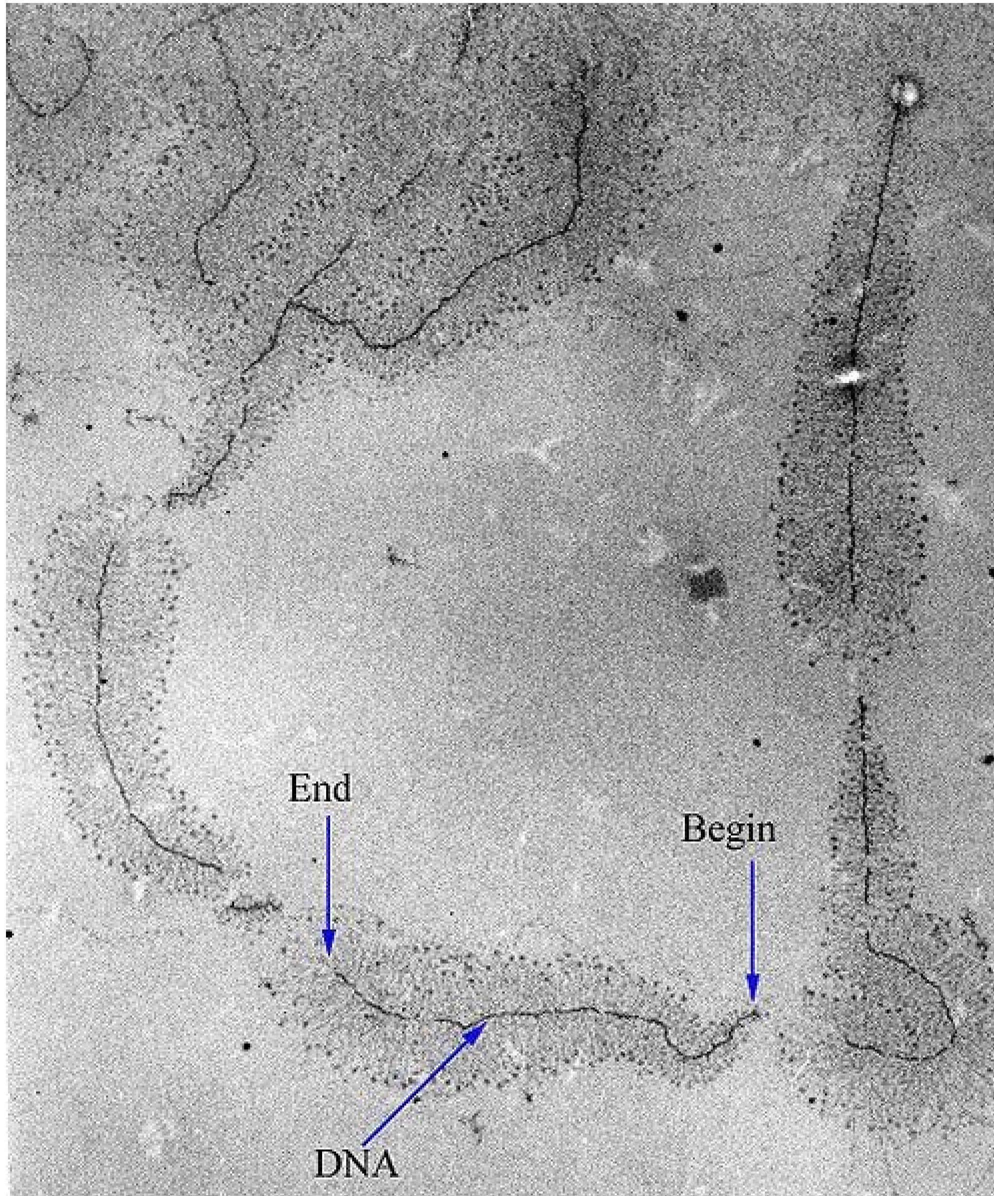
The nuclear envelope allows the nucleus to control its contents, and separate them from the rest of the cytoplasm where necessary. This is important for controlling processes on either side of the nuclear membrane. In some cases where a cytoplasmic process needs to be restricted, a key participant is removed to the nucleus, where it interacts with transcription factors to downregulate the production of certain enzymes in the pathway. This regulatory mechanism occurs in the case of glycolysis, a cellular pathway for breaking down glucose to produce energy. Hexokinase is an enzyme responsible for the first the step of glycolysis, forming glucose-6-phosphate from glucose. At high concentrations of fructose-6-phosphate, a molecule made later from glucose-6-phosphate, a regulator protein removes hexokinase to the nucleus, where it forms a transcriptional repressor complex with nuclear proteins to reduce the expression of genes involved in glycolysis.

In order to control which genes are being transcribed, the cell separates some transcription factor proteins responsible for regulating gene expression from physical access to the DNA until they are activated by other signaling pathways. This prevents even low levels of inappropriate gene expression. For example, in the case of NF-κB-controlled genes, which are involved in most inflammatory responses, transcription is induced in response to a signal pathway such as that initiated by the signaling molecule TNF-α, binds to a cell membrane receptor, resulting in the recruitment of signalling proteins, and eventually activating the transcription factor NF-κB. A nuclear localisation signal on the NF-κB protein allows it to be transported through the nuclear pore and into the nucleus, where it stimulates the transcription of the target genes.

The compartmentalization allows the cell to prevent translation of unspliced mRNA. Eukaryotic mRNA contains introns that must be removed before being translated to produce functional proteins. The splicing is done inside the nucleus before the mRNA can be accessed by ribosomes for translation. Without the nucleus, ribosomes would

translate newly transcribed (unprocessed) mRNA, resulting in misformed and nonfunctional proteins.

Gene expression



A micrograph of ongoing gene transcription of ribosomal RNA illustrating the growing primary transcripts. "Begin" indicates the 3' end of the DNA, where new RNA synthesis begins; "end" indicates the 5' end, where the primary transcripts are almost complete.

Gene expression first involves transcription, in which DNA is used as a template to produce RNA. In the case of genes encoding proteins, that RNA produced from this process is messenger RNA (mRNA), which then needs to be translated by ribosomes to form a protein. As ribosomes are located outside the nucleus, mRNA produced needs to be exported.

Since the nucleus is the site of transcription, it also contains a variety of proteins that either directly mediate transcription or are involved in regulating the process. These proteins include helicases, which unwind the double-stranded DNA molecule to facilitate access to it, RNA polymerases, which synthesize the growing RNA molecule, topoisomerases, which change the amount of supercoiling in DNA, helping it wind and unwind, as well as a large variety of transcription factors that regulate expression.

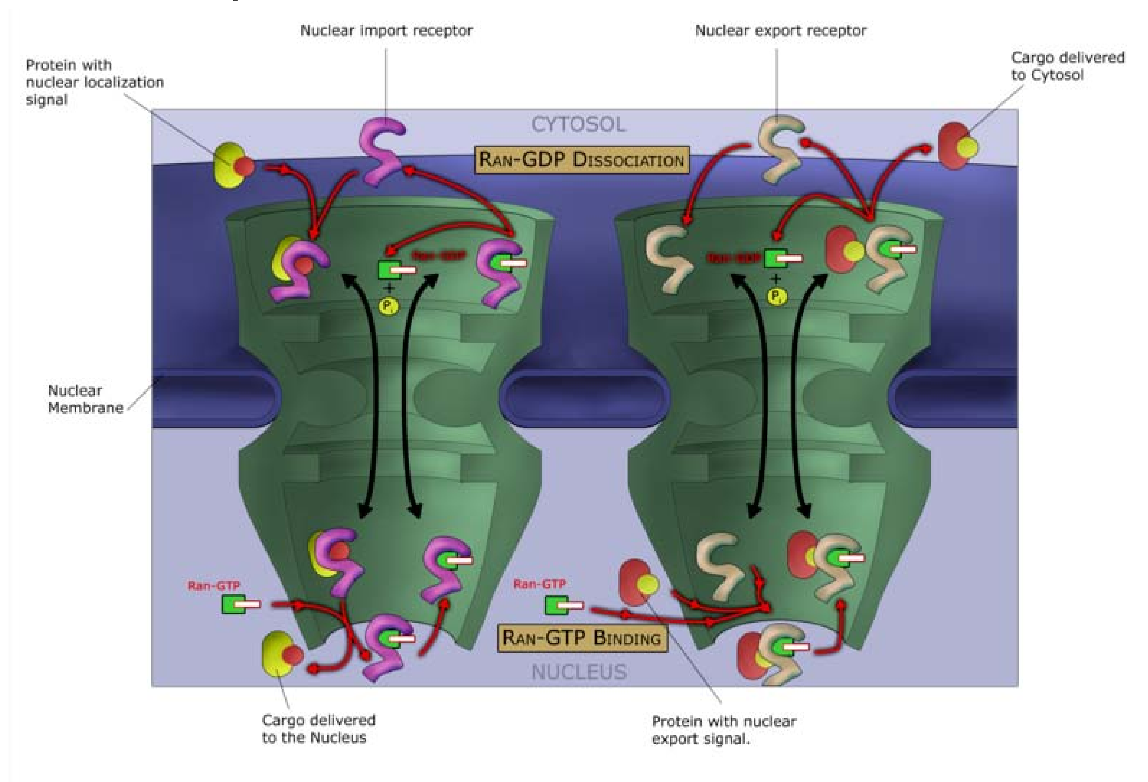
Processing of pre-mRNA

Newly synthesized mRNA molecules are known as primary transcripts or pre-mRNA. They must undergo post-transcriptional modification in the nucleus before being exported to the cytoplasm; mRNA that appears in the cytoplasm without these modifications is degraded rather than used for protein translation. The three main modifications are 5' capping, 3' polyadenylation, and RNA splicing. While in the nucleus, pre-mRNA is associated with a variety of proteins in complexes known as heterogeneous ribonucleoprotein particles (hnRNPs). Addition of the 5' cap occurs co-transcriptionally and is the first step in post-transcriptional modification. The 3' poly-adenine tail is only added after transcription is complete.

RNA splicing, carried out by a complex called the spliceosome, is the process by which introns, or regions of DNA that do not code for protein, are removed from the pre-mRNA and the remaining exons connected to re-form a single continuous molecule. This process normally occurs after 5' capping and 3' polyadenylation but can begin before synthesis is complete in transcripts with many exons. Many pre-mRNAs, including those encoding antibodies, can be spliced in multiple ways to produce different mature mRNAs that encode different protein sequences. This process is known as alternative splicing, and allows production of a large variety of proteins from a limited amount of DNA.

Dynamics and regulation

Nuclear transport



Macromolecules, such as RNA and proteins, are actively transported across the nuclear membrane in a process called the Ran-GTP nuclear transport cycle.

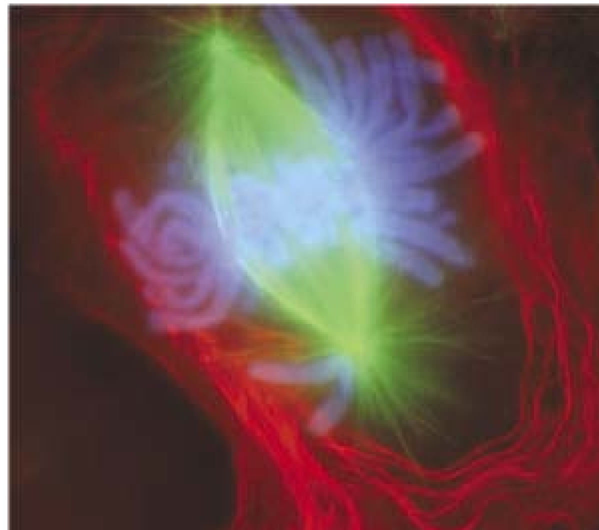
The entry and exit of large molecules from the nucleus is tightly controlled by the nuclear pore complexes. Although small molecules can enter the nucleus without regulation, macromolecules such as RNA and proteins require association karyopherins called importins to enter the nucleus and exportins to exit. "Cargo" proteins that must be translocated from the cytoplasm to the nucleus contain short amino acid sequences known as nuclear localization signals, which are bound by importins, while those transported from the nucleus to the cytoplasm carry nuclear export signals bound by exportins. The ability of importins and exportins to transport their cargo is regulated by GTPases, enzymes that hydrolyze the molecule guanosine triphosphate to release energy. The key GTPase in nuclear transport is Ran, which can bind either GTP or GDP (guanosine diphosphate), depending on whether it is located in the nucleus or the cytoplasm. Whereas importins depend on RanGTP to dissociate from their cargo, exportins require RanGTP in order to bind to their cargo.

Nuclear import depends on the importin binding its cargo in the cytoplasm and carrying it through the nuclear pore into the nucleus. Inside the nucleus, RanGTP acts to separate the cargo from the importin, allowing the importin to exit the nucleus and be reused. Nuclear export is similar, as the exportin binds the cargo inside the nucleus in a process facilitated

by RanGTP, exits through the nuclear pore, and separates from its cargo in the cytoplasm.

Specialized export proteins exist for translocation of mature mRNA and tRNA to the cytoplasm after post-transcriptional modification is complete. This quality-control mechanism is important due to the these molecules' central role in protein translation; mis-expression of a protein due to incomplete excision of exons or mis-incorporation of amino acids could have negative consequences for the cell; thus, incompletely modified RNA that reaches the cytoplasm is degraded rather than used in translation.

Assembly and disassembly



An image of a newt lung cell stained with fluorescent dyes during metaphase. The mitotic spindle can be seen, stained green, attached to the two sets of chromosomes, stained light blue. All chromosomes but one are already at the metaphase plate.

During its lifetime, a nucleus may be broken down, either in the process of cell division or as a consequence of apoptosis, a regulated form of cell death. During these events, the structural components of the nucleus—the envelope and lamina—are systematically degraded.

During the cell cycle the cell divides to form two cells. In order for this process to be possible, each of the new daughter cells must have a full set of genes, a process requiring replication of the chromosomes as well as segregation of the separate sets. This occurs by the replicated chromosomes, the sister chromatids, attaching to microtubules, which in turn are attached to different centrosomes. The sister chromatids can then be pulled to separate locations in the cell. In many cells, the centrosome is located in the cytoplasm, outside the nucleus; the microtubules would be unable to attach to the chromatids in the presence of the nuclear envelope. Therefore the early stages in the cell cycle, beginning in prophase and until around prometaphase, the nuclear membrane is dismantled. Likewise, during the same period, the nuclear lamina is also disassembled, a process regulated by phosphorylation of the lamins. Towards the end of the cell cycle, the nuclear

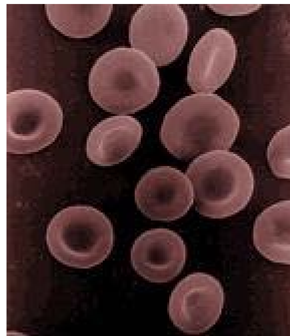
membrane is reformed, and around the same time, the nuclear lamina are reassembled by dephosphorylating the lamins.

However, in dinoflagellates, the nuclear envelope remains intact, the centrosomes are located in the cytoplasm, and the microtubules come in contact with chromosomes, whose centromeric regions are incorporated into the nuclear envelope (the so-called closed mitosis with extranuclear spindle). In many other protists (e.g., ciliates, sporozoans) and fungi, the centrosomes are intranuclear, and their nuclear envelope also does not disassemle during cell division.

Apoptosis is a controlled process in which the cell's structural components are destroyed, resulting in death of the cell. Changes associated with apoptosis directly affect the nucleus and its contents, for example, in the condensation of chromatin and the disintegration of the nuclear envelope and lamina. The destruction of the lamin networks is controlled by specialized apoptotic proteases called caspases, which cleave the lamin proteins and, thus, degrade the nucleus' structural integrity. Lamin cleavage is sometimes used as a laboratory indicator of caspase activity in assays for early apoptotic activity. Cells that express mutant caspase-resistant lamins are deficient in nuclear changes related to apoptosis, suggesting that lamins play a role in initiating the events that lead to apoptotic degradation of the nucleus. Inhibition of lamin assembly itself is an inducer of apoptosis.

The nuclear envelope acts as a barrier that prevents both DNA and RNA viruses from entering the nucleus. Some viruses require access to proteins inside the nucleus in order to replicate and/or assemble. DNA viruses, such as herpesvirus replicate and assemble in the cell nucleus, and exit by budding through the inner nuclear membrane. This process is accompanied by disassembly of the lamina on the nuclear face of the inner membrane.

Anucleated and polynucleated cells



Human red blood cells, like those of other mammals, lack nuclei. This occurs as a normal part of the cells' development.

Although most cells have a single nucleus, some eukaryotic cell types have no nucleus, and others have many nuclei. This can be a normal process, as in the maturation of mammalian red blood cells, or a result of faulty cell division.

Anucleated cells contain no nucleus and are, therefore, incapable of dividing to produce daughter cells. The best-known anucleated cell is the mammalian red blood cell, or erythrocyte, which also lacks other organelles such as mitochondria, and serves primarily as a transport vessel to ferry oxygen from the lungs to the body's tissues. Erythrocytes mature through erythropoiesis in the bone marrow, where they lose their nuclei, organelles, and ribosomes. The nucleus is expelled during the process of differentiation from an erythroblast to a reticulocyte, which is the immediate precursor of the mature erythrocyte. The presence of mutagens may induce the release of some immature "micronucleated" erythrocytes into the bloodstream. Anucleated cells can also arise from flawed cell division in which one daughter lacks a nucleus and the other has two nuclei.

Polynucleated cells contain multiple nuclei. Most Acantharean species of protozoa and some fungi in mycorrhizae have naturally polynucleated cells. Other examples include the intestinal parasites in the genus *Giardia*, which have two nuclei per cell. In humans, skeletal muscle cells, called myocytes, become polynucleated during development; the resulting arrangement of nuclei near the periphery of the cells allows maximal intracellular space for myofibrils. Multinucleated cells can also be abnormal in humans; for example, cells arising from the fusion of monocytes and macrophages, known as giant multinucleated cells, sometimes accompany inflammation and are also implicated in tumor formation.

Evolution

As the major defining characteristic of the eukaryotic cell, the nucleus' evolutionary origin has been the subject of much speculation. Four major theories have been proposed to explain the existence of the nucleus, although none have yet earned widespread support.

The theory known as the "syntrophic model" proposes that a symbiotic relationship between the archaea and bacteria created the nucleus-containing eukaryotic cell. (Organisms of the Archaea domain have no cell nucleus.) It is hypothesized that the symbiosis originated when ancient archaea, similar to modern methanogenic archaea, invaded and lived within bacteria similar to modern myxobacteria, eventually forming the early nucleus. This theory is analogous to the accepted theory for the origin of eukaryotic mitochondria and chloroplasts, which are thought to have developed from a similar endosymbiotic relationship between proto-eukaryotes and aerobic bacteria. The archaeal origin of the nucleus is supported by observations that archaea and eukarya have similar genes for certain proteins, including histones. Observations that myxobacteria are motile, can form multicellular complexes, and possess kinases and G proteins similar to eukarya, support a bacterial origin for the eukaryotic cell.

A second model proposes that proto-eukaryotic cells evolved from bacteria without an endosymbiotic stage. This model is based on the existence of modern planctomycetes bacteria that possess a nuclear structure with primitive pores and other compartmentalized membrane structures. A similar proposal states that a eukaryote-like

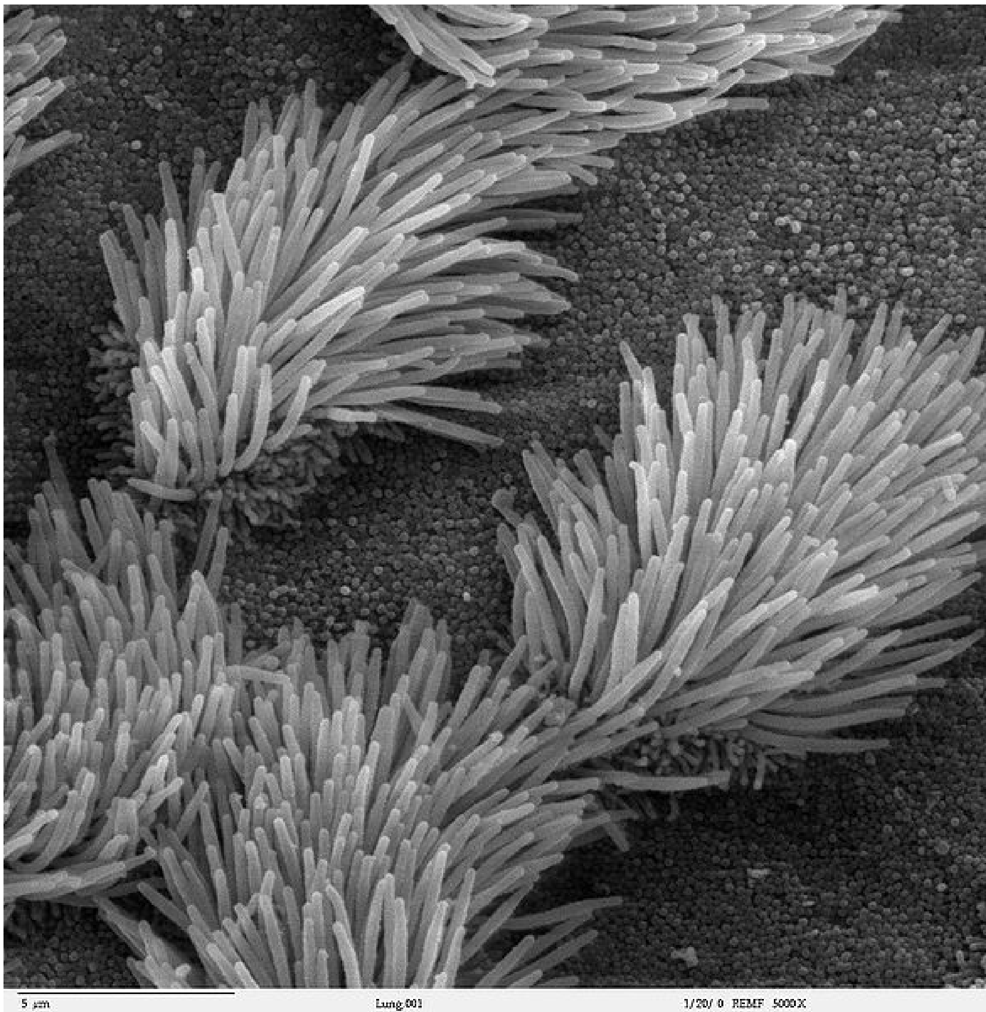
cell, the chronocyte, evolved first and phagocytosed archaea and bacteria to generate the nucleus and the eukaryotic cell.

The most controversial model, known as *viral eukaryogenesis*, posits that the membrane-bound nucleus, along with other eukaryotic features, originated from the infection of a prokaryote by a virus. The suggestion is based on similarities between eukaryotes and viruses such as linear DNA strands, mRNA capping, and tight binding to proteins (analogizing histones to viral envelopes). One version of the proposal suggests that the nucleus evolved in concert with phagocytosis to form an early cellular "predator". Another variant proposes that eukaryotes originated from early archaea infected by poxviruses, on the basis of observed similarity between the DNA polymerases in modern poxviruses and eukaryotes. It has been suggested that the unresolved question of the evolution of sex could be related to the viral eukaryogenesis hypothesis.

A very recent proposal suggests that traditional variants of the endosymbiont theory are insufficiently powerful to explain the origin of the eukaryotic nucleus. This model, termed the *exomembrane hypothesis*, suggests that the nucleus instead originated from a single ancestral cell that evolved a second exterior cell membrane; the interior membrane enclosing the original cell then became the nuclear membrane and evolved increasingly elaborate pore structures for passage of internally synthesized cellular components such as ribosomal subunits.

Chapter 8

Cilium



SEM micrograph of the cilia projecting from respiratory epithelium in the lungs

A **cilium** (Latin for eyelash; the plural is *cilia*) is an organelle found in eukaryotic cells. Cilia are slender protuberances that project from the much larger cell body.

There are two types of cilia: *motile* cilia and *non-motile*, or **primary cilia**, which typically serve as sensory organelles. In eukaryotes, cilia and flagella together make up a group of organelles known as undulipodia. Eukaryotic cilia are structurally identical to Eukaryotic flagella, although distinctions are sometimes made according to function and/or length.

Types and distribution

Cilia are rare in most plants, occurring most notably in cycads.

Cilia can be divided into primary and motile forms.

Motile cilia

Larger eukaryotes, such as mammals, have *motile* cilia as well. Motile cilia are usually present on a cell's surface in large numbers and beat in coordinated waves.

- In humans, for example, motile cilia are found in the lining of the trachea (windpipe), where they sweep mucus and dirt out of the lungs.
- In female mammals, the beating of cilia in the Fallopian tubes moves the ovum from the ovary to the uterus.

Ciliates are microscopic organisms that possess *motile* cilia exclusively and use them for either locomotion or to simply move liquid over their surface.

Primary/immotile cilium

In humans, *primary* cilia are found on nearly every cell in the body.

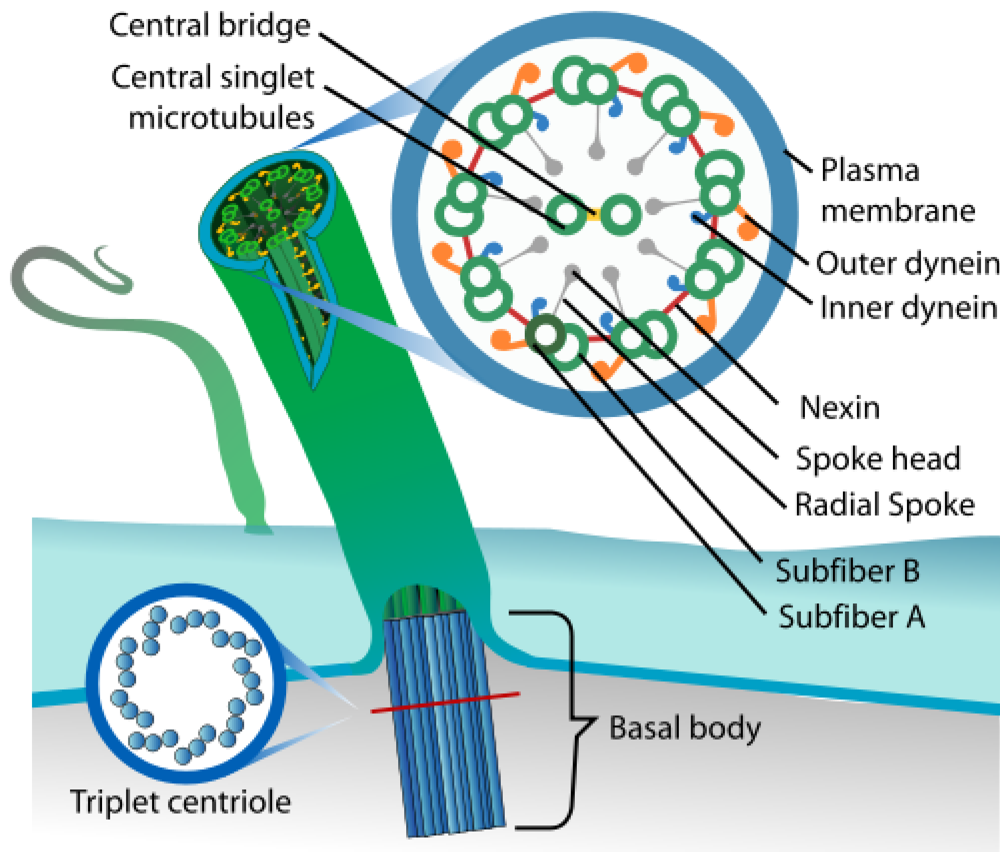
In comparison to *motile* cilia, non-motile (or *primary*) cilia usually occur one per cell; nearly all mammalian cells have a single non-motile *primary cilium.* In addition, examples of specialized primary cilia can be found in human sensory organs such as the eye and the nose:

- The outer segment of the rod photoreceptor cell in the human eye is connected to its cell body with a specialized non-motile cilium.
- The dendritic knob of the olfactory neuron, where the odorant receptors are located, also contains non-motile cilia (about 10 cilia per dendritic knob).

Although the primary cilium was discovered in 1898, it was largely ignored for a century. Only recently has great progress been made in understanding the function of the primary cilium. Until the 1990s, the prevailing view of the primary cilium was that it was merely a vestigial organelle, without important function. Recent findings regarding its physiological roles in chemical sensation, signal transduction, and control of cell growth, have led scientists to acknowledge its importance in cell function, with the discovery of its role in diseases not previously recognized to involve the dysgenesis and dysfunction

of cilia, such as polycystic kidney disease congenital heart disease, and an emerging group of genetic ciliopathies. The primary cilium is now known to play an important role in the function of many human organs. The current scientific understanding of primary cilia views them as "sensory cellular antennae that coordinate a large number of cellular signaling pathways, sometimes coupling the signaling to ciliary motility or alternatively to cell division and differentiation."

Structure, assembly and maintenance, and function



Eukaryotic motile cilium

"Inside cilia and flagella is a microtubule-based cytoskeleton called the axoneme. The axoneme of primary cilia typically has a ring of nine outer microtubule doublets (called a 9+0 axoneme), and the axoneme of a motile cilium has two central microtubule singlets in addition to the nine outer doublets (called a 9+2 axoneme). The axonemal cytoskeleton acts as a scaffolding for various protein complexes and provides binding sites for molecular motor proteins such as kinesin II, that help carry proteins up and down the microtubules."

The building blocks of the cilia such as tubulins and other partially assembled axonemal proteins are added to the ciliary tips which point away from the cell body. In most species bi-directional motility called intraflagellar transport (IFT) plays an essential role to move these building materials from the cell body to the assembly site. IFT also carries the

disassembled material to be recycled from the ciliary tip back to the cell body. By regulating the equilibrium between these two IFT processes, the length of cilia can be maintained dynamically. The disassembly of the cilia requires the action of the protein kinase Aurora A .

Exceptions where IFT is not present include *Plasmodium falciparum* which is one of the species of *Plasmodium* that cause malaria in humans. In this parasite, cilia assemble in the cytoplasm.

At the base of the cilium where the cilia attaches to the cell body is the microtubule organizing center, the basal body. Some basal body proteins as CEP164, ODF2 and CEP170, regulate the formation and the stability of the cilium. A transition zone between the basal body and the axoneme "serves as a docking station for intraflagellar transport and motor proteins."

"In effect, the [cilium] is a nanomachine composed of perhaps over 600 proteins in molecular complexes, many of which also function independently as nanomachines."

Sensing the extracellular environment

"Some epithelial cells are ciliated, and they commonly exist as a sheet of polarised cells forming a tube or tubule with cilia projecting into the lumen." Primary cilia on epithelial cells provide chemosensation, thermosensation and mechanosensation of the extracellular environment by playing "a sensory role mediating specific signalling cues, including soluble factors in the external cell environment, a secretory role in which a soluble protein is released to have an effect downstream of the fluid flow, and mediation of fluid flow if the cilia are motile."

Cilium-related disease

Ciliary defects can lead to a number of human diseases. Genetic mutations compromising the proper functioning of cilia, ciliopathies, can cause chronic disorders such as primary ciliary dyskinesia (PCD), nephronophthisis or Senior-Loken syndrome. In addition, a defect of the primary cilium in the renal tube cells can lead to polycystic kidney disease (PKD). In another genetic disorder called Bardet-Biedl syndrome (BBS), the mutant gene products are the components in the basal body and cilia.

Lack of functional cilia in female Fallopian tubes can cause ectopic pregnancy. A fertilized ovum may not reach the uterus if the cilia are unable to move it there. In such a case, the ovum will implant in the Fallopian tubes, causing a tubal pregnancy, the most common form of ectopic pregnancy.

Since the flagellum of human sperm is actually a modified cilium, ciliary dysfunction can also be responsible for male infertility.

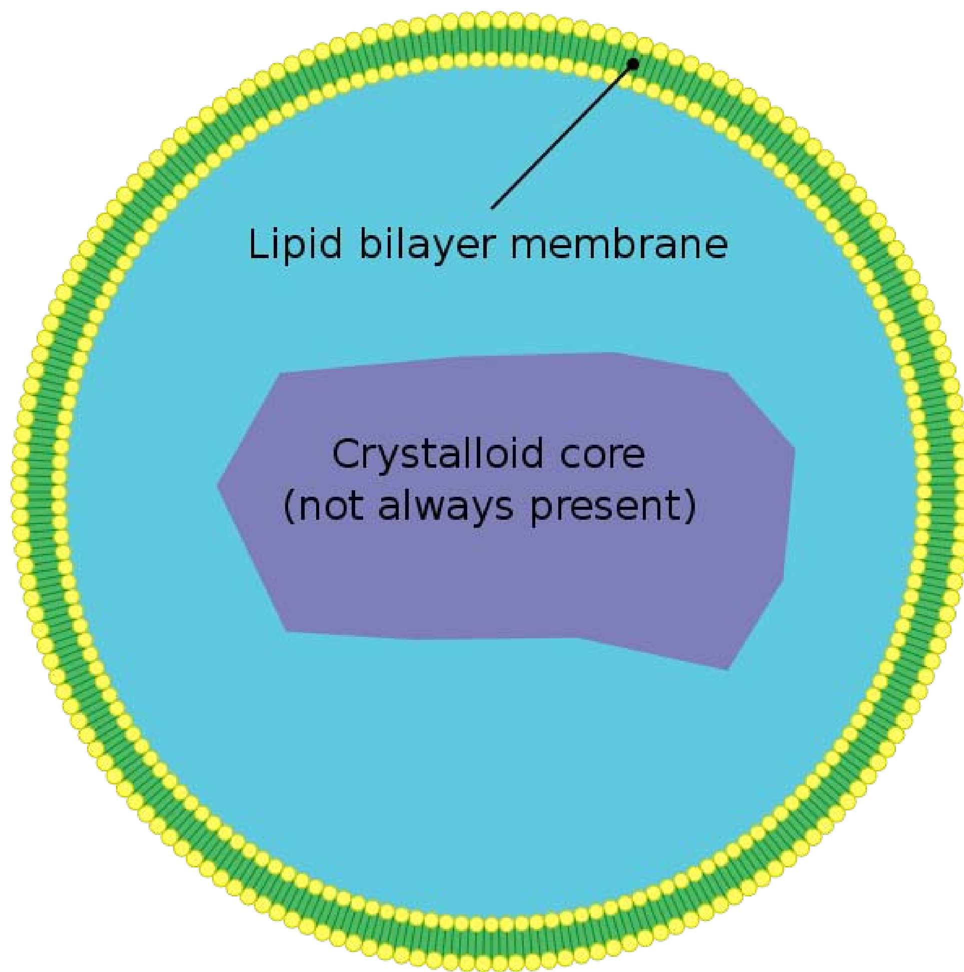
Of interest, there is an association of primary ciliary dyskinesia with left-right anatomic abnormalities such as situs inversus (a combination of findings known as Kartagener's syndrome) and other heterotaxic defects. These left-right anatomic abnormalities can also result in congenital heart disease. In fact, it has been shown that proper cilial function is responsible for the normal left-right asymmetry in mammals.

Ciliopathy as an origin for many multi-symptom genetic diseases

Recent findings in genetic research have suggested that a large number of genetic disorders, both genetic syndromes and genetic diseases, that were not previously related in the medical literature, may be, in fact, highly related in the root cause of the widely-varying set of medical symptoms that are clinically visible in the disorder. These have been grouped as an emerging class of diseases called ciliopathies. The underlying cause may be a dysfunctional molecular mechanism in the primary cilia structures, organelles which are present in many diverse cellular types throughout the human body. Cilia defects adversely affect "numerous critical developmental signaling pathways" essential to cellular development and thus offer a plausible hypothesis for the often multi-symptom nature of a large set of syndromes and diseases. Known ciliopathies include primary ciliary dyskinesia, Bardet-Biedl syndrome, polycystic kidney and liver disease, nephronophthisis, Alstrom syndrome, Meckel-Gruber syndrome and some forms of retinal degeneration.

Chapter 9

Peroxisome



Basic structure of a peroxisome

Peroxisomes (also called **microbodies**) are organelles found in virtually all eukaryotic cells. They are involved in the catabolism of very long chain fatty acids, branched chain fatty acids, D-amino acids, polyamines, and biosynthesis of plasmalogens, etherphospholipids critical for the normal function of mammalian brains and lungs. They

also contain approximately 10% of the total activity of two enzymes in the pentose phosphate pathway, which is important for energy metabolism. It is rigorously debated if peroxisomes are involved in isoprenoid and cholesterol synthesis in animals. Other known peroxisomal functions include the glyoxylate cycle in germinating seeds ("glyoxysomes"), photorespiration in leaves, glycolysis in trypanosomes ("glycosomes"), and methanol and/or amine oxidation and assimilation in some yeasts.

Peroxisomes were identified as organelles by the Belgian cytologist Christian de Duve in 1967 after they had been first described in a PhD thesis of Rhodin a decade earlier.

Metabolic functions

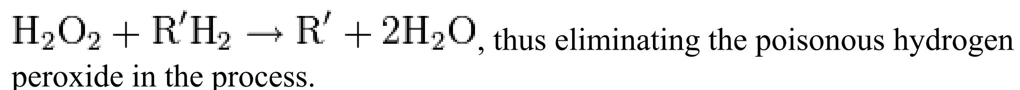
A major function of the peroxisome is the breakdown of very long chain fatty acids through beta-oxidation. In animal cells, the very long fatty acids are converted to medium chain fatty acids, which are subsequently shuttled to mitochondria where they are eventually broken down to carbon dioxide and water. In yeast and plant cells, this process is exclusive for the peroxisome.

The first reactions in the formation of plasmalogen in animal cells also occur in peroxisomes. Plasmalogen is the most abundant phospholipid in myelin. Deficiency of plasmalogens causes profound abnormalities in the myelination of nerve cells, which is one reason why many peroxisomal disorders affect the nervous system. Peroxisomes also play a role in the production of bile acids important for the absorption of fats and fat-soluble vitamins, such as vitamin K.

Peroxisomes contain oxidative enzymes, such as catalase, D-amino acid oxidase, and uric acid oxidase. However the last enzyme is absent in humans, explaining the disease known as gout, caused by the accumulation of uric acid. Certain enzymes within the peroxisome, by using molecular oxygen, remove hydrogen atoms from specific organic substrates (labeled as R), in an oxidative reaction, producing hydrogen peroxide (H_2O_2, itself toxic):

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

Catalase, another peroxisomal enzyme, uses this H_2O_2 to oxidize other substrates, including phenols, formic acid, formaldehyde, and alcohol, by means of the peroxidation reaction:



$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$, thus eliminating the poisonous hydrogen peroxide in the process.

This reaction is important in liver and kidney cells, where the peroxisomes detoxify various toxic substances that enter the blood. About 25% of the ethanol humans drink is oxidized to acetaldehyde in this way. In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through this reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

In higher plants, peroxisomes contain also a complex battery of antioxidative enzymes such as superoxide dismutase, the components of the ascorbate-glutathione cycle, and the NADP-dehydrogenases of the pentose-phosphate pathway. It has been demonstrated the generation of superoxide ($O_2^{\bullet-}$) and nitric oxide ($^{\bullet}NO$) radicals.

The peroxisome of plant cells is polarised when fighting fungal penetration. Infection causes a glucosinolate molecule to play an antifungal role to be made and delivered to the outside of the cell through the action of the peroxisomal proteins (PEN2 and PEN3).

Peroxisome assembly

Peroxisomes can be derived from the endoplasmic reticulum and replicate by fission. Peroxisome matrix proteins are translated in the cytoplasm prior to import. Specific amino acid sequences (PTS or peroxisomal targeting signal) at the *C-terminus* (PTS1) or *N-terminus* (PTS2) of peroxisomal matrix proteins signals them to be imported into the organelle. There are at least 32 known peroxisomal proteins, called peroxins, which participate in the process of peroxisome assembly. Proteins do not have to unfold to be imported into the peroxisome. The protein receptors, the peroxins *PEX5* and *PEX7*, accompany their cargoes (containing a PTS1 or a PTS2 amino acid sequence, respectively) all the way into the peroxisome where they release the cargo and then return to the cytosol - a step named *recycling*. A model describing the import cycle is referred to as the *extended shuttle mechanism*. There is now evidence that ATP hydrolysis is required for the recycling of receptors to the cytosol. Also, ubiquitination appears to be crucial for the export of PEX5 from the peroxisome, to the cytosol.

Associated medical conditions

Peroxisomal disorders are a class of medical conditions that typically affect the human nervous system as well as many other organ systems. Two common examples are X-linked adrenoleukodystrophy and peroxisome biogenesis disorders.

Genes

PEX genes encode the protein machinery ("peroxins") required for proper peroxisome assembly, as described above. Membrane assembly and maintenance requires three of these (peroxins 3, 16, and 19) and may occur without the import of the matrix (lumen) enzymes. Proliferation of the organelle is regulated by Pex11p.

Genes that encode peroxin proteins include: PEX1, PEX2 - PXMP3, PEX3, PEX5, PEX6, PEX7, PEX10, PEX11A, PEX11B, PEX11G, PEX12, PEX13, PEX14, PEX16, PEX19, PEX26, PEX28, PEX30, and PEX31

Evolutionary origins

The protein content of peroxisomes varies across species, but the presence of proteins common to many species has been used to suggest an endosymbiotic origin; that is, peroxisomes evolved from bacteria that invaded larger cells as parasites, and very gradually evolved a symbiotic relationship. However, this view has been challenged by recent discoveries. For example, peroxisome-less mutants can restore peroxisomes upon introduction of the wild-type gene.

Two independent evolutionary analyses of the peroxisomal proteome found homologies between the peroxisomal import machinery and the ERAD pathway in the endoplasmic reticulum, along with a number of metabolic enzymes that were likely recruited from the mitochondria. Recently, it has been suggested that the peroxisome may have had an actinobacterial origin, however, this is controversial.

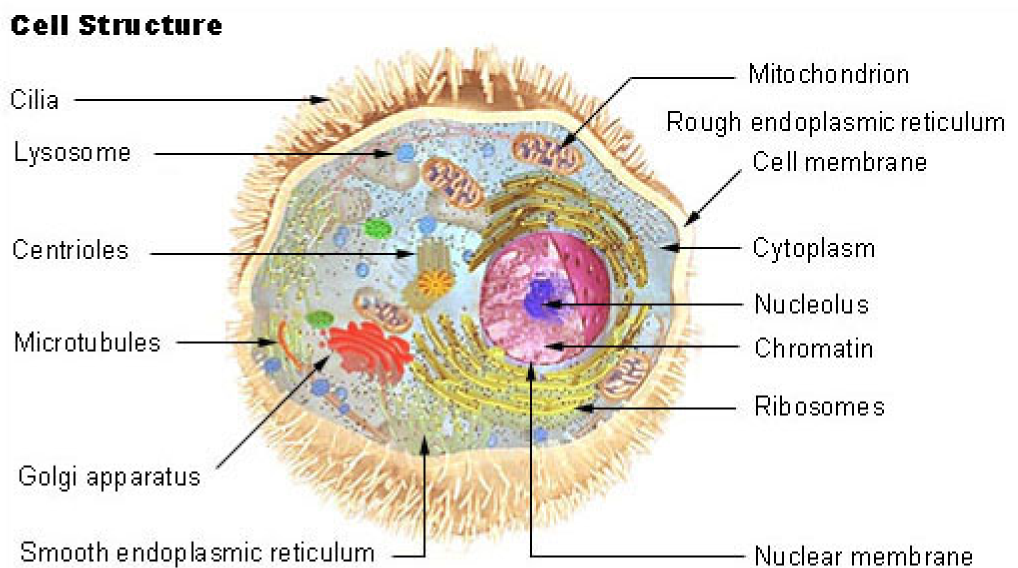
Other related organelles

Other organelles of the microbody family related to peroxisomes include glyoxysomes of plants and filamentous fungi, glycosomes of kinetoplastids and Woronin bodies of filamentous fungi.

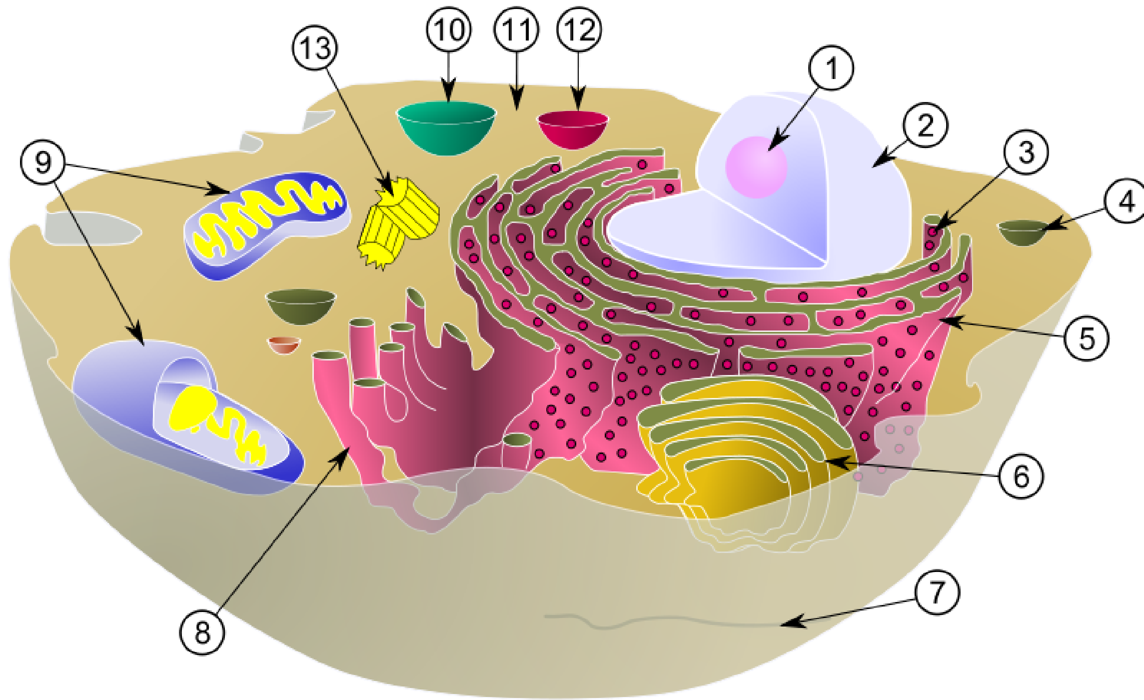
Chapter 10

Lysosome, Melanosome and Myofibril

Lysosome



Various organelles labeled. The **lysosome** is labeled in the upper left.



Schematic of typical animal cell, showing subcellular components. Organelles:
(1) nucleolus
(2) nucleus
(3) ribosomes (little dots)
(4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) smooth endoplasmic reticulum
(9) mitochondria
(10) vacuole
(11) cytosol
(12) lysosome
(13) centrioles within centrosome

Lysosomes are cellular organelles that contain acid hydrolase enzymes to break up waste materials and cellular debris. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles. Lysosomes digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are created by the addition of hydrolytic enzymes to early endosomes from the Golgi apparatus. The name *lysosome* derives from the Greek words **lysis**, *to separate*, and **soma**, *body*. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their role in autolysis. Lysosomes were discovered by the Belgian cytologist Christian de Duve in the 1950s.

The size of lysosomes varies from 0.1–1.2 μm. At pH 4.8, the interior of the lysosomes is acidic compared to the slightly alkaline cytosol (pH 7.2). The lysosome maintains this pH differential by pumping protons (H^+ ions) from the cytosol across the membrane via proton pumps and chloride ion channels. The lysosomal membrane protects the cytosol, and therefore the rest of the cell, from the degradative enzymes within the lysosome. The cell is additionally protected from any lysosomal acid hydrolases that leak into the cytosol, as these enzymes are pH-sensitive and do not function as well in the alkaline environment of the cytosol.

Enzymes

Some important enzymes found within lysosomes include:

- Lipase, which digests lipids
- Amylase, which digests amylose, starch, and maltodextrins
- Proteases, which digest proteins
- Nucleases, which digest nucleic acids
- Phosphoric acid monoesters.

Lysosomal enzymes are synthesized in the cytosol and the endoplasmic reticulum, where they receive a mannose-6-phosphate tag that targets them for the lysosome . Aberrant lysosomal targeting causes inclusion-cell disease, whereby enzymes do not properly reach the lysosome, resulting in accumulation of waste within these organelles.

Functions

Lysosomes are the cell's waste disposal system and can break up anything. They digest almost everything. One exception is asbestos. They are used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracellular material, like foreign invading microbes), endocytosis (where receptor proteins are recycled from the cell surface), and autophagy (where in old or unneeded organelles or proteins, or microbes that have invaded the cytoplasm are delivered to the lysosome). Autophagy may also lead to autophagic cell death, a form of programmed self-destruction, or autolysis, of the cell, which means that the cell is digesting itself.

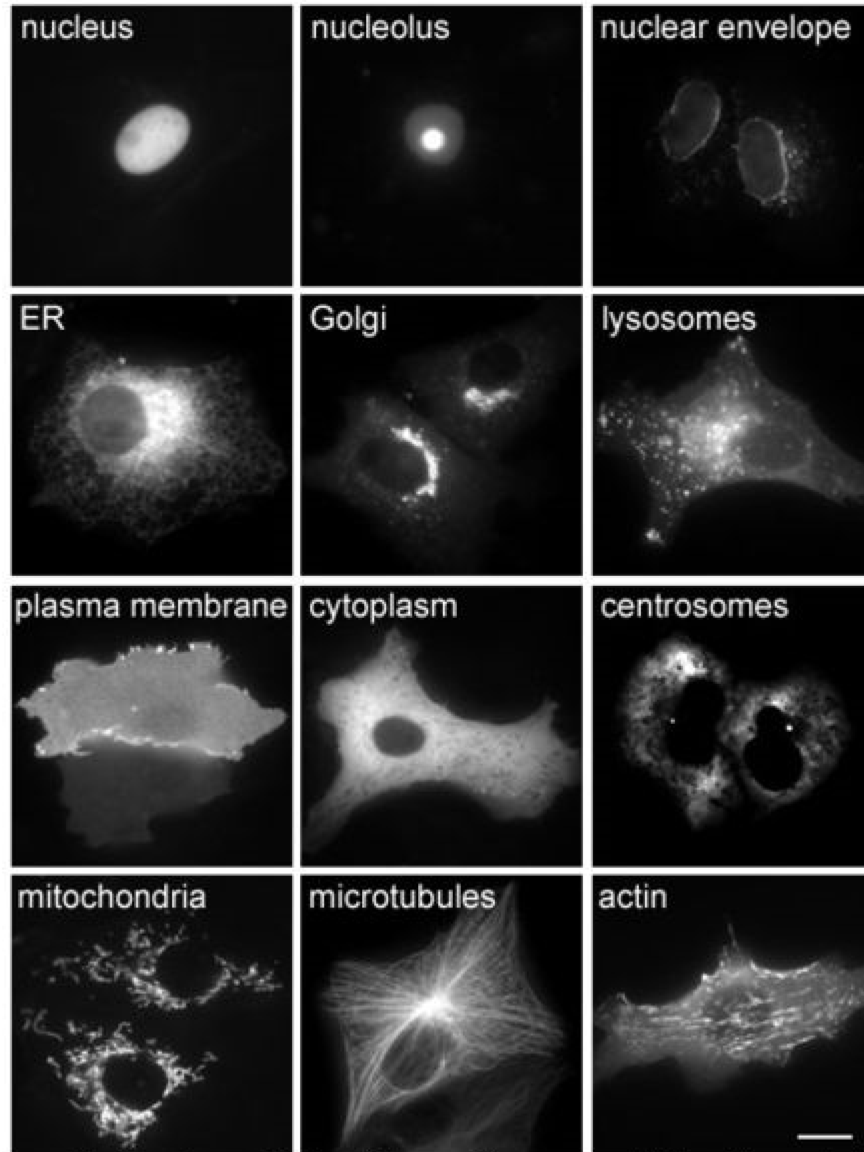
Other functions include digesting foreign bacteria (or other forms of waste) that invade a cell and helping repair damage to the plasma membrane by serving as a membrane patch, sealing the wound. In the past, lysosomes were thought to kill cells that are no longer wanted, such as those in the tails of tadpoles or in the web from the fingers of a 3- to 6-month-old fetus. While lysosomes digest some materials in this process, it is actually accomplished through programmed cell death, called apoptosis.

Clinical relevance

There are a number of **lysosomal storage diseases** that are caused by the malfunction of the lysosomes or one of their digestive proteins; examples include Tay-Sachs disease and

Pompe's disease. These diseases are caused by a defective or missing digestive protein, which leads to the accumulation of substrates within the cell, impairing metabolism.

In the broad sense, these can be classified as mucopolysaccharidoses, GM_2 gangliosidoses, lipid storage disorders, glycoproteinoses, mucolipidoses, or leukodystrophies.



Proteins in different cellular compartments and structures tagged with green fluorescent protein.

Melanosome

In a biological cell, a **melanosome** is an organelle containing melanin, the most common light-absorbing pigment found in the animal kingdom.

Cells that synthesize melanins are called melanocytes, and also the retinal pigment epithelium cells, whereas cells that have merely engulfed the melanosomes are called melanophages.

Shape

Melanosomes are bound by a lipid membrane and are, in general, rounded, sausage-like, or cigar-like in shape. The shape is constant for a given species and cell type.

They have a characteristic ultrastructure on electron microscopy, which varies according to the maturity of the melanosome, and, for research purposes, a numeric staging system is sometimes used.

Synthesis of melanin

They are dependent for their pigment on a set of enzymes within the cell (especially tyrosinase) that synthesise the large polymers generically known as melanin.

Before it contains much pigment (sufficient to be seen on light microscopy), it is known as a pre-melanosome.

Dysfunction or absence of the melanin-synthesising enzymes leads to various patterns of albinism.

Pseudopodia

In some melanocytes, the melanosomes remain static within the cell. In other types of melanocyte, the cell can extend its surface as long pseudopodia, carrying melanosomes away from the center of the cell and increasing the cell's effectiveness in absorbing light.

This happens slowly in dermal melanocytes in response to ultraviolet light, as well as production of new melanosomes and increased 'donation' of melanosomes to adjacent keratinocytes, the normal skin surface cells. This donation comes about because some keratinocytes may engulf the end of the melanocyte pseudopodia, which contain many melanosomes. Cytoplasmic dynein will carry the vesicles containing the melanin to the center of the cell. This causes melanosomes to become sequestered around the keratinocyte's nucleus, providing optimal protection from UV rays.

These changes, together, are responsible for *tanning* after exposure to UV or sunlight.

In animals

In many species of fish, amphibians, crustaceans, and reptiles, melanosomes can be highly mobile within the cell in response to hormonal (or sometimes neural) control, and this leads to visible changes in colour that are used for behavioural signaling. Melanosomes found in certain fish species contain pigments that control the color of the fish's scales. Molecular motors, when signaled, will either carry melanosomes containing pigments out to the periphery of the cell, or concentrate them at the center. The motors responsible for concentrating the melanosomes at the center are dynein, which move the melanosomes along microtubule tracts towards the minus end (i.e. the center of the cell). The motors responsible for dispersing the melanosomes to the periphery are kinesin, which are plus end directed motors. Since the plus end of microtubules are oriented towards the periphery, kinesin will carry melanosomes to the periphery. Dispersion of melanosomes to the periphery causes the cell to appear darker. Concentration of melanosomes towards the center will cause the cell to appear lighter color. This is how a protective system works for the fish on a molecular level.

The beautiful and rapid colour changes seen in many cephalopods (octopuses and squid) are based, however, on a different system, the chromatophore organ.

In fossils

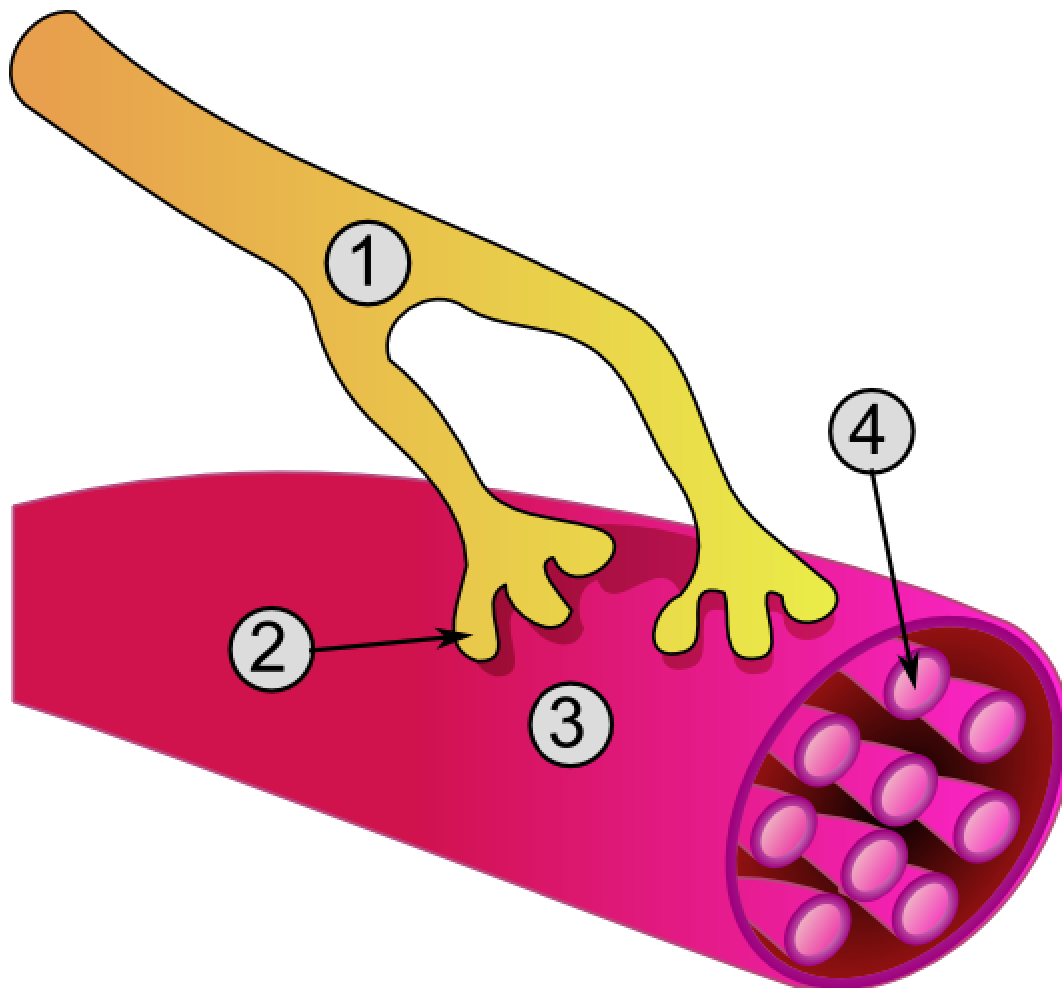
Recent (2008) discoveries by Xu Xing, a Chinese paleontologists, have found fossilized feathers in rock formations dating from the Jurassic period (200 to 150 million years ago) to the late Tertiary period (65 to 2 million years ago). The feathers contain preserved residues of carbon that were previously thought to be traces of bacteria that decomposed feather tissues but are in fact microscopic organic imprints of fossilized melanosomes. Some of these structures still maintain an iridescent color typical of feather and fur tissues. It is conjectured that these microscopic structures could be further studied to reveal the original colors and textures of softer tissues in fossils. "The discovery of ultra-structural detail in feather fossils opens up remarkable possibilities for the investigation of other features in soft-bodied fossils, like fur and even internal organs," said Derek Briggs of the Yale University study team.

Melanosomes were used to discover the true colors of fossil *Anchiornis huxleyi* by the Beijing Museum of Natural History.

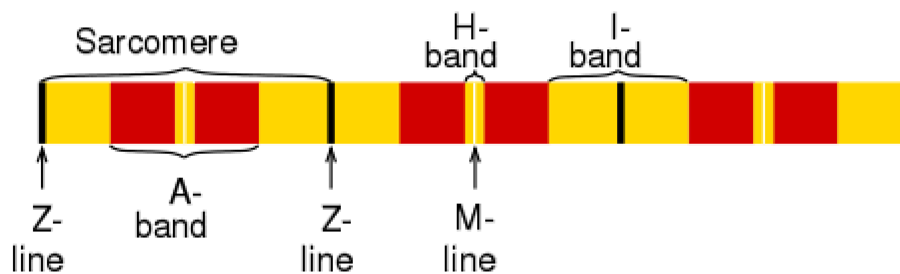
Templating

Melanosomes are believed to template melanin polymerization by way of amyloidogenesis of the protein Pmel17, which is present in abundant quantities in melanosomes.

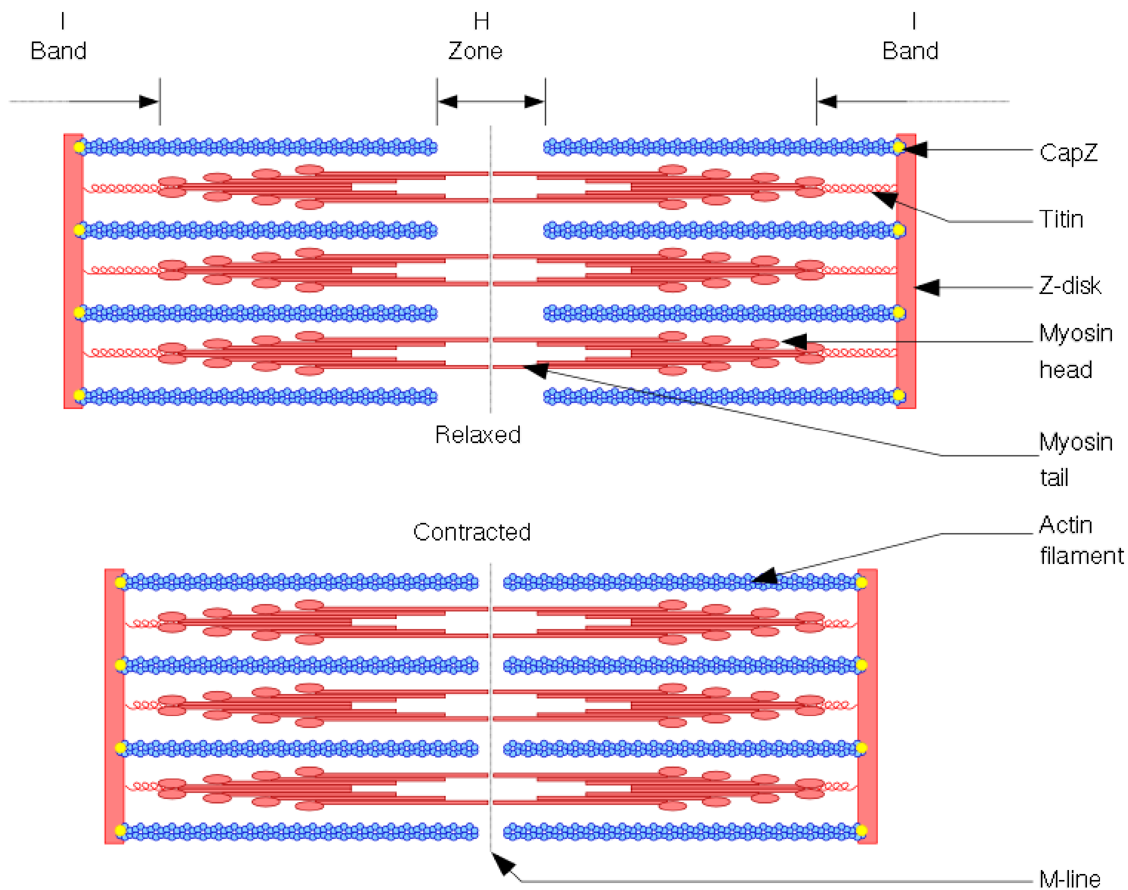
Myofibril



1. Axon
2. Neuromuscular junction
3. Muscle fiber
4. **Myofibril**



A diagram of the structure of a Myofibril



Sliding filament model of muscle contraction

Myofibrils (obsolete term: **sarcostyles**) are cylindrical organelles. They are found within muscle cells. They are bundles of **actomyosin** filaments that run from one end of the cell to the other and are attached to the cell surface membrane at each end.

Actomyosin motors are important in muscle contraction (relying in this case on "classical myosins") as well as other processes like retraction of membrane blebs, filiopod retraction, and uropodium advancement (relying in this case on "nonclassical myosins").

Structure

The filaments of myofibrils, myofilaments, consist of two types, thick and thin.

- Thin filaments consist primarily of the protein actin, coiled with nebulin filaments.
- Thick filaments consist primarily of the protein myosin, held in place by titin filaments.

The protein complex composed of actin and myosin is sometimes referred to as "actomyosin."

In striated muscle, such as skeletal and cardiac muscle, the actin and myosin filaments each have a specific and constant length on the order of a few micrometers, far less than the length of the elongated muscle cell (a few millimeters in the case of human skeletal muscle cells). The filaments are organized into repeated subunits along the length of the myofibril. These subunits are called sarcomeres. The muscle cell is nearly filled with myofibrils running parallel to each other on the long axis of the cell. The sarcomeric subunits of one myofibril are in nearly perfect alignment with those of the myofibrils next to it. This alignment gives rise to certain optical properties which cause the cell to appear striped or striated. In smooth muscle cells, this alignment is absent, hence there are no apparent striations and the cells are called smooth.

Appearance

The names of the various sub-regions of the sarcomere are based on their relatively lighter or darker appearance when viewed through the light microscope. Each sarcomere is delimited by two very dark colored bands called Z-discs or Z-lines (from the German *zwischen* meaning between). These Z-discs are dense protein discs that do not easily allow the passage of light. The T-tubule is present in this area. The area between the Z-discs is further divided into two lighter colored bands at either end called the I-bands, and a darker, grayish band in the middle called the A band.

The I bands appear lighter because these regions of the sarcomere mainly contain the thin actin filaments, whose smaller diameter allows the passage of light between them. The A band, on the other hand, contains mostly myosin filaments whose larger diameter restricts the passage of light. A stands for anisotropic and I for isotropic, referring to the optical properties of living muscle as demonstrated with polarized light microscopy.

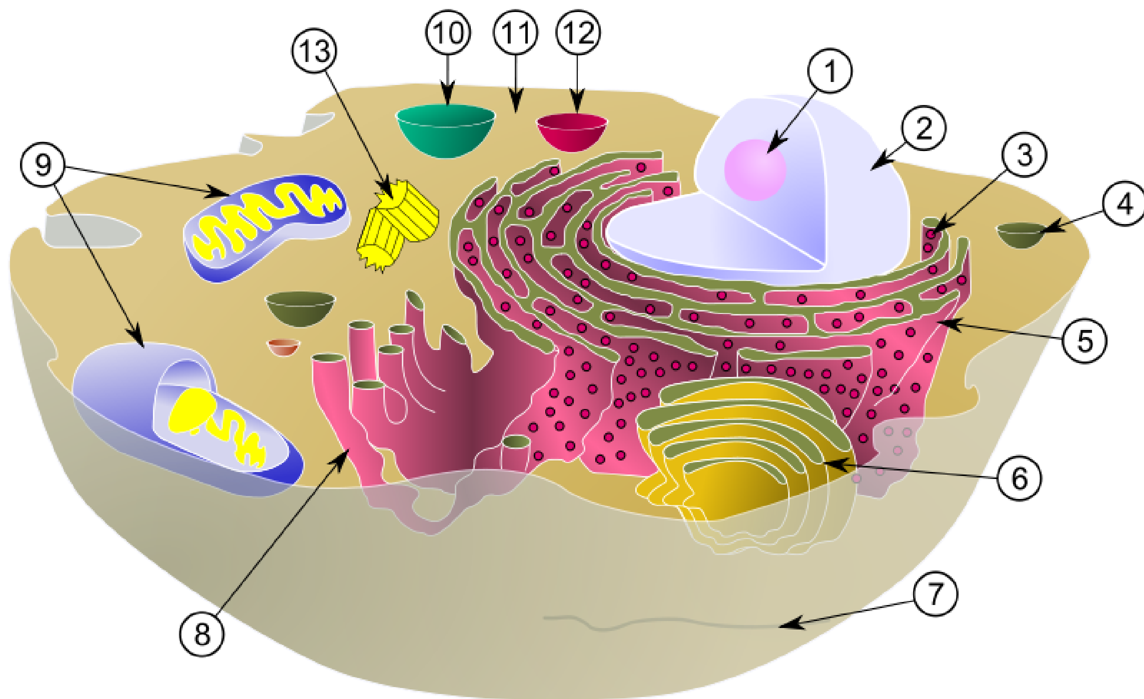
The parts of the A band that abut the I bands are occupied by the both actin and myosin filaments (where they interdigitate as described above). Also within the A band is a relatively brighter central region called the H-zone (from the German *helle*, meaning bright) in which there is no actin/myosin overlap when the muscle is in a relaxed state. Finally, the A band is bisected by a dark central line called the M-line (from the German *mittel* meaning middle).

Action

When a muscle contracts, the actin is pulled along myosin toward the center of the sarcomere until the actin and myosin filaments are completely overlapped. The H zone becomes smaller and smaller due to the increasing overlap of actin and myosin filaments, and the muscle shortens. Thus when the muscle is fully contracted, the H zone is no longer visible (as in the bottom diagram, left). Note that the actin and myosin filaments themselves do not change length, but instead slide past each other. This is known as the sliding filament theory of muscle contraction.

Chapter 11

Centrosome

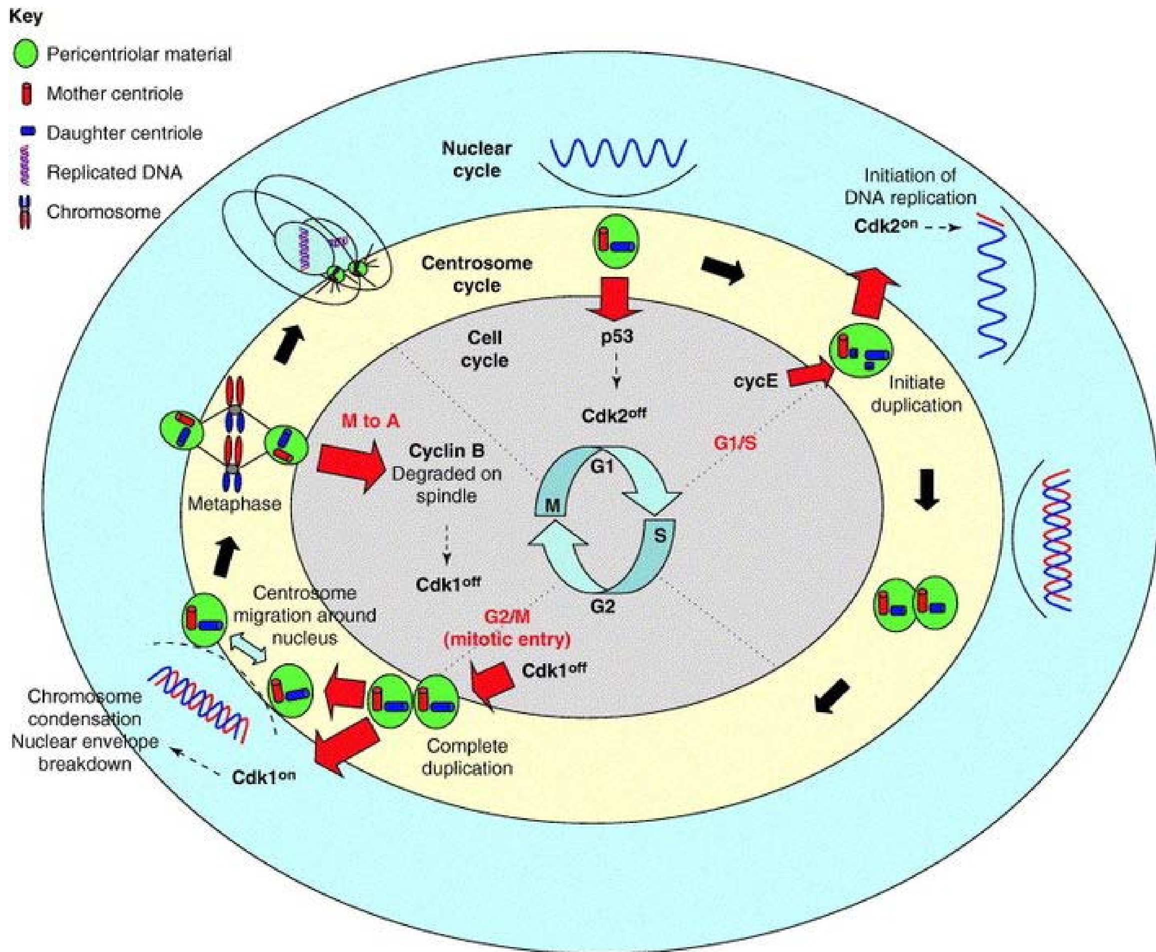


Schematic of typical animal cell, showing subcellular components. Organelles:
(1) Nucleolus
(2) Nucleus
(3) Ribosomes (little dots)
(4) Vesicle
(5) Rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(10) Vacuole
(11) Cytoplasm
(12) Lysosome
(13) Centrioles within **Centrosome**

In cell biology, the **centrosome** is an organelle that serves as the main microtubule organizing center (MTOC) of the animal cell as well as a regulator of cell-cycle progression. It was discovered by Edouard Van Beneden in 1883 and was described and named in 1888 by Theodor Boveri. The centrosome is thought to have evolved only in the metazoan lineage of eukaryotic cells. Fungi and plants use other MTOC structures to organize their microtubules. Although the centrosome has a key role in efficient mitosis in animal cells, it is not essential.

Centrosomes are composed of two orthogonally arranged centrioles surrounded by an amorphous mass of protein termed the pericentriolar material (PCM). The PCM contains proteins responsible for microtubule nucleation and anchoring including γ-tubulin, pericentrin and ninein. In general, each centriole of the centrosome is based on a nine triplet microtubule assembled in a cartwheel structure, and contains centrin, cenexin and tektin.

Roles of the centrosome

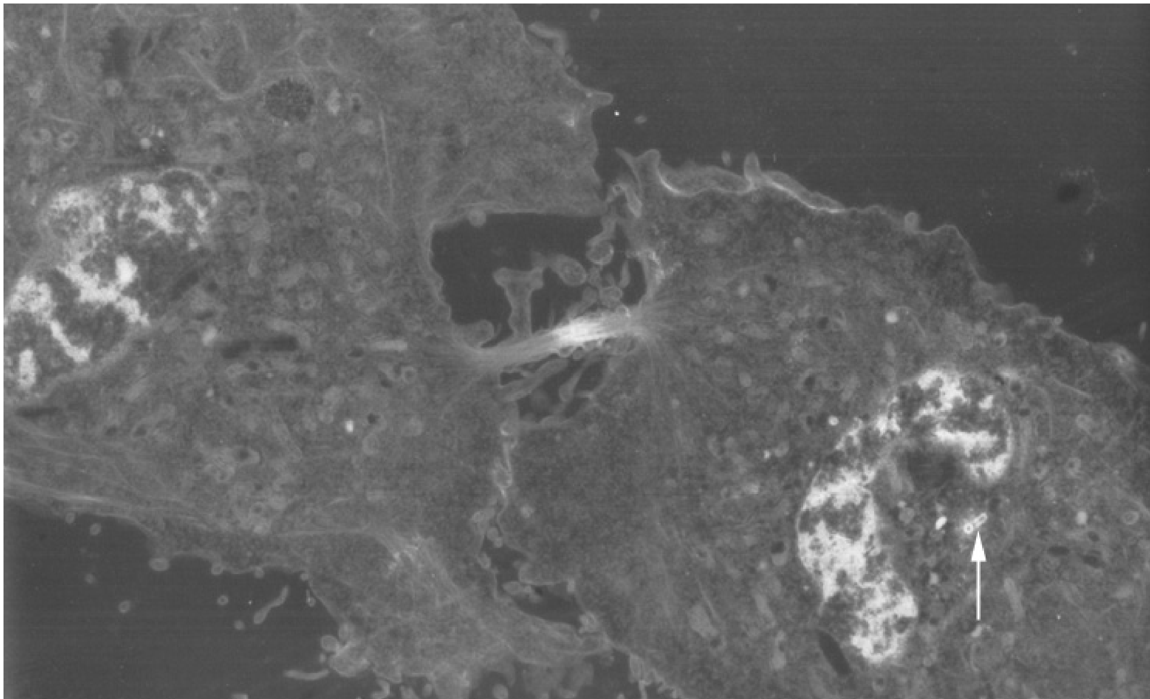


Role of the centrosome in cell cycle progression

Centrosomes are often associated with the nuclear membrane during interphase of the cell cycle. In mitosis the nuclear membrane breaks down and the centrosome nucleated microtubules can interact with the chromosomes to build the mitotic spindle.

The mother centriole, the one that was inherited from the mother cell, also has a central role in making cilia and flagella.

The centrosome is copied only once per cell cycle so that each daughter cell inherits one centrosome, containing two centrioles. The centrosome replicates during the S phase of the cell cycle. During the prophase in the process of cell division called mitosis, the centrosomes migrate to opposite poles of the cell. The mitotic spindle then forms between the two centrosomes. Upon division, each daughter cell receives one centrosome. Aberrant numbers of centrosomes in a cell have been associated with cancer. Doubling of a centrosome is similar to DNA replication in two respects: the semiconservative nature of the process and the action of cdk2 as a regulator of the process. But the processes are essentially different in that centrosome doubling does not occur by template reading and assembly. The mother centriole just aids in the accumulation of materials required for the assembly of the daughter centriole.



Centrosome (shown by arrow) next to nucleus

In animal cells, centrosomes contain two structures called centrioles. Interestingly, centrioles are not required for the progression of mitosis. When the centrioles are irradiated by a laser, mitosis proceeds normally with a morphologically normal spindle. Moreover, development of the fruit fly *Drosophila* is largely normal when centrioles are absent due to a mutation in a gene required for their duplication. In the absence of the

centrioles the microtubules of the spindle are focused by motors allowing the formation of a bipolar spindle. Many cells can completely undergo interphase without centrioles. Unlike centrioles, centrosomes are required for survival of the organism. Acentrosomal cells lack radial arrays of astral microtubules. They are also defective in spindle positioning and in ability to establish a central localization site in cytokinesis. The function of centrosome in this context is hypothesized to ensure the fidelity of cell division because it greatly increases the efficacy. Some cell types arrest in the following cell cycle when centrosomes are absent. This is not a universal phenomenon.

When the nematode *C. elegans* egg is fertilized the sperm delivers a pair of centrioles. These centrioles will form the centrosomes which will direct the first cell division of the zygote and this will determine its polarity. It is not yet clear whether the role of the centrosome in polarity determination is microtubule dependent or independent.

Evolution of the centrosome

The evolutionary history of the centrosome and the centriole has been traced for some of the signature genes, e.g. the centrins. Centrins participate in calcium signaling and are required for centriole duplication. There exist two main subfamilies of centrins, both of which are present in the early-branching eukaryote Giardia intestinalis. Centrins have therefore been present in the common ancestor of eukaryotes. Conversely, they have no recognizable homologs in archea and bacteria and are thus part of the "eukaryotic signature genes." Although there are studies on the evolution of the centrins and centrioles, no studies have been published on the evolution of the pericentriolar material.

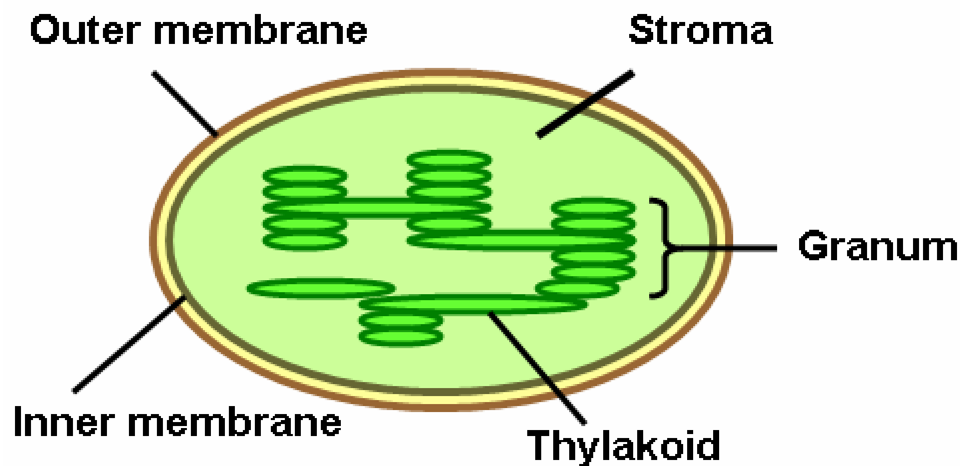
It is evident that some parts of the centrosome are highly diverged in the model species Drosophila melanogaster and Caenorhabditis elegans. For example, both species have lost one of the centrin subfamilies that are usually associated with centriole duplication. Drosophila melanogaster mutants that lack centrosomes can even develop to morphologically normal adult flies, which then die shortly after birth because their sensory neurons lack cilia. Thus, these flies have evolved functionally redundant machinery, which is independent of the centrosomes.

Centrosome associated nucleotides

Research in 2006 indicated that centrosomes from Surf clam eggs contain RNA sequences. The sequences identified were found in "few to no" other places in the cell, and do not appear in existing genome databases. One identified RNA sequence contains a putative RNA polymerase, leading to the hypothesis of an RNA based genome within the centrosome. However, subsequent research has shown that centrosome do not contain their own DNA-based genomes. While it was confirmed that RNA molecules associate with centrosomes, the sequences have still been found within the nucleus. Furthermore, centrosomes can form *de novo* after having been removed (e.g. by laser irradiation) from normal cells.

Chapter 12

Thylakoid



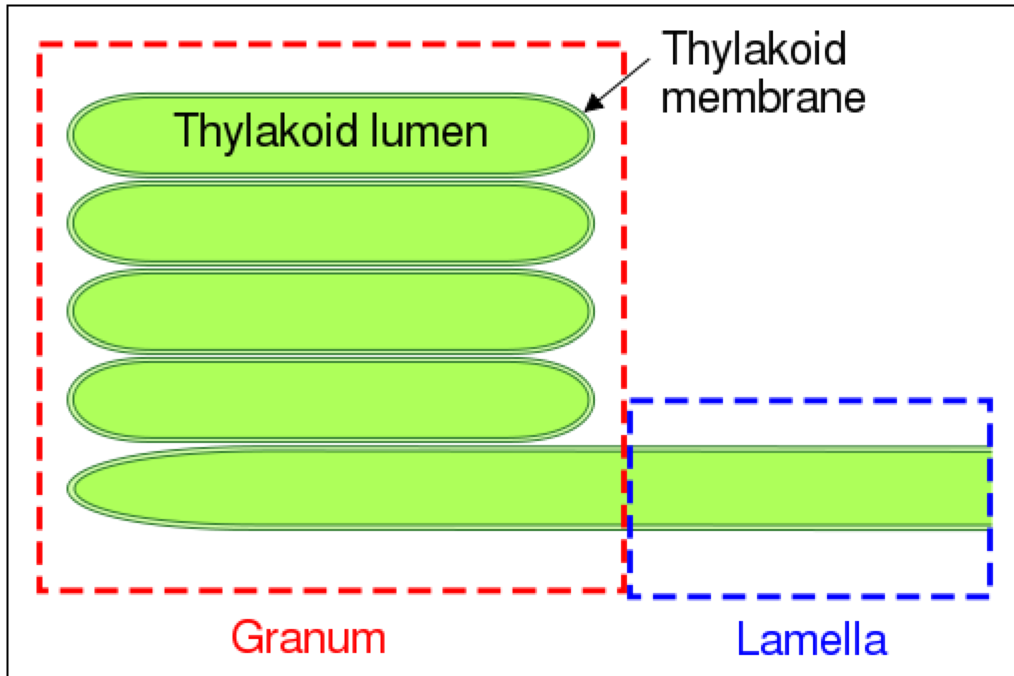
Thylakoids (green) inside a chloroplast

A **thylakoid** is a membrane-bound compartment inside chloroplasts and cyanobacteria. They are the site of the light-dependent reactions of photosynthesis. Thylakoids consist of a **thylakoid membrane** surrounding a **thylakoid lumen**. Chloroplast thylakoids frequently form stacks of disks referred to as **grana** (singular: **granum**). Grana are connected by **intergrana** or **stroma** thylakoids, which join granum stacks together as a single functional compartment.

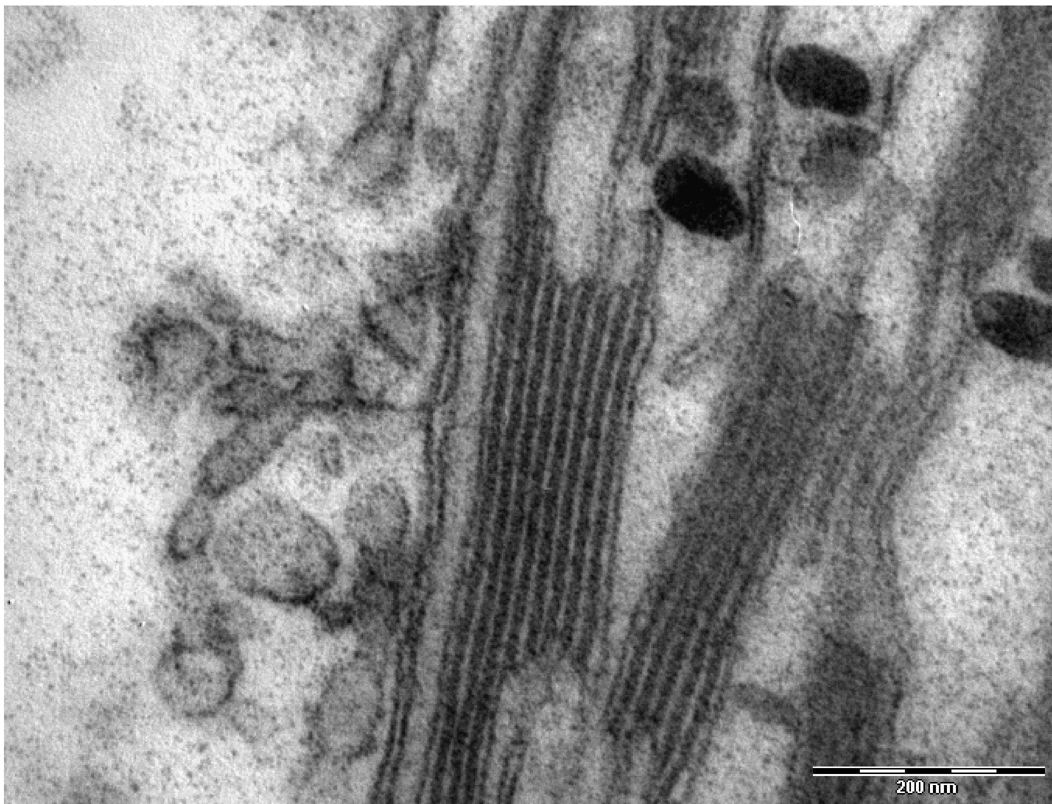
Etymology

The word *thylakoid* come via Latin from Greek *thylakos* meaning *sac* or *pouch*. Thus, *thylakoid* means *sac-like* or *pouch-like*.

Thylakoid structure



Thylakoid structures



TEM image of grana

Thylakoids are membrane-bound structures embedded into the chloroplast stroma.

Membrane

The **thylakoid membrane** is the site of the light-dependent reactions of photosynthesis with the photosynthetic pigments embedded directly in the membrane. It is an alternating pattern of dark and light bands measuring each 1 nanometre. The thylakoid lipid bilayer shares characteristic features with prokaryotic membranes and the inner chloroplast membrane. For example, acidic lipids can be found in thylakoid membranes, cyanobacteria and other photosynthetic bacteria and are involved in the functional integrity of the photosystems. The thylakoid membranes of higher plants are composed primarily of phospholipids and galactolipids that are asymmetrically arranged along and across the membranes. The lipids for the thylakoid membranes are synthesized in a complex pathway involving exchange of lipid precursors between the endoplasmic reticulum and inner membrane of the plastid envelope and transported from the inner membrane to the thylakoids via vesicles.

Lumen

The **thylakoid lumen** is the compartment bounded by the thylakoid membrane. It plays a vital role for photophosphorylation during photosynthesis. During the light-dependent reaction, protons are pumped across the thylakoid membrane into the lumen making it acidic down to pH 4.

Granum

A **granum** (plural **grana**) is a stack of thylakoid discs. Chloroplasts can have from 10 to 100 grana. Grana are connected by stroma thylakoids, also called intergrana thylakoids or **lamellae**. Grana thylakoids and stroma thylakoids can be distinguished by their different protein composition. Grana contribute to chloroplasts' large surface area to volume ratio. Different interpretations of electron tomography imaging of thylakoid membranes has resulted in two models for grana structure. Both posit that lamellae intersect grana stacks in parallel sheets, though whether these sheets intersect in planes perpendicular to the grana stack axis, or are arranged in a right-handed helix is debated.

Thylakoid formation

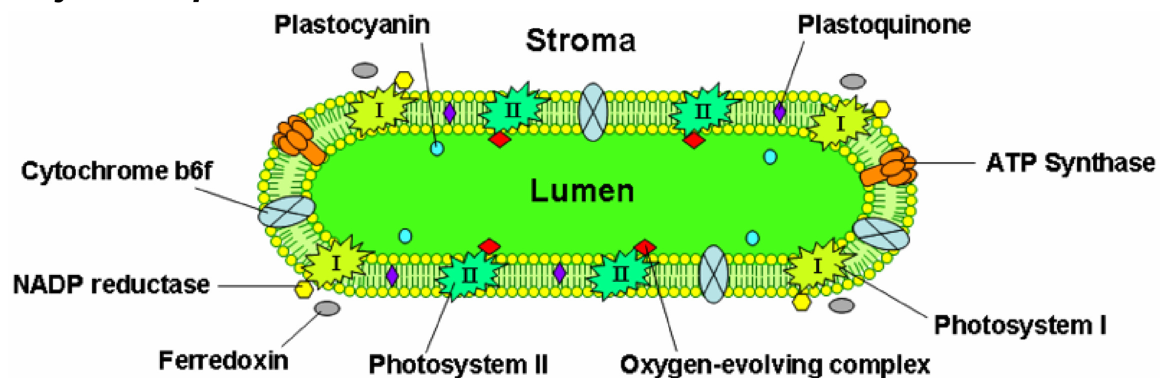
Chloroplasts develop from proplastids when seedlings emerge from the ground. Thylakoid formation requires light. In the plant embryo and in the absence of light, proplastids develop into etioplasts that contain semicrystalline membrane structures called prolamellar bodies. When exposed to light, these prolamellar bodies develop into thylakoids. This does not happen in seedlings grown in the dark, which undergo etiolation. An underexposure to light can cause the thylakoids to fail. This causes the chloroplasts to fail resulting in the death of the plant.

Thylakoid formation requires the action of *vesicle-inducing protein in plastids 1* (VIPP1). Plants cannot survive without this protein, and reduced VIPP1 levels lead to slower growth and paler plants with reduced ability to photosynthesize. VIPP1 appears to be required for basic thylakoid membrane formation, but not for the assembly of protein complexes of the thylakoid membrane. It is conserved in all organisms containing thylakoids, including cyanobacteria, green algae, such as Chlamydomonas, and higher plants, such as *Arabidopsis thaliana*.

Thylakoid isolation and fractionation

Thylakoids can be purified from plant cells using a combination of differential and gradient centrifugation. Disruption of isolated thylakoids, for example by mechanical shearing, releases the lumenal fraction. Peripheral and integral membrane fractions can be extracted from the remaining membrane fraction. Treatment with sodium carbonate (Na_2CO_3) detaches peripheral membrane proteins, whereas treatment with detergents and organic solvents solubilizes integral membrane proteins.

Thylakoid proteins



Thylakoid disc with embedded and associated proteins

Thylakoids contain many integral and peripheral membrane proteins, as well as lumenal proteins. Recent proteomics studies of thylakoid fractions have provided further details on the protein composition of the thylakoids. These data have been summarized in several plastid protein databases that are available online.

According to these studies, the thylakoid proteome consists of at least 335 different proteins. Out of these, 89 are in the lumen, 116 are integral membrane proteins, 62 are peripheral proteins on the stroma side, and 68 peripheral proteins on the lumenal side. Additional low-abundance lumenal proteins can be predicted through computational methods. Of the thylakoid proteins with known functions, 42% are involved in photosynthesis. The next largest functional groups include proteins involved in protein targeting, processing and folding with 11%, oxidative stress response (9%) and translation (8%).

Integral membrane proteins

Thylakoid membranes contain integral membrane proteins which play an important role in light harvesting and the light-dependent reactions of photosynthesis. There are four major protein complexes in the thylakoid membrane:

- Photosystems I and II
- Cytochrome b6f complex
- ATP synthase

Photosystem II is located mostly in the grana thylakoids, whereas photosystem I and ATP synthase are mostly located in the stroma thylakoids and the outer layers of grana. The cytochrome b6f complex is distributed evenly throughout thylakoid membranes. Due to the separate location of the two photosystems in the thylakoid membrane system, mobile electron carriers are required to shuttle electrons between them. These carriers are plastoquinone and plastocyanin. Plastoquinone shuttles electrons from photosystem II to the cytochrome b6f complex, whereas plastocyanin carries electrons from the cytochrome b6f complex to photosystem I.

Together, these proteins make use of light energy to drive electron transport chains that generate a chemiosmotic potential across the thylakoid membrane and NADPH, a product of the terminal redox reaction. The ATP synthase uses the chemiosmotic potential to make ATP during photophosphorylation.

Photosystems

These photosystems are light-driven redox centers, each consisting of an antenna complex that uses chlorophylls and accessory photosynthetic pigments such as carotenoids and phycobiliproteins to harvest light at a variety of wavelengths. Each antenna complex has between 250 and 400 pigment molecules and the energy they absorb is shuttled by resonance energy transfer to a specialized chlorophyll *a* at the reaction center of each photosystem. When either of the two chlorophyll *a* molecules at the reaction center absorb energy, an electron is excited and transferred to an electron-acceptor molecule. **Photosystem I** contains a pair of chlorophyll *a* molecules, designated P700, at its reaction center that maximally absorbs 700 nm light. **Photosystem II** contains P680 chlorophyll that absorbs 680 nm light best (note that these wavelengths correspond to deep red). The P is short for pigment and the number is the specific absorption peak in nanometers for the chlorophyll molecules in each reaction center.

Cytochrome b6f complex

The cytochrome b6f complex is part of the thylakoid electron transport chain and couples electron transfer to the pumping of protons into the thylakoid lumen. Energetically, it is situated between the two photosystems and transfers electrons from photosystem II-plastoquinone to plastocyanin-photosystem I.

ATP synthase

The thylakoid ATP synthase is a CF1FO-ATP synthase similar to the mitochondrial ATPase. It is integrated into the thylakoid membrane with the CF1-part sticking into stroma. Thus, ATP synthesis occurs on the stromal side of the thylakoids where the ATP is needed for the light-independent reactions of photosynthesis.

Thylakoid lumen proteins

The electron transport protein plastocyanin is present in the lumen and shuttles electrons from the cytochrome b6f protein complex to photosystem I. While plastoquinones are lipid-soluble and therefore move within the thylakoid membrane, plastocyanin moves through the thylakoid lumen.

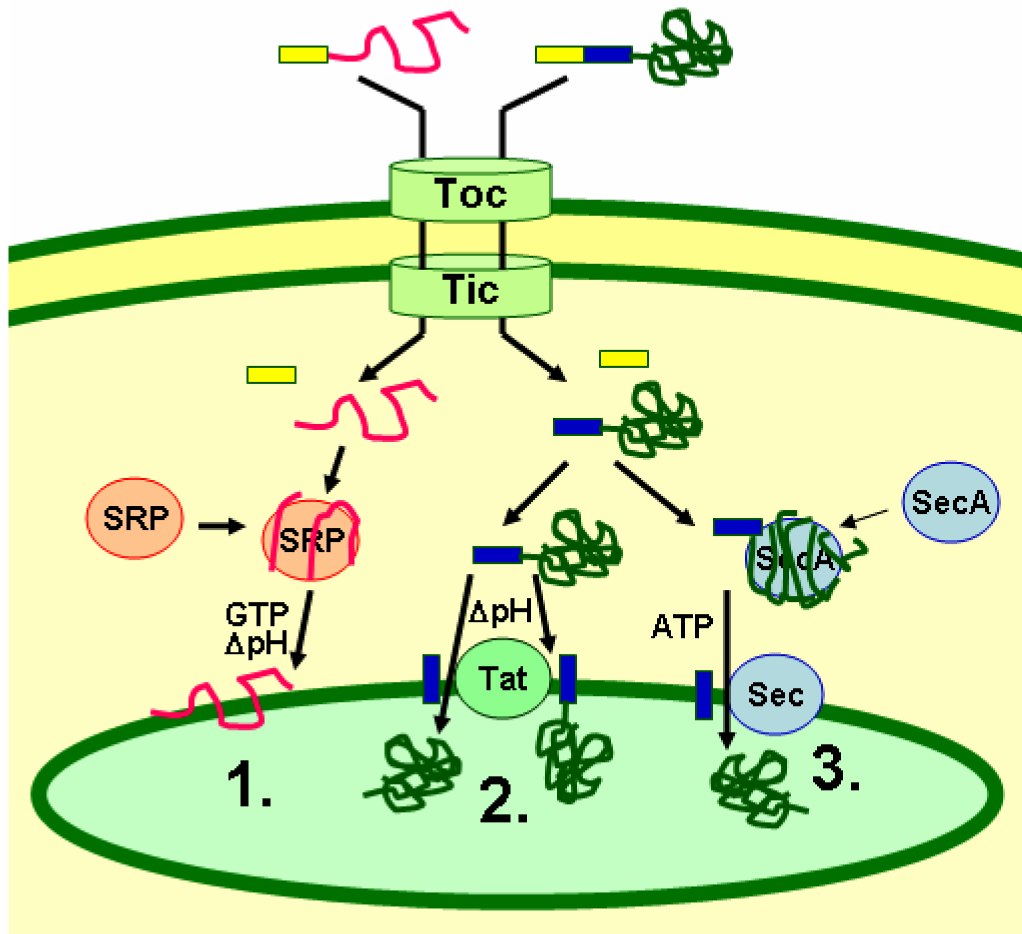
The lumen of the thylakoids is also the site of water oxidation by the oxygen evolving complex associated with the lumenal side of photosystem II.

Lumenal proteins can be predicted computationally based on their targeting signals. In Arabidopsis, out of the predicted lumenal proteins possessing the Tat signal, the largest groups with known functions are 19% involved in protein processing (proteolysis and folding), 18% in photosynthesis, 11% in metabolism, and 7% redox carriers and defense.

Thylakoid protein expression

Chloroplasts have their own genome, which encodes a number of thylakoid proteins. However, during the course of plastid evolution from their cyanobacterial endosymbiotic ancestors, extensive gene transfer from the chloroplast genome to the cell nucleus took place. This results in the four major thylakoid protein complexes being encoded in part by the chloroplast genome and in part by the nuclear genome. Plants have developed several mechanisms to co-regulate the expression of the different subunits encoded in the two different organelles to assure the proper stoichiometry and assembly of these protein complexes. For example, transcription of nuclear genes encoding parts of the photosynthetic apparatus is regulated by light. Biogenesis, stability and turnover of thylakoid protein complexes is regulated by phosphorylation via redox-sensitive kinases in the thylakoid membranes. The translation rate of chloroplast-encoded proteins is controlled by the presence or absence of assembly partners (control by epistasy of synthesis). This mechanism involves negative feedback through binding of excess protein to the 5' untranslated region of the chloroplast mRNA. Chloroplasts also need to balance the ratios of photosystem I and II for the electron transfer chain. The redox state of the electron carrier plastoquinone in the thylakoid membrane directly affects the transcription of chloroplast genes encoding proteins of the reaction centers of the photosystems, thus counteracting imbalances in the electron transfer chain.

Protein targeting to the thylakoids

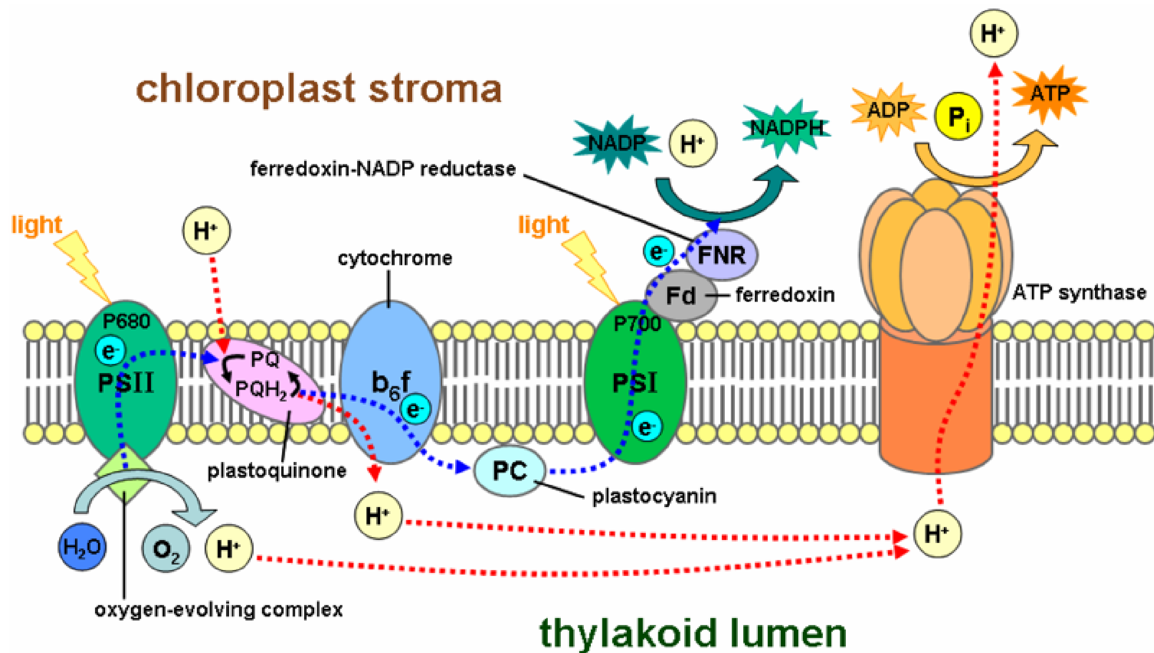


Schematic representation of thylakoid protein targeting pathways.

Thylakoid proteins are targeted to their destination via signal peptides and prokaryotic-type secretory pathways inside the chloroplast. Most thylakoid proteins encoded by a plant's nuclear genome need two targeting signals for proper localization: An N-terminal chloroplast targeting peptide (shown in yellow in the figure), followed by a thylakoid targeting peptide (shown in blue). Proteins are imported through the translocon of outer and inner membrane (Toc and Tic) complexes. After entering the chloroplast, the first targeting peptide is cleaved off by a protease processing imported proteins. This unmasks the second targeting signal and the protein is exported from the stroma into the thylakoid in a second targeting step. This second step requires the action of protein translocation components of the thylakoids and is energy-dependent. Proteins are inserted into the membrane via the SRP-dependent pathway (1), the Tat-dependent pathway (2), or spontaneously via their transmembrane domains (not shown in figure). Lumenal proteins are exported across the thylakoid membrane into the lumen by either the Tat-dependent pathway (2) or the Sec-dependent pathway (3) and released by cleavage from the thylakoid targeting signal. The different pathways utilize different signals and energy sources. The Sec (secretory) pathway requires ATP as energy source and consists of SecA, which binds to the imported protein, and a Sec membrane complex to shuttle the

protein across. Proteins with a twin arginine motif in their thylakoid signal peptide are shuttled through the Tat (twin arginine translocation) pathway, which requires a membrane-bound Tat complex and the pH gradient as an energy source. Some other proteins are inserted into the membrane via the SRP (signal recognition particle) pathway. The chloroplast SRP can interact with its target proteins either post-translationally or co-translationally, thus transporting imported proteins as well as those that are translated inside the chloroplast. The SRP pathway requires GTP and the pH gradient as energy sources. Some transmembrane proteins may also spontaneously insert into the membrane from the stromal side without energy requirement.

Thylakoid function



Light-dependent reactions of photosynthesis at the thylakoid membrane

The thylakoids are the site of the light-dependent reactions of photosynthesis. These include light-driven water oxidation and oxygen evolution, the pumping of protons across the thylakoid membranes coupled with the electron transport chain of the photosystems and cytochrome b6f complex, and ATP synthesis by the ATP synthase utilizing the generated proton gradient.

Water photolysis

The first step in photosynthesis is the light-driven oxidation (splitting) of water to provide the electrons for the photosynthetic electron transport chains as well as protons for the establishment of a proton gradient. The water-splitting reaction occurs on the lumenal side of the thylakoid membrane and is driven by the light energy captured by the photosystems. It is interesting to note that this oxidation of water conveniently produces

the waste product O_2 that is vital for cellular respiration. The molecular oxygen formed by the reaction is released into the atmosphere.

Electron transport chains

Two different variations of electron transport are used during photosynthesis:

- **Noncyclic electron transport** or **Non-cyclic photophosphorylation** produces NADPH + H^+ and ATP.
- **Cyclic electron transport** or **Cyclic photophosphorylation** produces only ATP.

The noncyclic variety involves the participation of both photosystems, while the cyclic electron flow is dependent on only photosystem I.

- **Photosystem I** uses light energy to reduce $NADP^+$ to NADPH + H^+, and is active in both noncyclic and cyclic electron transport. In cyclic mode, the energized electron is passed down a chain that ultimately returns it (in its base state) to the chlorophyll that energized it.
- **Photosystem II** uses light energy to oxidize water molecules, producing electrons (e^-), protons (H^+), and molecular oxygen (O_2), and is only active in noncyclic transport. Electrons in this system are not conserved, but are rather continually entering from oxidized $2H_2O$ (O_2 + 4 H^+ + 4 e^-) and exiting with $NADP^+$ when it is finally reduced to NADPH.

Chemiosmosis

A major function of the thylakoid membrane and its integral photosystems is the establishment of chemiosmotic potential. The carriers in the electron transport chain use some of the electron's energy to actively transport protons from the stroma to the lumen. During photosynthesis, the lumen becomes acidic, as low as pH 4, compared to pH 8 in the stroma. This represents a 10,000 fold concentration gradient for protons across the thylakoid membrane.

Source of proton gradient

The protons in the lumen come from three primary sources.

- Photolysis by photosystem II oxidises water to oxygen, protons and electrons in the lumen.
- The transfer of electrons from photosystem II to plastoquinone during non-cyclic electron transport consumes two protons from the stroma. These are released in the lumen when the reduced plastoquinol is oxidized by the cytochrome b6f protein complex on the lumen side of the thylakoid membrane. From the plastoquinone pool, electrons pass through the cytochrome b6f complex. This integral membrane assembly resembles cytochrome bc1.

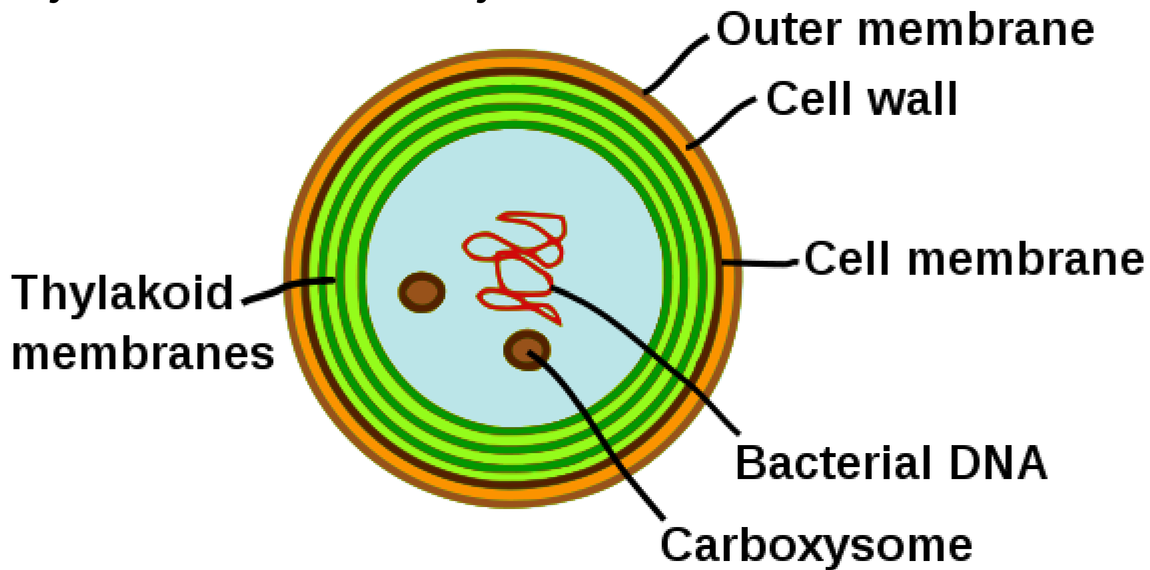
- The reduction of plastoquinone by ferredoxin during cyclic electron transport also transfers two protons from the stroma to the lumen.

The proton gradient is also caused by the consumption of protons in the stroma to make NADPH from NADP+ at the NADP reductase.

ATP generation

The molecular mechanism of ATP generation in chloroplasts is similar to that in mitochondria and takes the required energy from the proton motive force (PMF). However, chloroplasts rely more on the chemical potential of the PMF to generate the potential energy required for ATP synthesis. The PMF is the sum of a proton chemical potential (given by the proton concentration gradient) and a transmembrane electrical potential (given by charge separation across the membrane). Compared to the inner membranes of mitochondria, which have a significantly higher membrane potential due to charge separation, thylakoid membranes lack a charge gradient. To compensate for this, the 10,000 fold proton concentration gradient across the thylakoid membrane is much higher compared to a 10 fold gradient across the inner membrane of mitochondria. The resulting chemiosmotic potential between the lumen and stroma is high enough to drive ATP synthesis using the ATP synthase. As the protons travel back down the gradient through channels in ATP synthase, ADP + P_i is combined into ATP. In this manner, the light-dependent reactions are coupled to the synthesis of ATP via the proton gradient.

Thylakoid Membranes in Cyanobacteria



Thylakoids (green) inside a cyanobacterium (*Synechocystis*)

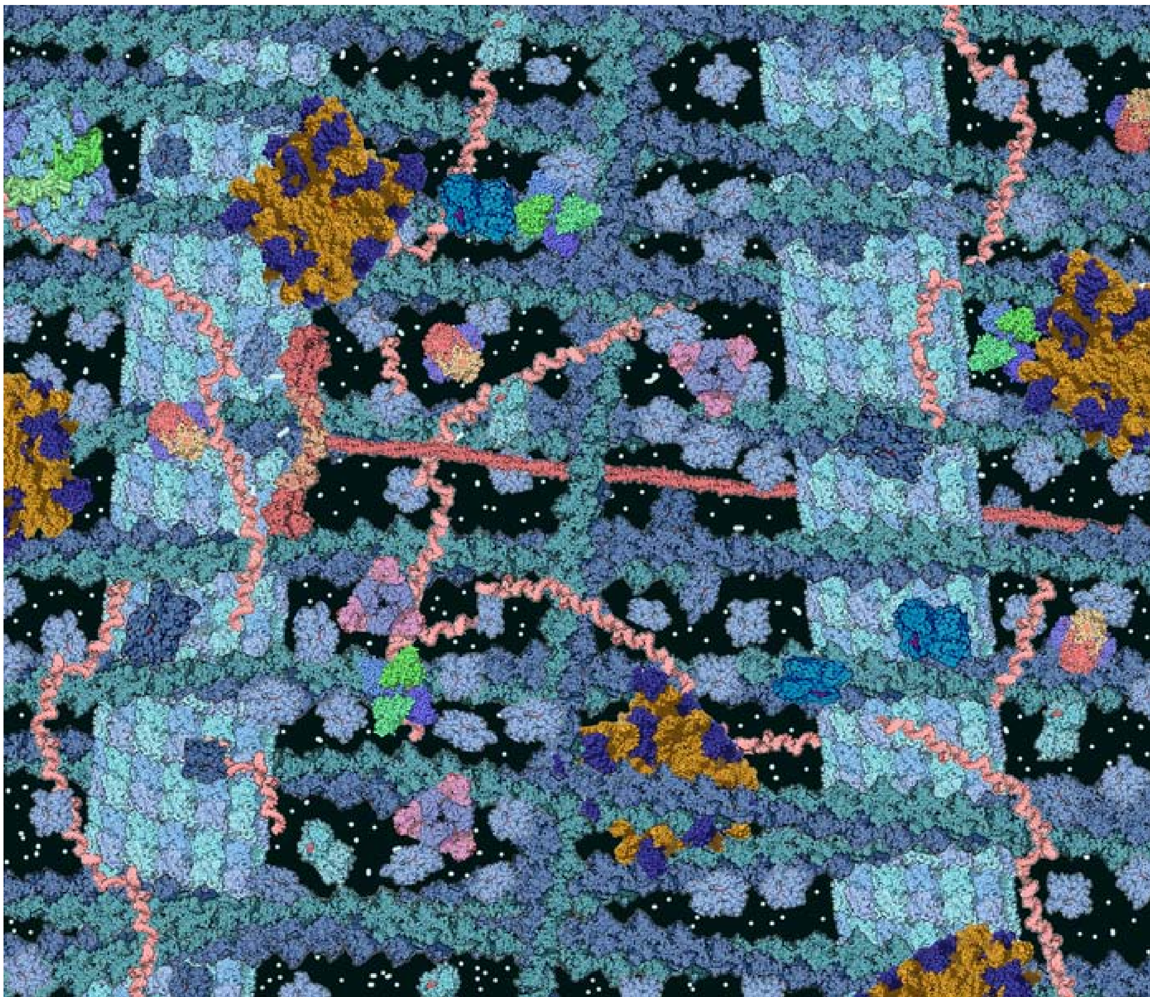
Cyanobacteria are photosynthetic prokaryotes with highly differentiated membrane systems. Cyanobacteria have an internal system of thylakoid membranes where the fully

functional electron transfer chains of photosynthesis and respiration reside. The presence of different membrane systems lends these cells a unique complexity among bacteria. Cyanobacteria must be able to reorganize the membranes, synthesize new membrane lipids, and properly target proteins to the correct membrane system. The outer membrane, plasma membrane, and thylakoid membranes each have specialized roles in the cyanobacterial cell. Understanding the organization, functionality, protein composition and dynamics of the membrane systems remains a great challenge in cyanobacterial cell biology.

WORLD TECHNOLOGIES

Chapter 13

Cytosol



The cytosol is a crowded solution of many different types of molecules that fills much of the volume of cells.

The **cytosol** or **intracellular fluid** (or **cytoplasmic matrix**) is the liquid found inside cells. It is the liquid of a cell, that is parted from other parts of the cell by cell walls, such

as the mitochondrial matrix inside the mitochondrion. The entire contents of a eukaryotic cell within cell membrane, minus the contents of the cell nucleus, are referred to as the cytoplasm. In prokaryotes, most of the chemical reactions of metabolism take place in the cytosol, while a few take place in membranes or in the periplasmic space. In eukaryotes, while many metabolic pathways still occur in the cytosol, others are contained within organelles.

The cytosol is a complex mixture of substances dissolved in water. Although water forms the large majority of the cytosol, its structure and properties within cells is not well understood. The concentrations of ions such as sodium and potassium are different in the cytosol than in the extracellular fluid; these differences in ion levels are important in processes such as osmoregulation and cell signaling. The cytosol also contains large amounts of macromolecules, which can alter how molecules behave, through macromolecular crowding.

Although once thought to be a simple solution of molecules, multiple levels of organization exist in the cytosol. These include concentration gradients of small molecules such as calcium, large complexes of enzymes that act together to carry out metabolic pathways, and protein complexes such as proteasomes and carboxysomes that enclose and separate parts of the cytosol.

Definition

The term cytosol was first introduced in 1965 by H.A. Lardy, and initially referred to the liquid that was produced by breaking cells apart and pelleting all the insoluble components by ultracentrifugation. Such a soluble cell extract is not identical to the soluble part of the cell cytoplasm and is usually called a cytoplasmic fraction. The term *cytosol* is now used to refer to the liquid phase of the cytoplasm in an intact cell, this excludes any part of the cytoplasm that is contained within organelles. Due to the possibility of confusion between the use of the word "cytosol" to refer to both extracts of cells and the soluble part of the cytoplasm in intact cells, the phrase "aqueous cytoplasm" has been used to describe the liquid contents of the cytoplasm of living cells.

Properties and composition

The proportion of cell volume that is cytosol varies: for example while this compartment forms the bulk of cell structure in bacteria, in plant cells the main compartment is the large central vacuole. The cytosol consists mostly of water, dissolved ions, small molecules, and large water-soluble molecules (such as proteins). The majority of these non-protein molecules have a molecular mass of less than 300 Da. This mixture of small molecules is extraordinarily complex, as the variety of molecules that are involved in metabolism (the metabolites) is immense. For example up to 200,000 different small molecules might be made in plants, although not all these will be present in the same species, or in a single cell. Indeed, estimates of the number of metabolites in single cells such as *E. coli* and baker's yeast predict that under 1,000 are made.

Water

Most of the cytosol is water, which makes up about 70% of the total volume of a typical cell. The pH of the extracellular fluid is 7.4. while human cytosolic pH ranges between 7.0 - 7.4, and is usually higher if a cell is growing. The viscosity of cytoplasm is roughly the same as pure water, although diffusion of small molecules through this liquid is about fourfold slower than in pure water, due mostly to collisions with the large numbers of macromolecules in the cytosol. Studies in the brine shrimp have examined how water affects cell functions; these found that reducing the amount of water in a cell below 80% of the normal level inhibits metabolism, with this decreasing progressively as the cell dries out and all metabolism halting at a water level about 30% of normal.

Although water is vital for life, the structure of this water in the cytosol is not well understood, mostly because methods such as nuclear magnetic resonance only give information on the average structure of water, and cannot measure local variations at the microscopic scale. Even the structure of pure water is poorly understood, due to the ability of water to form structures such as water clusters through hydrogen bonds.

The classic view of water in cells is that about 5% of this water is strongly bound in by solutes or macromolecules as water of solvation, while the majority has the same structure as pure water. This water of solvation is not active in osmosis and may have different solvent properties, so that some dissolved molecules are excluded, while others become concentrated. However, others argue that the effects of the high concentrations of macromolecules in cells extend throughout the cytosol and that water in cells behaves very differently from the water in dilute solutions. These ideas include the proposal that cells contain zones of low and high-density water, which could have widespread effects on the structures and functions of the other parts of the cell. However, the use of advanced nuclear magnetic resonance methods to directly measure the mobility of water in living cells contradicts this idea, as it suggests that 85% of cell water acts like that pure water, while the remainder is less mobile and probably bound to macromolecules.

Ions

The concentrations of the other ions in cytosol are quite different from those in extracellular fluid and the cytosol also contains much higher amounts of charged macromolecules such as proteins and nucleic acids than the outside of the cell.

Typical ion concentrations in mammalian cytosol and blood.

Ion	Concentration in cytosol (millimolar)	Concentration in blood (millimolar)
Potassium	139	4
Sodium	12	145
Chloride	4	116
Bicarbonate	12	29
Amino acids in	138	9

proteins		
Magnesium	0.8	1.5
Calcium	<0.0002	1.8

In contrast to extracellular fluid, cytosol has a high concentration of potassium ions and a low concentration of sodium ions. This difference in ion concentrations is critical for osmoregulation, since if the ion levels were the same inside a cell as outside, water would enter constantly by osmosis - since the levels of macromolecules inside cells are higher than their levels outside. Instead, sodium ions are expelled and potassium ions taken up by the Na^+/K^+-ATPase, potassium ions then flow down their concentration gradient through potassium-selection ion channels, this loss of positive charge creates a negative membrane potential. To balance this potential difference, negative chloride ions also exit the cell, through selective chloride channels. The loss of sodium and chloride ions compensates for the osmotic effect of the higher concentration of organic molecules inside the cell.

Cells can deal with even larger osmotic changes by accumulating osmoprotectants such as betaines or trehalose in their cytosol. Some of these molecules can allow cells to survive being completely dried out and allow an organism to enter a state of suspended animation called cryptobiosis. In this state the cytosol and osmoprotectants become a glass-like solid that helps stabilize proteins and cell membranes from the damaging effects of desiccation.

The low concentration of calcium in the cytosol allows this ion to function as a second messenger in calcium signaling. Here, a signal such as a hormone or an action potential opens calcium channels so that calcium floods into the cytosol. This sudden increase in cytosolic calcium activates other signalling molecules, such as calmodulin and protein kinase C. Other ions such as chloride and potassium may also have signaling functions in the cytosol, but these are not well understood.

Macromolecules

Protein molecules that do not bind to cell membranes or the cytoskeleton are dissolved in the cytosol. The amount of protein in cells is extremely high, and approaches 200 mg/ml, occupying about 20-30% of the volume of the cytosol. However, measuring precisely how much protein is dissolved in cytosol in intact cells is difficult, since some proteins appear to be weakly-associated with membranes or organelles in whole cells and are released into solution upon cell lysis. Indeed, in experiments where the plasma membrane of cells were carefully disrupted using saponin, without damaging the other cell membranes, only about one quarter of cell protein was released. These cells were also able to synthesize proteins if given ATP and amino acids, implying that many of the enzymes in cytosol are bound to the cytoskeleton. However, the idea that the majority of the proteins in cells are tightly bound in a network called the microtrabecular lattice is now seen as unlikely.

In prokaryotes the cytosol contains the cell's genome, within a structure known as a nucleoid. This is an irregular mass of DNA and associated proteins that control the transcription and replication of the bacterial chromosome and plasmids. In eukaryotes the genome is held within the cell nucleus, which is separated from the cytosol by nuclear pores that block the free diffusion of any molecule larger than about 10 nanometres in diameter.

This high concentration of macromolecules in cytosol causes an effect called macromolecular crowding, which is when the effective concentration of other macromolecules is increased, since they have less volume to move in. This crowding effect can produce large changes in both the rates and the position of chemical equilibrium of reactions in the cytosol. It is particularly important in its ability to alter dissociation constants by favoring the association of macromolecules, such as when multiple proteins come together to form protein complexes, or when DNA-binding proteins bind to their targets in the genome.

Organization

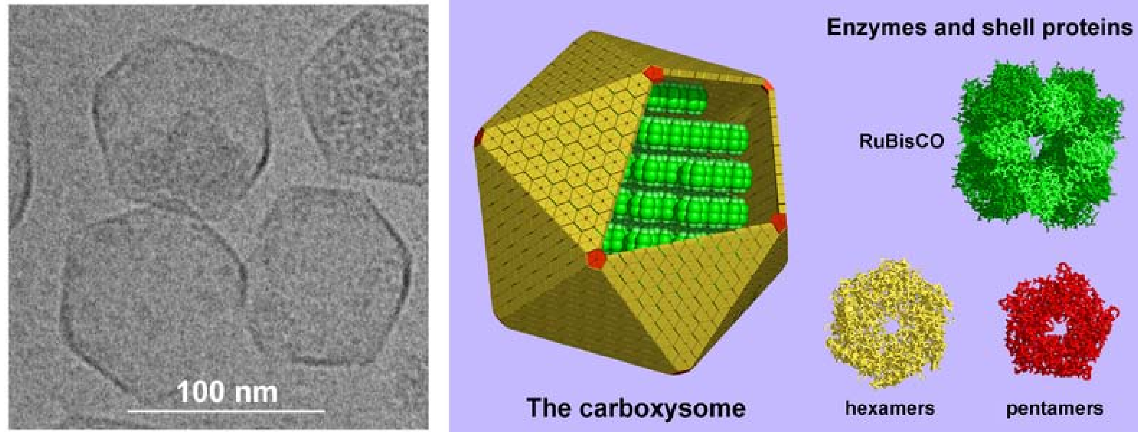
Although the components of the cytosol are not separated into regions by cell membranes, these components do not always mix randomly and several levels of organization can localize specific molecules to defined sites within the cytosol.

Concentration gradients

Although small molecules diffuse rapidly in the cytosol, concentration gradients can still be produced within this compartment. A well-studied example of these are the "calcium sparks" that are produced for a short period in the region around an open calcium channel. These are about 2 micrometres in diameter and last for only a few milliseconds, although several sparks can merge together to form larger gradients, called "calcium waves". Concentration gradients of other small molecules, such as oxygen and adenosine triphosphate may be produced in cells around clusters of mitochondria, although these are less well understood.

Protein complexes

Proteins can associate to form protein complexes, these often contain a set of proteins with similar functions, such as enzymes that carry out several steps in the same metabolic pathway. This organization can allow substrate channeling, which is when the product of one enzyme is passed directly to the next enzyme in a pathway without being released into solution. Channeling can make a pathway more rapid and efficient than it would be if the enzymes were randomly distributed in the cytosol, and can also prevent the release of unstable reaction intermediates. Although a wide variety of metabolic pathways involve enzymes that are tightly bound to each other, others may involve more loosely-associated complexes that are very difficult to study outside the cell. Consequently, the importance of these complexes for metabolism in general remains unclear.



Carboxysomes are protein-enclosed bacterial microcompartments within the cytosol. On the left is an electron microscope image of carboxysomes, and on the right a model of their structure.

Protein compartments

Some protein complexes contain a large central cavity that is isolated from the remainder of the cytosol. One example of such an enclosed compartment is the proteasome. Here, a set of subunits form a hollow barrel containing proteases that degrade cytosolic proteins. Since these would be damaging if they mixed freely with the remainder of the cytosol, the barrel is capped by a set of regulatory proteins that recognize proteins with a signal directing them for degradation (a ubiquitin tag) and feed them into the proteolytic cavity.

Another large class of protein compartments are bacterial microcompartments, which are made of a protein shell that encapsulates various enzymes. These compartments are typically about 100-200 nanometres across and made of interlocking proteins. A well-understood example is the carboxysome, which contains enzymes involved in carbon fixation such as RuBisCO.

Cytoskeletal sieving

Although the cytoskeleton is not part of the cytosol, the presence of this network of filaments restricts the diffusion of large particles in the cell. For example, in several studies tracer particles larger than about 25 nanometres (about the size of a ribosome) were excluded from parts of the cytosol around the edges of the cell and next to the nucleus. These "excluding compartments" may contain a much denser meshwork of actin fibres than the remainder of the cytosol. These microdomains could influence the distribution of large structures such as ribosomes and organelles within the cytosol by excluding them from some areas and concentrating them in others.

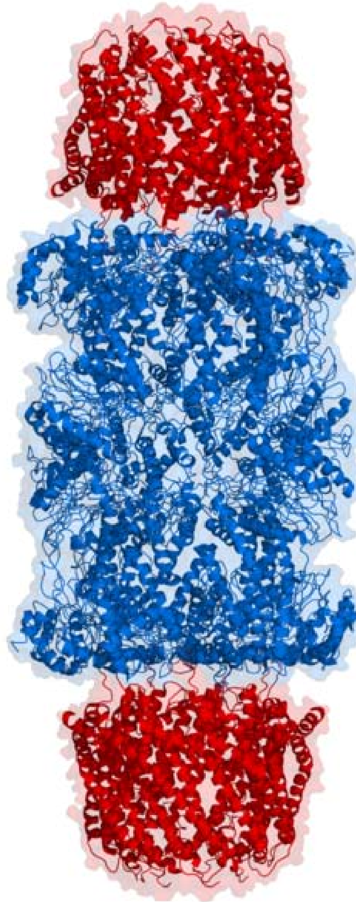
Function

The cytosol has no single function and is instead the site of multiple cell processes. Examples of these processes include signal transduction from the cell membrane to sites within the cell, such as the cell nucleus, or organelles. This compartment is also the site of many of the processes of cytokinesis, after the breakdown of the nuclear membrane in mitosis. Another major function of cytosol is to transport metabolites from their site of production to where they are used. This is relatively simple for water-soluble molecules, such as amino acids, which can diffuse rapidly through the cytosol. However, hydrophobic molecules, such as fatty acids or sterols, can be transported through the cytosol by specific binding proteins, which shuttle these molecules between cell membranes. Molecules taken into the cell by endocytosis or on their way to be secreted can also be transported through the cytosol inside vesicles, which are small spheres of lipids that are moved along the cytoskeleton by motor proteins.

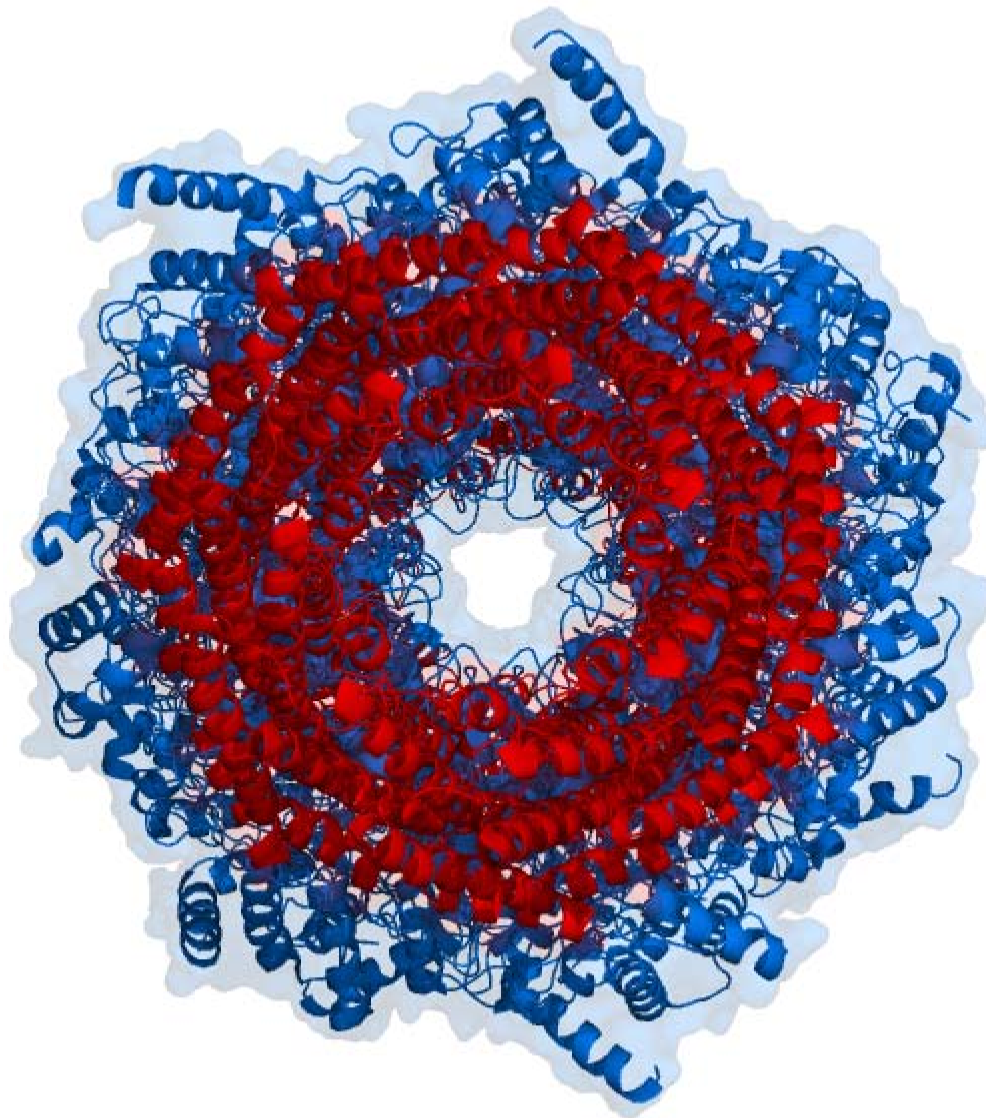
The cytosol is the site of most metabolism in prokaryotes, and a large proportion of the metabolism of eukaryotes. For instance, in mammals about half of the proteins in the cell are localized to the cytosol. The most complete data are available in yeast, where metabolic reconstructions indicate that the majority of both metabolic processes and metabolites occur in the cytosol. Major metabolic pathways that occur in the cytosol in animals are protein biosynthesis, the pentose phosphate pathway, glycolysis and gluconeogenesis. The localization of pathways can be different in other organisms, for instance fatty acid synthesis occurs in chloroplasts in plants and in apicoplasts in apicomplexa.

Chapter 14

Proteasome



Cartoon representation of a proteasome. Its active sites are sheltered inside the tube (blue). The caps (red; in this case, 11S regulatory particles) on the ends regulate entry into the destruction chamber, where the protein is degraded.



Top view of the proteasome above.

Proteasomes are very large protein complexes inside all eukaryotes and archaea, and in some bacteria. In eukaryotes, they are located in the nucleus and the cytoplasm. The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis, a chemical reaction that breaks peptide bonds. Enzymes that carry out such reactions are called proteases. Proteasomes are part of a major mechanism by which cells regulate the concentration of particular proteins and degrade misfolded proteins. The degradation process yields peptides of about seven to eight amino acids long, which can then be further degraded into amino acids and used in synthesizing new proteins. Proteins are tagged for degradation with a small protein called ubiquitin. The tagging reaction is catalyzed by enzymes called ubiquitin ligases. Once a protein is tagged with a single ubiquitin molecule, this is a signal to other ligases to attach additional ubiquitin

molecules. The result is a *polyubiquitin chain* that is bound by the proteasome, allowing it to degrade the tagged protein.

In structure, the proteasome is a cylindrical complex containing a "core" of four stacked rings around a central pore. Each ring is composed of seven individual proteins. The inner two rings are made of seven *β subunits* that contain the six protease active sites. These sites are located on the interior surface of the rings, so that the target protein must enter the central pore before it is degraded. The outer two rings each contain seven *α subunits* whose function is to maintain a "gate" through which proteins enter the barrel. These α subunits are controlled by binding to "cap" structures or *regulatory particles* that recognize polyubiquitin tags attached to protein substrates and initiate the degradation process. The overall system of ubiquitination and proteasomal degradation is known as the *ubiquitin-proteasome system*.

The proteasomal degradation pathway is essential for many cellular processes, including the cell cycle, the regulation of gene expression, and responses to oxidative stress. The importance of proteolytic degradation inside cells and the role of ubiquitin in proteolytic pathways was acknowledged in the award of the 2004 Nobel Prize in Chemistry to Aaron Ciechanover, Avram Hershko and Irwin Rose.

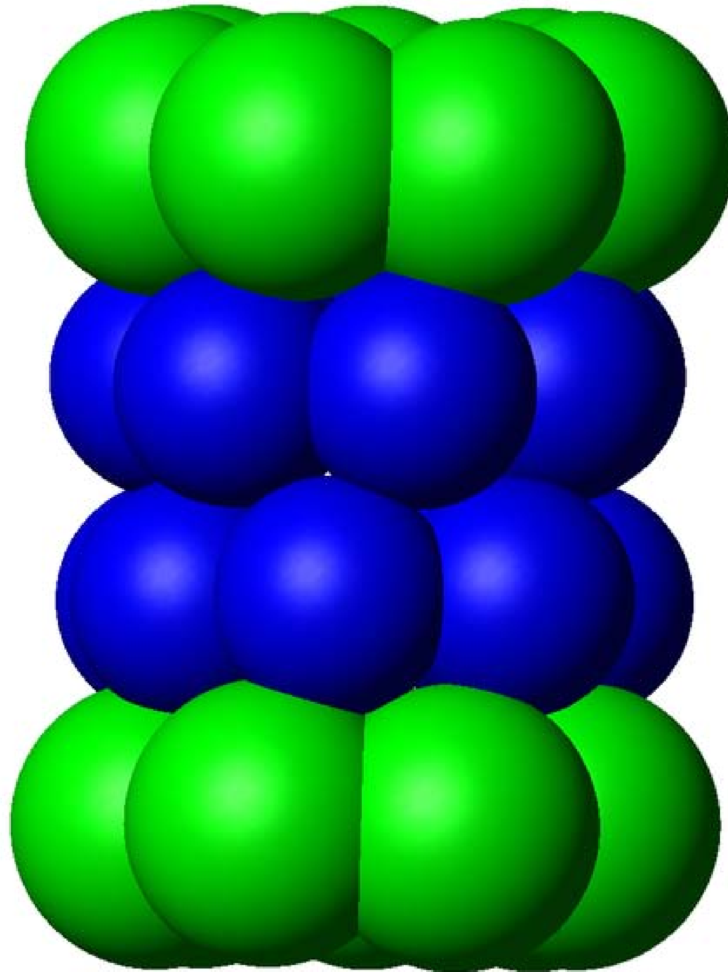
Discovery

Before the discovery of the ubiquitin proteasome system, protein degradation in cells was thought to rely mainly on lysosomes, membrane-bound organelles with acidic and protease-filled interiors that can degrade and then recycle exogenous proteins and aged or damaged organelles. However, work by Alfred Goldberg in 1977 on ATP-dependent protein degradation in reticulocytes, which lack lysosomes, suggested the presence of a second intracellular degradation mechanism. This was shown in 1978 to be composed of several distinct protein chains, a novelty among proteases at the time. Later work on modification of histones led to the identification of an unexpected covalent modification of the histone protein by a bond between a lysine side chain of the histone and the C-terminal glycine residue of ubiquitin, a protein which had no known function. It was then discovered that a previously identified protein associated with proteolytic degradation, known as ATP-dependent proteolysis factor 1 (APF-1), was the same protein as ubiquitin. Later, the ATP-dependent proteolytic complex that was responsible for ubiquitin-dependent protein degradation was discovered and was called the 26S proteasome.

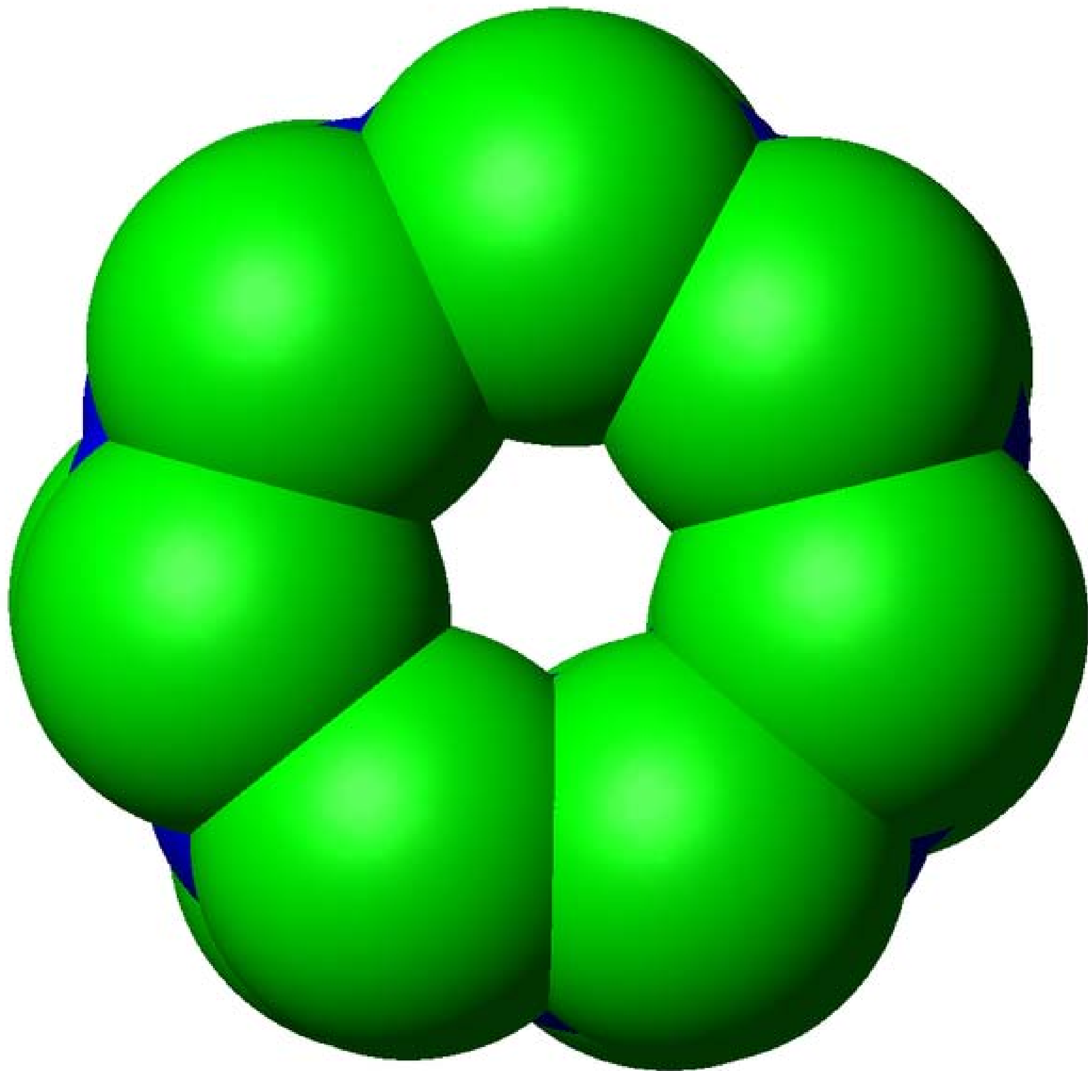
Much of the early work leading up to the discovery of the ubiquitin proteasome system occurred in the late 1970s and early 1980s at the Technion in the laboratory of Avram Hershko, where Aaron Ciechanover worked as a graduate student. Hershko's year-long sabbatical in the laboratory of Irwin Rose at the Fox Chase Cancer Center provided key conceptual insights, though Rose later downplayed his role in the discovery. The three shared the 2004 Nobel Prize in Chemistry for their work in discovering this system.

Although electron microscopy data revealing the stacked-ring structure of the proteasome became available in the mid-1980s, the first structure of the proteasome core particle was not solved by X-ray crystallography until 1994. As of 2006, no structure has been solved of the core particle in complex with the most common form of regulatory cap.

Structure and organization



A schematic diagram of the proteasome 20S core particle viewed from one side. The α subunits that make up the outer two rings are shown in green, and the β subunits that make up the inner two rings are shown in blue.



Top view of the same schematic, illustrating the seven-fold symmetry of the rings.

The proteasome subcomponents are often referred to by their Svedberg sedimentation coefficient (denoted *S*). The most common form of the proteasome is known as the 26S proteasome, which is about 2000 kilodaltons (kDa) in molecular mass and contains one 20S core particle structure and two 19S regulatory caps. The core is hollow and provides an enclosed cavity in which proteins are degraded; openings at the two ends of the core allow the target protein to enter. Each end of the core particle associates with a 19S regulatory subunit that contains multiple ATPase active sites and ubiquitin binding sites; it is this structure that recognizes polyubiquitinated proteins and transfers them to the catalytic core. An alternative form of regulatory subunit called the 11S particle can associate with the core in essentially the same manner as the 19S particle; the 11S may play a role in degradation of foreign peptides such as those produced after infection by a virus.

20S core particle

The number and diversity of subunits contained in the 20S core particle depends on the organism; the number of distinct and specialized subunits is larger in multicellular than unicellular organisms and larger in eukaryotes than in prokaryotes. All 20S particles consist of four stacked heptameric ring structures that are themselves composed of two different types of subunits; α subunits are structural in nature, whereas β subunits are predominantly catalytic. The outer two rings in the stack consist of seven α subunits each, which serve as docking domains for the regulatory particles and the alpha subunits N-termini form a gate that blocks unregulated access of substrates to the interior cavity. The inner two rings each consist of seven β subunits and contain the protease active sites that perform the proteolysis reactions. The size of the proteasome is relatively conserved and is about 150 angstroms (Å) by 115 Å. The interior chamber is at most 53 Å wide, though the entrance can be as narrow as 13 Å, suggesting that substrate proteins must be at least partially unfolded to enter.

In archaea such as *Thermoplasma acidophilum*, all the α and all the β subunits are identical, while eukaryotic proteasomes such as those in yeast contain seven distinct types of each subunit. In mammals, the β1, β2, and β5 subunits are catalytic; although they share a common mechanism, they have three distinct substrate specificities considered chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide-hydrolyzing (PHGH). Alternative β forms denoted β1i, β2i, and β5i can be expressed in hematopoietic cells in response to exposure to pro-inflammatory signals such as cytokines, in particular, interferon gamma. The proteasome assembled with these alternative subunits is known as the *immunoproteasome*, whose substrate specificity is altered relative to the normal proteasome.

19S regulatory particle

The 19S particle in eukaryotes consists of 19 individual proteins and is divisible into two subassemblies, a 10-protein base that binds directly to the α ring of the 20S core particle, and a 9-protein lid where polyubiquitin is bound. Six of the ten base proteins are ATPase subunits from the AAA Family, and an evolutionary homolog of these ATPases exists in archaea, called PAN (Proteasome Activating Nucleotidase). The association of the 19S and 20S particles requires the binding of ATP to the 19S ATPase subunits, and ATP hydrolysis is required for the assembled complex to degrade folded and ubiquitinated proteins. Interestingly, only the step of substrate unfolding requires energy from ATP hydrolysis, while ATP-binding alone can support all the other steps required for protein degradation (e.g. complex assembly, gate opening, translocation and proteolysis). In fact, ATP binding to the ATPases by itself supports the rapid degradation of unfolded proteins. However, while ATP hydrolysis is required for unfolding only it is not yet clear whether this energy may be used in the coupling of some of these steps. As of 2011, the atomic structure of the 26S proteasome has not been solved, despite massive efforts to do so. Nevertheless, it is understood generally how the 19S associates with and regulates the 20S core particle. In fact, the 19S and 11S particles bind to the same sites in the α rings of the 20S core particle although, they each induce gate opening by different mechanism.

Regulation of the 20S by the 19S

The 19S regulatory particle is responsible for stimulating the 20S to degrade proteins. A primary function of the 19S regulatory ATPases is to open the gate in the 20S that blocks the entry of substrates into the degradation chamber. The mechanism by which the proteasomal ATPase open this gate has been recently elucidated. 20S gate opening, and thus substrate degradation, requires the C-termini of the proteasomal ATPases, which contains a specific motif (i.e. HbYX motif). The ATPases C-termini bind into pockets in the top of the 20S, and tether the ATPase complex to the 20S proteolytic complex thus joining the substrate unfolding equipment with the 20S degradation machinery. Binding of these C-termini into these 20S pockets by themselves stimulates opening of the gate in the 20S much like a "key-in-a-lock" opens a door. The precise mechanism by which this "key-in-a-lock" mechanism functions has been structurally elucidated.

11S regulatory particle

20S proteasomes can also associate with a second type of regulatory particle, the 11S regulatory particle, a heptameric structure that does not contain any ATPases and can promote the degradation of short peptides, but not of complete proteins. It is presumed that this is because the complex cannot unfold larger substrates. This structure is also known as PA28 or REG. The mechanisms by which it binds to the core particle through the C-terminal tails of its subunits and induces α-ring conformational changes to open the 20S gate suggest a similar mechanism for the 19S particle. The expression of the 11S particle is induced by interferon gamma and is responsible, in conjunction with the immunoproteasome β subunits, for the generation of peptides that bind to the major histocompatibility complex.

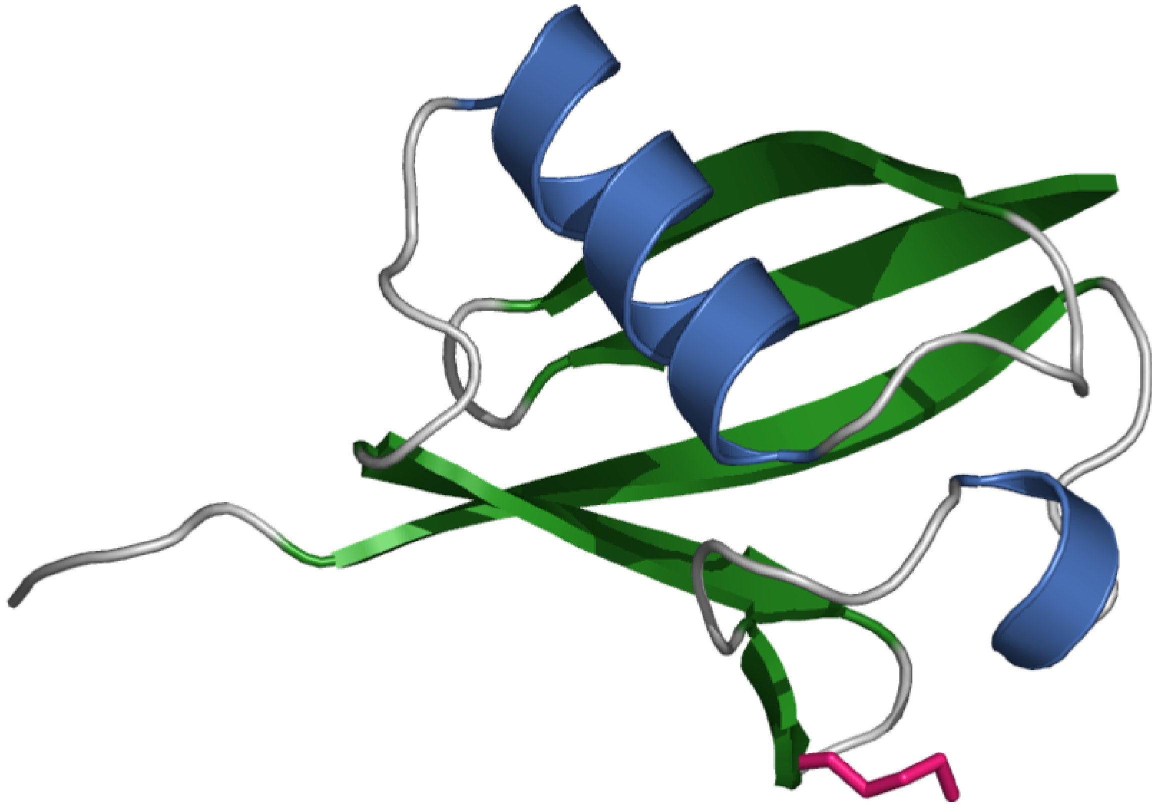
Assembly

The assembly of the proteasome is a complex process due to the number of subunits that must associate to form an active complex. The β subunits are synthesized with N-terminal "propeptides" that are post-translationally modified during the assembly of the 20S particle to expose the proteolytic active site. The 20S particle is assembled from two half-proteasomes, each of which consists of a seven-membered pro-β ring attached to a seven-membered α ring. The association of the β rings of the two half-proteasomes triggers threonine-dependent autolysis of the propeptides to expose the active site. These β interactions are mediated mainly by salt bridges and hydrophobic interactions between conserved alpha helices whose disruption by mutation damages the proteasome's ability to assemble. The assembly of the half-proteasomes, in turn, is initiated by the assembly of the α subunits into their heptameric ring, forming a template for the association of the corresponding pro-β ring. The assembly of α subunits has not been characterized.

In general, less is known about the assembly and maturation of the 19S regulatory particles. They are believed to assemble as two distinct subcomponents, the ATPase-containing base and the ubiquitin-recognizing lid. The six ATPases in the base may assemble in a pairwise manner mediated by coiled-coil interactions. The order in which

the nineteen subunits of the regulatory particle are bound is a likely regulatory mechanism that prevents exposure of the active site before assembly is complete.

The protein degradation process



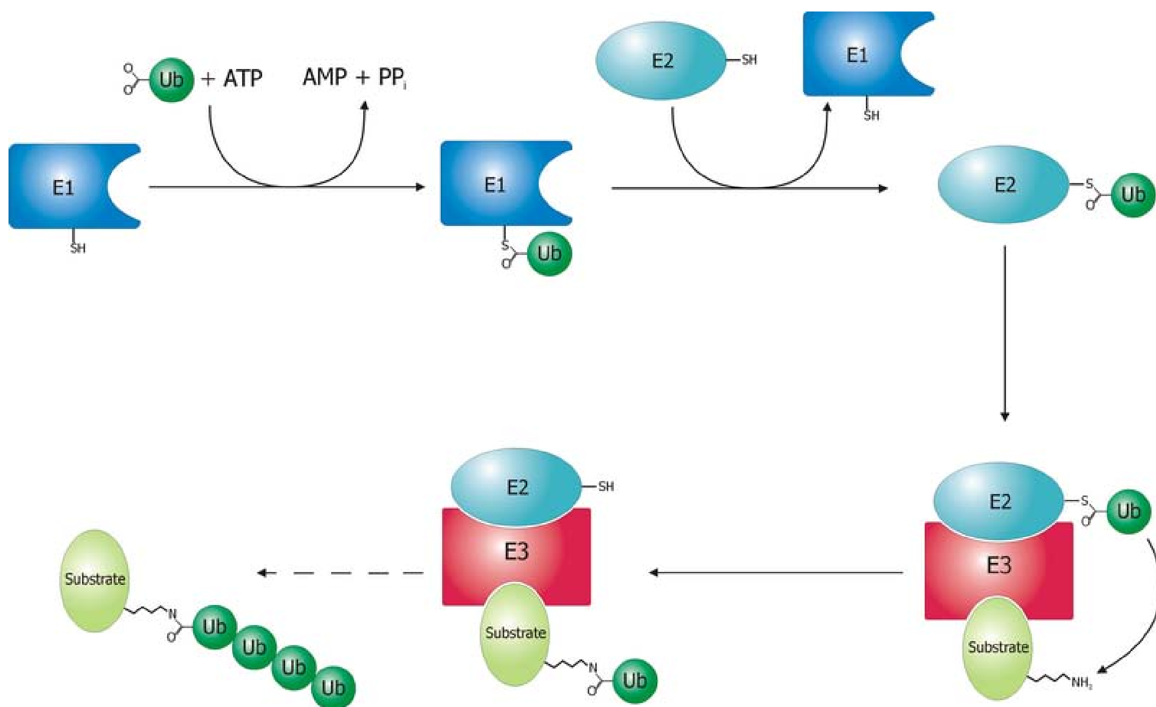
Ribbon diagram of ubiquitin, the highly conserved protein that serves as a molecular tag targeting proteins for degradation by the proteasome

Ubiquitination and targeting

Proteins are targeted for degradation by the proteasome by covalent modification of a lysine residue that requires the coordinated reactions of three enzymes. In the first step, a ubiquitin-activating enzyme (known as E1) hydrolyzes ATP and adenylates a ubiquitin molecule. This is then transferred to E1's active-site cysteine residue in concert with the adenylation of a second ubiquitin. This adenylated ubiquitin is then transferred to a cysteine of a second enzyme, ubiquitin-conjugating enzyme (E2). In the last step, a member of a highly diverse class of enzymes known as ubiquitin ligases (E3) recognizes the specific protein to be ubiquitinated and catalyzes the transfer of ubiquitin from E2 to this target protein. A target protein must be labeled with at least four ubiquitin monomers (in the form of a polyubiquitin chain) before it is recognized by the proteasome lid. It is therefore the E3 that confers substrate specificity to this system. The number of E1, E2, and E3 proteins expressed depends on the organism and cell type, but there are many different E3 enzymes present in humans, indicating that there is a huge number of targets for the ubiquitin proteasome system.

The mechanism by which a polyubiquitinated protein is targeted to the proteasome is not fully understood. Ubiquitin-receptor proteins have an N-terminal ubiquitin-like (UBL) domain and one or more ubiquitin-associated (UBA) domains. The UBL domains are recognized by the 19S proteasome caps and the UBA domains bind ubiquitin via three-helix bundles. These receptor proteins may escort polyubiquitinated proteins to the proteasome, though the specifics of this interaction and its regulation are unclear.

The ubiquitin protein itself is 76 amino acids long and was named due to its ubiquitous nature, as it has a highly conserved sequence and is found in all known eukaryotic organisms. The genes encoding ubiquitin in eukaryotes are arranged in tandem repeats, possibly due to the heavy transcription demands on these genes to produce enough ubiquitin for the cell. It has been proposed that ubiquitin is the slowest-evolving protein identified to date.



The ubiquitination pathway

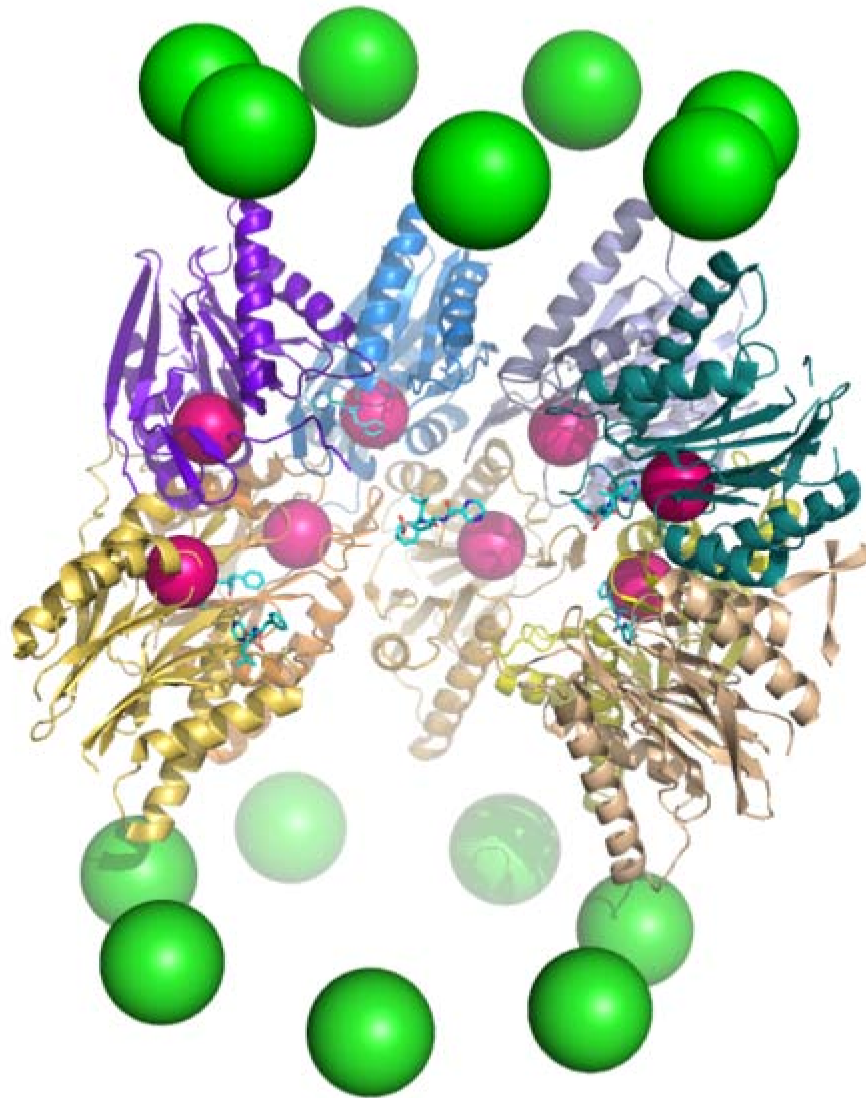
Unfolding and translocation

After a protein has been ubiquitinated, it is recognized by the 19S regulatory particle in an ATP-dependent binding step. The substrate protein must then enter the interior of the 20S particle to come in contact with the proteolytic active sites. Because the 20S particle's central channel is narrow and gated by the N-terminal tails of the α ring subunits, the substrates must be at least partially unfolded before they enter the core. The passage of the unfolded substrate into the core is called *translocation* and necessarily occurs after deubiquitination. However, the order in which substrates are deubiquitinated and unfolded is not yet clear. Which of these processes is the rate-limiting step in the

overall proteolysis reaction depends on the specific substrate; for some proteins, the unfolding process is rate-limiting, while deubiquitination is the slowest step for other proteins. The extent to which substrates must be unfolded before translocation is not known, but substantial tertiary structure, and in particular nonlocal interactions such as disulfide bonds, are sufficient to inhibit degradation.

The gate formed by the α subunits prevents peptides longer than about four residues from entering the interior of the 20S particle. The ATP molecules bound before the initial recognition step are hydrolyzed before translocation. While energy is needed for substrate unfolding it is not required for translocation. The assembled 26S proteasome can degrade unfolded proteins in the presence of a non-hydrolyzable ATP analog, but cannot degrade folded proteins, indicating that energy from ATP hydrolysis is used for substrate unfolding. Passage of the unfolded substrate through the opened gate occurs via facilitated diffusion if the 19S cap is in the ATP-bound state.

The mechanism for unfolding of globular proteins is necessarily general, but somewhat dependent on the amino acid sequence. Long sequences of alternating glycine and alanine have been shown to inhibit substrate unfolding decreasing the efficiency of proteasomal degradation; this results in the release of partially degraded byproducts, possibly due to the decoupling of the ATP hydrolysis and unfolding steps. Such glycine-alanine repeats are also found in nature, for example in silk fibroin; in particular, certain Epstein-Barr virus gene products bearing this sequence can stall the proteasome, helping the virus propagate by preventing antigen presentation on the major histocompatibility complex.



A cutaway view of the proteasome 20S core particle illustrating the locations of the active sites. The α subunits are represented as green spheres and the β subunits as protein backbones colored by individual polypeptide chain. The small pink spheres represent the location of the active-site threonine residue in each subunit. Light blue chemical structures are the inhibitor bortezomib bound to the active sites.

Proteolysis

The mechanism of proteolysis by the β subunits of the 20S core particle is through a threonine-dependent nucleophilic attack. This mechanism may depend on an associated water molecule for deprotonation of the reactive threonine hydroxyl. Degradation occurs within the central chamber formed by the association of the two β rings and normally does not release partially degraded products, instead reducing the substrate to short

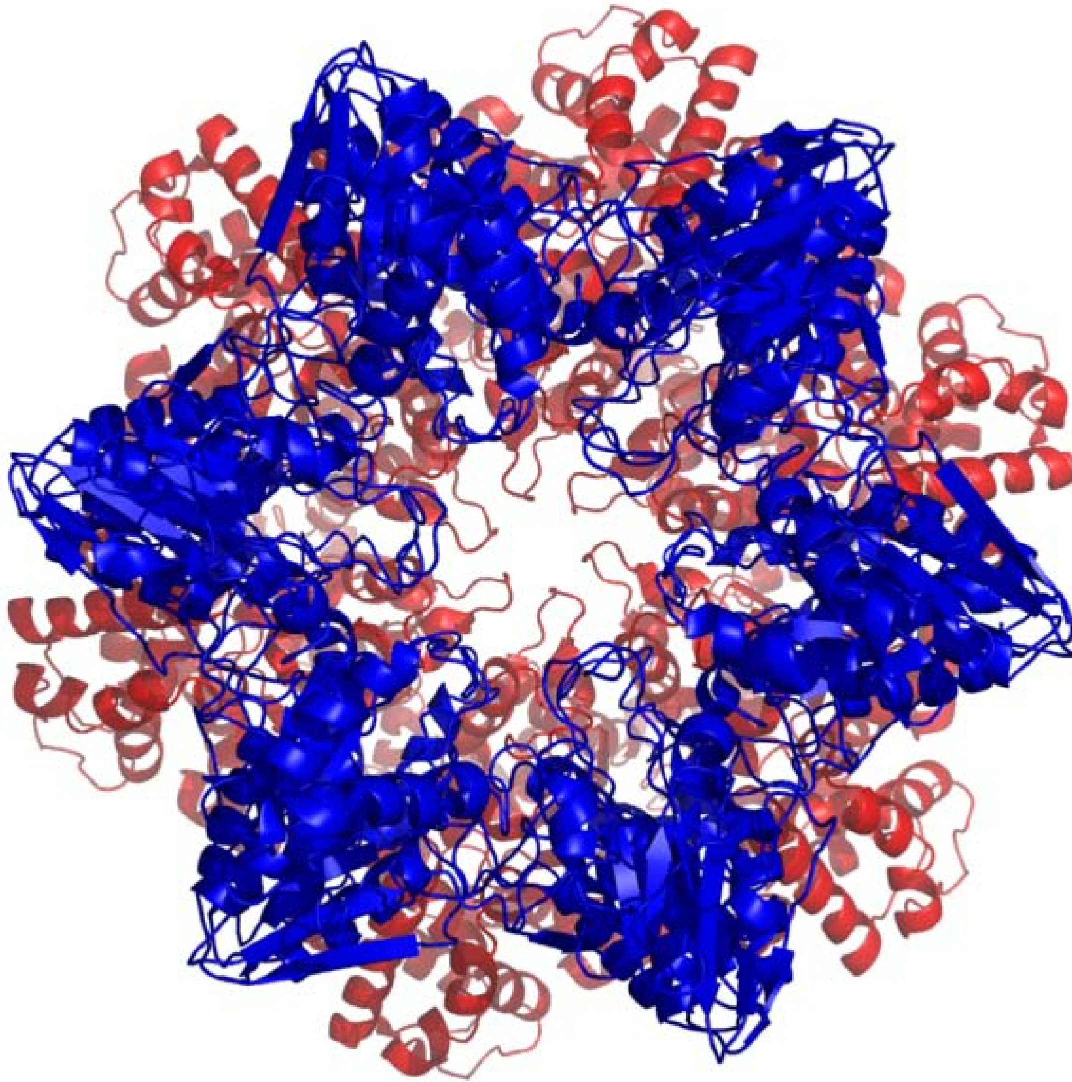
polypeptides typically 7–9 residues long, though they can range from 4 to 25 residues depending on the organism and substrate. The biochemical mechanism that determines product length is not fully characterized. Although the three catalytic β subunits have a common mechanism, they have slightly different substrate specificities, which are considered chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide-hydrolyzing (PHGH)-like. These variations in specificity are the result of interatomic contacts with local residues near the active sites of each subunit. Each catalytic β subunit also possesses a conserved lysine residue required for proteolysis.

Although the proteasome normally produces very short peptide fragments, in some cases these products are themselves biologically active and functional molecules. Certain transcription factors regulating the expression of specific genes, including one component of the mammalian complex NF-κB, are synthesized as inactive precursors whose ubiquitination and subsequent proteasomal degradation converts them to an active form. Such activity requires the proteasome to cleave the substrate protein internally: rather than processively degrading it from one terminus. It has been suggested that long loops on these proteins' surfaces serve as the proteasomal substrates and enter the central cavity, while the majority of the protein remains outside. Similar effects have been observed in yeast proteins; this mechanism of selective degradation is known as *regulated ubiquitin/proteasome dependent processing* (RUP).

Ubiquitin-independent degradation

Although most proteasomal substrates must be ubiquitinated before being degraded, there are some exceptions to this general rule, especially when the proteasome plays a normal role in the post-translational processing of the protein. The proteasomal activation of NF-κB by processing p105 into p50 via internal proteolysis is one major example. Some proteins that are hypothesized to be unstable due to intrinsically unstructured regions, are degraded in a ubiquitin-independent manner. The most well-known example of a ubiquitin-independent proteasome substrate is the enzyme ornithine decarboxylase. Ubiquitin-independent mechanisms targeting key cell cycle regulators such as p53 have also been reported, although p53 is also subject to ubiquitin-dependent degradation. Finally, structurally abnormal, misfolded, or highly oxidized proteins are also subject to ubiquitin-independent and 19S-independent degradation under conditions of cellular stress.

Evolution



The assembled complex of hslV (blue) and hslU (red) from *E. coli*. This complex of heat shock proteins is thought to resemble the ancestor of the modern proteasome.

The 20S proteasome is both ubiquitous and essential in eukaryotes. Some prokaryotes, including many archaea and the bacterial order Actinomycetales also share homologs of the 20S proteasome, whereas most bacteria possess heat shock genes hslV and hslU, whose gene products are a multimeric protease arranged in a two-layered ring and an ATPase. The hslV protein has been hypothesized to resemble the likely ancestor of the 20S proteasome. In general, HslV is not essential in bacteria, and not all bacteria possess it, whereas some protists possess both the 20S and the hslV systems.

Sequence analysis suggests that the catalytic β subunits diverged earlier in evolution than the predominantly structural α subunits. In bacteria that express a 20S proteasome, the β

subunits have high sequence identity to archaeal and eukaryotic β subunits, whereas the α sequence identity is much lower. The presence of 20S proteasomes in bacteria may result from lateral gene transfer, while the diversification of subunits among eukaryotes is ascribed to multiple gene duplication events.

Cell cycle control

Cell cycle progression is controlled by ordered action of cyclin-dependent kinases (CDKs), activated by specific cyclins that demarcate phases of the cell cycle. Mitotic cyclins, which persist in the cell for only a few minutes, have one of the shortest life spans of all intracellular proteins. After a CDK-cyclin complex has performed its function, the associated cyclin is polyubiquitinated and destroyed by the proteasome, which provides directionality for the cell cycle. In particular, exit from mitosis requires the proteasome-dependent dissociation of the regulatory component cyclin B from the mitosis promoting factor complex. In vertebrate cells, "slippage" through the mitotic checkpoint leading to premature M phase exit can occur despite the delay of this exit by the spindle checkpoint.

Earlier cell cycle checkpoints such as post-restriction point check between G_1 phase and S phase similarly involve proteasomal degradation of cyclin A, whose ubiquitination is promoted by the anaphase promoting complex (APC), an E3 ubiquitin ligase. The APC and the Skp1/Cul1/F-box protein complex (SCF complex) are the two key regulators of cyclin degradation and checkpoint control; the SCF itself is regulated by the APC via ubiquitination of the adaptor protein, Skp2, which prevents SCF activity before the G1-S transition.

Individual components of the 19S particle have their own regulatory roles. Gankyrin, a recently identified oncoprotein, is one of the 19S subcomponents that also tightly binds the cyclin-dependent kinase CDK4 and plays a key role in recognizing ubiquitinated p53, via its affinity for the ubiquitin ligase MDM2. Gankyrin is anti-apoptotic and has been shown to be overexpressed in some tumor cell types such as hepatocellular carcinoma.

Regulation of plant growth

In plants, signaling by auxins, or phytohormones that order the direction and tropism of plant growth, induces the targeting of a class of transcription factor repressors known as Aux/IAA proteins for proteasomal degradation. These proteins are ubiquitinated by SCFTIR1, or SCF in complex with the auxin receptor TIR1. Degradation of Aux/IAA proteins derepresses transcription factors in the auxin-response factor (ARF) family and induces ARF-directed gene expression. The cellular consequences of ARF activation depend on the plant type and developmental stage, but are involved in directing growth in roots and leaf veins. The specific response to ARF derepression is thought to be mediated by specificity in the pairing of individual ARF and Aux/IAA proteins.

Apoptosis

Both internal and external signals can lead to the induction of apoptosis, or programmed cell death. The resulting deconstruction of cellular components is primarily carried out by specialized proteases known as caspases, but the proteasome also plays important and diverse roles in the apoptotic process. The involvement of the proteasome in this process is indicated by both the increase in protein ubiquitination, and of E1, E2, and E3 enzymes that is observed well in advance of apoptosis, During apoptosis, proteasomes localized to the nucleus have also been observed to translocate to outer membrane blebs characteristic of apoptosis.

Proteasome inhibition has different effects on apoptosis induction in different cell types. In general, the proteasome is not required for apoptosis, although inhibiting it is pro-apoptotic in most cell types that have been studied. Apoptosis is mediated through disrupting the regulated degradation of pro-growth cell cycle proteins. However, some cell lines — in particular, primary cultures of quiescent and differentiated cells such as thymocytes and neurons — are prevented from undergoing apoptosis on exposure to proteasome inhibitors. The mechanism for this effect is not clear, but is hypothesized to be specific to cells in quiescent states, or to result from the differential activity of the pro-apoptotic kinase JNK. The ability of proteasome inhibitors to induce apoptosis in rapidly dividing cells has been exploited in several recently developed chemotherapy agents such as bortezomib and salinosporamide A.

Response to cellular stress

In response to cellular stresses – such as infection, heat shock, or oxidative damage – heat shock proteins that identify misfolded or unfolded proteins and target them for proteasomal degradation are expressed. Both Hsp27 and Hsp90—chaperone proteins have been implicated in increasing the activity of the ubiquitin-proteasome system, though they are not direct participants in the process. Hsp70, on the other hand, binds exposed hydrophobic patches on the surface of misfolded proteins and recruits E3 ubiquitin ligases such as CHIP to tag the proteins for proteasomal degradation. The CHIP protein (carboxyl terminus of Hsp70-interacting protein) is itself regulated via inhibition of interactions between the E3 enzyme CHIP and its E2 binding partner.

Similar mechanisms exist to promote the degradation of oxidatively damaged proteins via the proteasome system. In particular, proteasomes localized to the nucleus are regulated by PARP and actively degrade inappropriately oxidized histones. Oxidized proteins, which often form large amorphous aggregates in the cell, can be degraded directly by the 20S core particle without the 19S regulatory cap and do not require ATP hydrolysis or tagging with ubiquitin. However, high levels of oxidative damage increases the degree of cross-linking between protein fragments, rendering the aggregates resistant to proteolysis. Larger numbers and sizes of such highly oxidized aggregates are associated with aging.

Dysregulation of the ubiquitin proteasome system may contribute to several neural diseases. It may lead to brain tumors such as astrocytomas. In some of the late-onset

neurodegenerative diseases that share aggregation of misfolded proteins as a common feature, such as Parkinson's disease and Alzheimer's disease, large insoluble aggregates of misfolded proteins can form and then result in neurotoxicity, through mechanisms that are not yet well understood. Decreased proteasome activity has been suggested as a cause of aggregation and Lewy body formation in Parkinson's. This hypothesis is supported by the observation that yeast models of Parkinson's are more susceptible to toxicity from α-synuclein, the major protein component of Lewy bodies, under conditions of low proteasome activity. Impaired proteasomal activity may underlie cognitive disorders such as the autism spectrum disorders, and muscle and nerve diseases such as inclusion body myopathy.

Role in the immune system

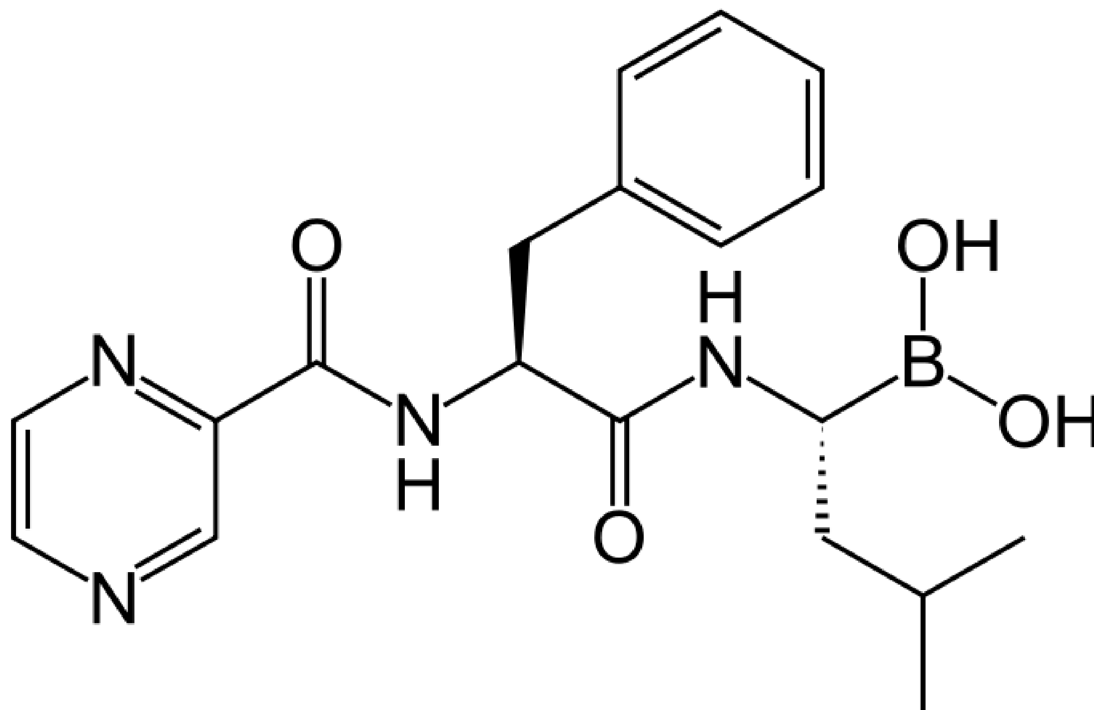
The proteasome plays a straightforward but critical role in the function of the adaptive immune system. Peptide antigens are displayed by the major histocompatibility complex class I (MHC) proteins on the surface of antigen-presenting cells. These peptides are products of proteasomal degradation of proteins originated by the invading pathogen. Although constitutively expressed proteasomes can participate in this process, a specialized complex composed of proteins whose expression is induced by interferon gamma produces peptides of the optimal size and composition for MHC binding. These proteins whose expression increases during the immune response include the 11S regulatory particle, whose main known biological role is regulating the production of MHC ligands, and specialized β subunits called β1i, β2i, and β5i with altered substrate specificity. The complex formed with the specialized β subunits is known as the *immunoproteasome*. Another β5i variant subunit, β5t, is expressed in the thymus, leading to a thymus-specific "thymoproteasome" whose function is as yet unclear.

The strength of MHC class I ligand binding is dependent on the composition of the ligand C-terminus, as peptides bind by hydrogen bonding and by close contacts with a region called the "B pocket" on the MHC surface. Many MHC class I alleles prefer hydrophobic C-terminal residues, and the immunoproteasome complex is more likely to generate hydrophobic C-termini.

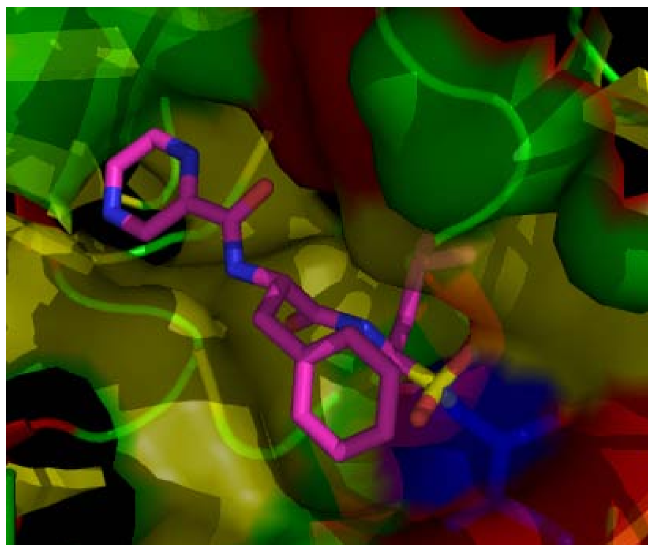
Due to its role in generating the activated form of NF-κB, an anti-apoptotic and pro-inflammatory regulator of cytokine expression, proteasomal activity has been linked to inflammatory and autoimmune diseases. Increased levels of proteasome activity correlate with disease activity and have been implicated in autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis.

The proteasome is also involved in Intracellular antibody-mediated proteolysis of antibody bound virions. In this neutralisation pathway, TRIM21 (a protein of the tripartite motif family) binds with immunoglobulin G to direct the virion to the proteasome where it is degraded.

Proteasome inhibitors



Chemical structure of bortezomib, a proteasome inhibitor used in chemotherapy that is particularly effective against multiple myeloma



Bortezomib bound to the core particle in a yeast proteasome. The bortezomib molecule is in the center colored by atom type (carbon = pink, nitrogen = blue, oxygen = red, boron = yellow), surrounded by the local protein surface. The blue patch is the catalytic threonine residue whose activity is blocked by the presence of bortezomib.

Proteasome inhibitors have effective anti-tumor activity in cell culture, inducing apoptosis by disrupting the regulated degradation of pro-growth cell cycle proteins. This approach of selectively inducing apoptosis in tumor cells has proven effective in animal models and human trials. Bortezomib, a molecule developed by Millennium Pharmaceuticals and marketed as Velcade, is the first proteasome inhibitor to reach clinical use as a chemotherapy agent. Bortezomib is used in the treatment of multiple myeloma. Notably, multiple myeloma has been observed to result in increased proteasome levels in blood serum that decrease to normal levels in response to successful chemotherapy. Studies in animals have indicated that bortezomib may also have clinically significant effects in pancreatic cancer. Preclinical and early clinical studies have been started to examine bortezomib's effectiveness in treating other B-cell-related cancers, particularly some types of non-Hodgkin's lymphoma.

The molecule ritonavir, marketed as Norvir, was developed as a protease inhibitor and used to target HIV infection. However, it has been shown to inhibit proteasomes as well as free proteases; to be specific, the chymotrypsin-like activity of the proteasome is inhibited by ritonavir, while the trypsin-like activity is somewhat enhanced. Studies in animal models suggest that ritonavir may have inhibitory effects on the growth of glioma cells.

Proteasome inhibitors have also shown promise in treating autoimmune diseases in animal models. For example, studies in mice bearing human skin grafts found a reduction in the size of lesions from psoriasis after treatment with a proteasome inhibitor. Inhibitors also show positive effects in rodent models of asthma.

Labeling and inhibition of the proteasome is also of interest in laboratory settings for both *in vitro* and *in vivo* study of proteasomal activity in cells. The most commonly used laboratory inhibitors are lactacystin, a natural product synthesized by *Streptomyces* bacteria, and peptide MG132. Fluorescent inhibitors have also been developed to specifically label the active sites of the assembled proteasome.