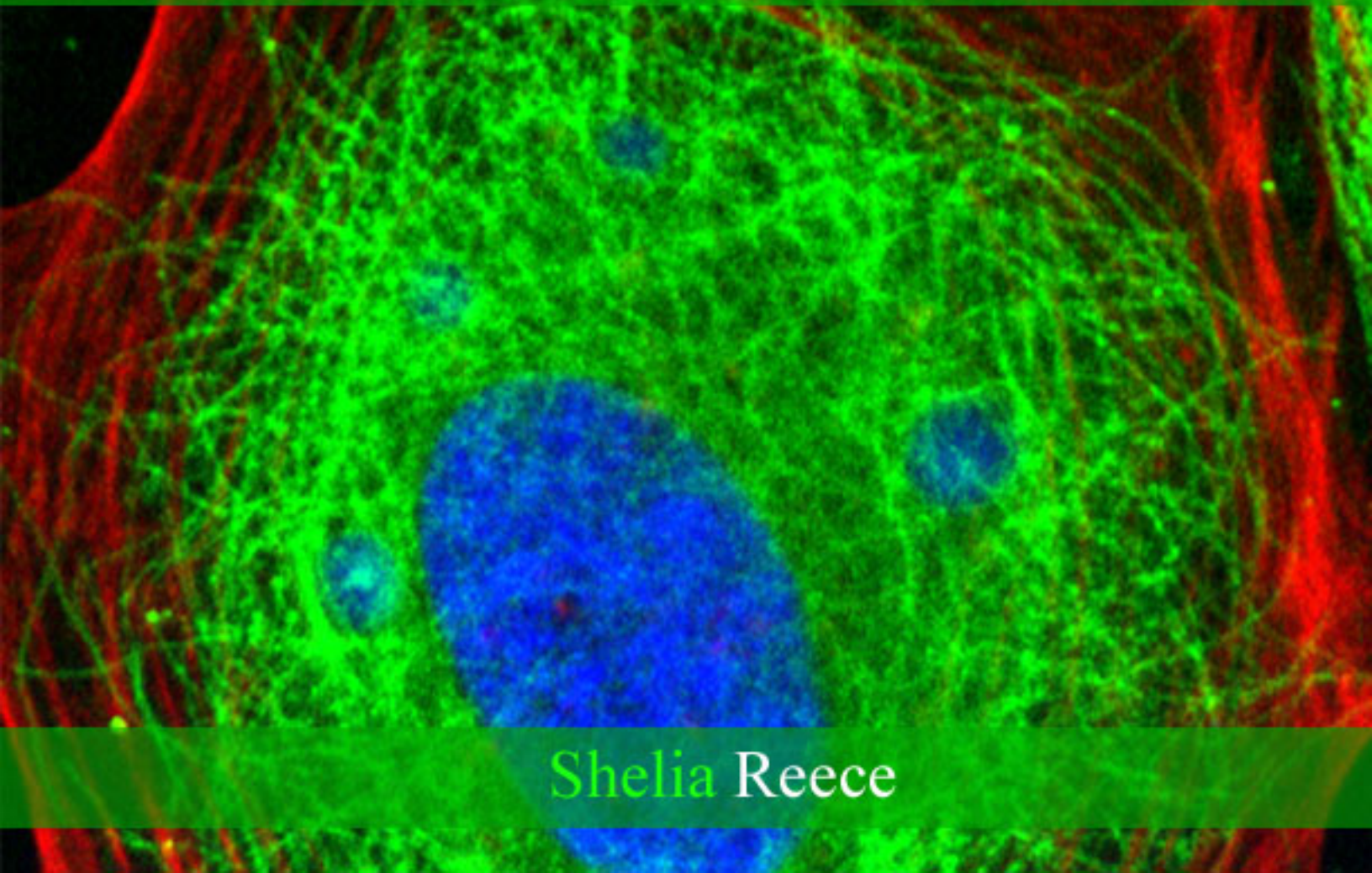
Advanced Cell Biology



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First Edition, 2012

ISBN 978-81-323-3117-9

Published by:
Research World
4735/22 Prakashdeep Bldg,
Ansari Road, Darya Ganj,
Delhi - 110002
Email: info@wtbooks.com

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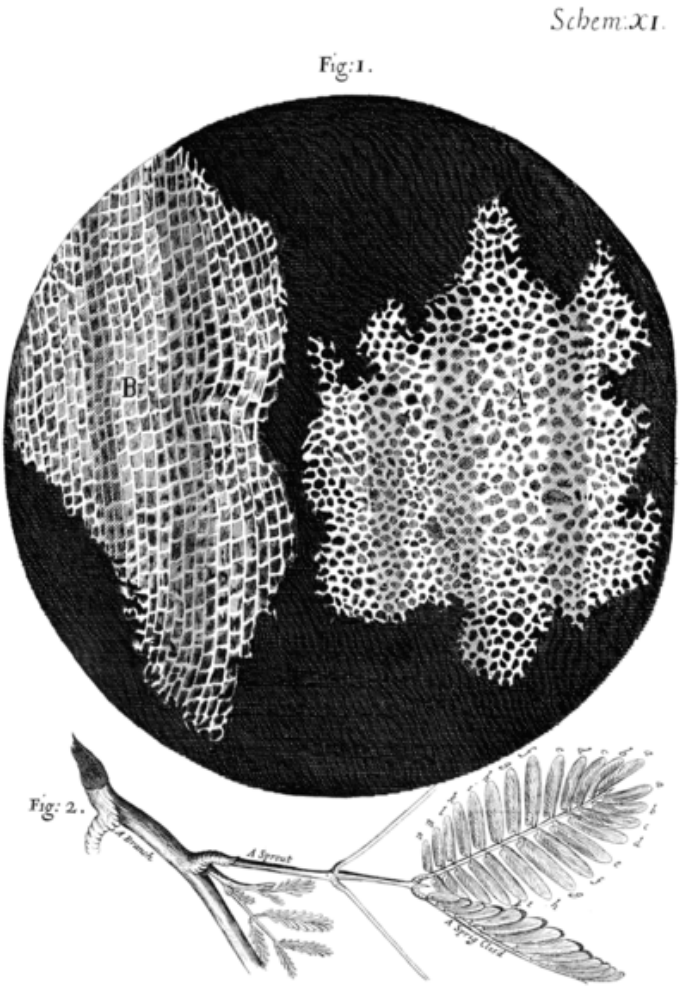
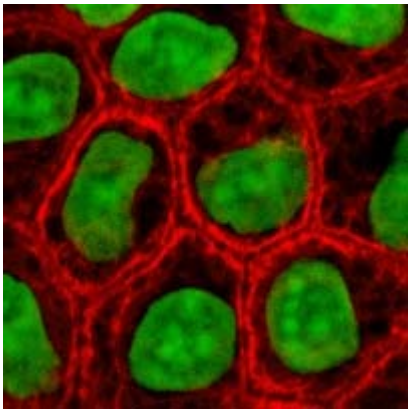
Cell (Biology)



Drawing of the structure of cork as it appeared under the microscope to Robert Hooke from Micrographia which is the origin of the word "**cell**" being used to describe the smallest unit of a living organism.



Cells in culture, stained for keratin (red) and DNA (green)

The **cell** is the functional basic unit of life. It was discovered by Robert Hooke and is the functional unit of all known living organisms. It is the smallest unit of life that is classified as a living thing, and is often called the building block of life. Some organisms, such as most bacteria, are unicellular (consist of a single cell). Other organisms, such as humans, are multicellular. Humans have about 100 trillion or 10^{14} cells; a typical cell size is 10 µm and a typical cell mass is 1 nanogram. The largest cells are about 135 µm in the anterior horn in the spinal cord while granule cells in the cerebellum, the smallest, can be some 4 µm and the longest cell can reach from the toe to the lower brain stem (Pseudounipolar cells). The largest known cells are unfertilised ostrich egg cells which weigh 3.3 pounds.

In 1835, before the final cell theory was developed, Jan Evangelista Purkyně observed small "granules" while looking at the plant tissue through a microscope. The cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that all cells come from preexisting cells, that vital functions of an organism occur within cells, and that all cells contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells.

The word *cell* comes from the Latin *cellula*, meaning, a small room. The descriptive term for the smallest living biological structure was coined by Robert Hooke in a book he published in 1665 when he compared the cork cells he saw through his microscope to the small rooms monks lived in.

Anatomy

There are two types of cells: eukaryotic and prokaryotic. Prokaryotic cells are usually independent, while eukaryotic cells are often found in multicellular organisms.

Prokaryotic cells

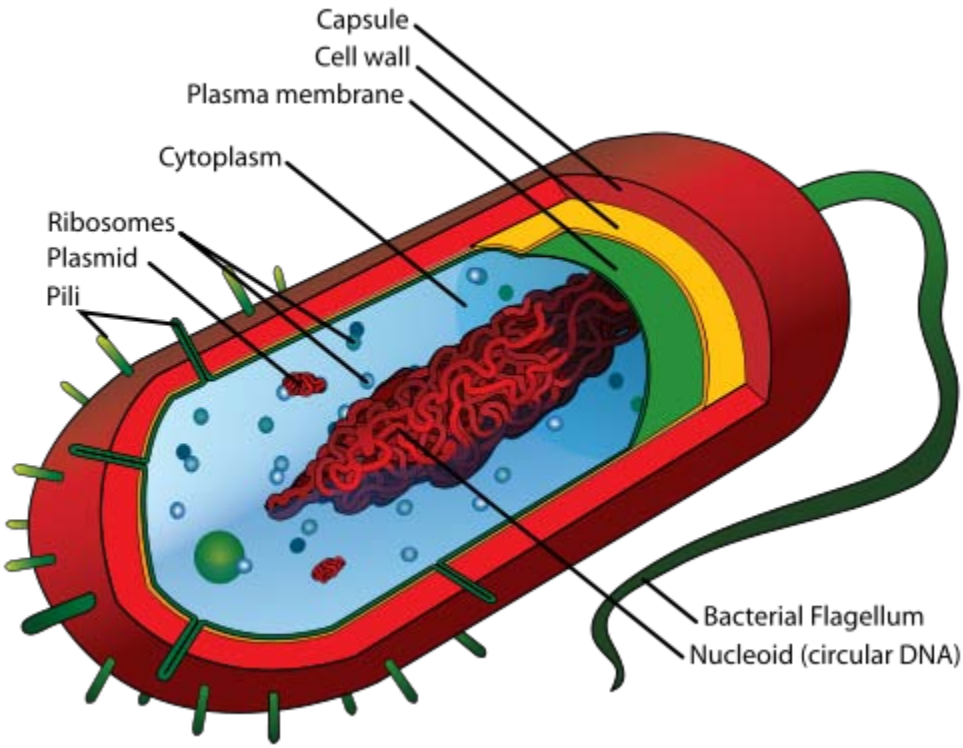


Diagram of a typical prokaryotic cell

The prokaryote cell is simpler, and therefore smaller, than a eukaryote cell, lacking a nucleus and most of the other organelles of eukaryotes. There are two kinds of prokaryotes: bacteria and archaea; these share a similar structure.

Nuclear material of prokaryotic cell consist of a single chromosome which is in direct contact with cytoplasm. Here the undefined nuclear region in the cytoplasm is called nucleoid.

A prokaryotic cell has three architectural regions:

- On the outside, flagella and pili project from the cell's surface. These are structures (not present in all prokaryotes) made of proteins that facilitate movement and communication between cells;
- Enclosing the cell is the cell envelope – generally consisting of a cell wall covering a plasma membrane though some bacteria also have a further covering layer called a capsule. The envelope gives rigidity to the cell and separates the interior of the cell from its environment, serving as a protective filter. Though most prokaryotes have a cell wall, there are exceptions such as *Mycoplasma* (bacteria) and *Thermoplasma* (archaea). The cell wall consists of *peptidoglycan* in bacteria, and acts as an additional barrier against exterior forces. It also prevents

the cell from expanding and finally bursting (cytolysis) from osmotic pressure against a hypotonic environment. Some eukaryote cells (plant cells and fungi cells) also have a cell wall;

- Inside the cell is the cytoplasmic region that contains the cell genome (DNA) and ribosomes and various sorts of inclusions. A prokaryotic chromosome is usually a circular molecule (an exception is that of the bacterium *Borrelia burgdorferi*, which causes Lyme disease). Though not forming a *nucleus*, the DNA is condensed in a *nucleoid*. Prokaryotes can carry extrachromosomal DNA elements called *plasmids*, which are usually circular. Plasmids enable additional functions, such as antibiotic resistance.

Eukaryotic cells

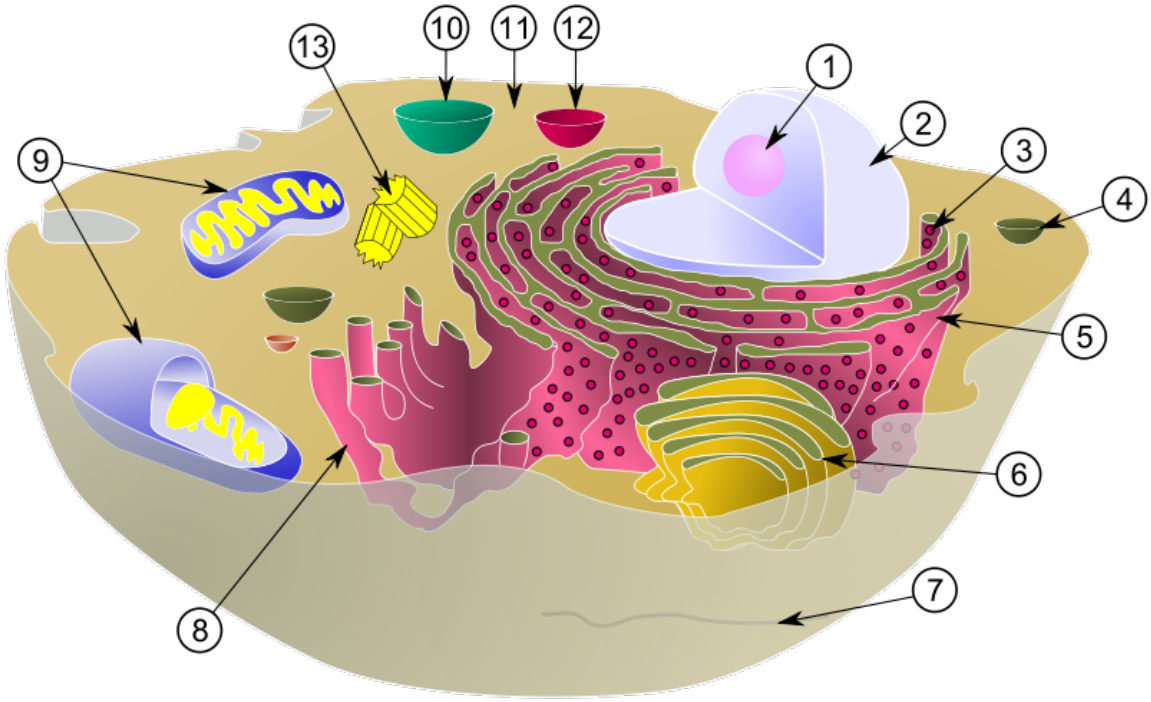


Diagram of a typical animal (eukaryotic) cell, showing subcellular components.
Organelles:
(1) nucleolus
(2) nucleus
(3) ribosome
(4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) smooth endoplasmic reticulum
(9) mitochondria
(10) vacuole
(11) cytoplasm

(12) lysosome
(13) centrioles within centrosome

Eukaryotic cells are about 15 times wider than a typical prokaryote and can be as much as 1000 times greater in volume. The major difference between prokaryotes and eukaryotes is that eukaryotic cells contain membrane-bound compartments in which specific metabolic activities take place. Most important among these is a cell nucleus, a membrane-delineated compartment that houses the eukaryotic cell's DNA. This nucleus gives the eukaryote its name, which means "true nucleus." Other differences include:

- The plasma membrane resembles that of prokaryotes in function, with minor differences in the setup. Cell walls may or may not be present.
- The eukaryotic DNA is organized in one or more linear molecules, called chromosomes, which are associated with histone proteins. All chromosomal DNA is stored in the *cell nucleus*, separated from the cytoplasm by a membrane. Some eukaryotic organelles such as mitochondria also contain some DNA.
- Many eukaryotic cells are ciliated with *primary cilia*. Primary cilia play important roles in chemosensation, mechanosensation, and thermosensation. Cilia may thus be "viewed 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."
- Eukaryotes can move using *motile cilia* or *flagella*. The flagella are more complex than those of prokaryotes.

Table 1: Comparison of features of prokaryotic and eukaryotic cells

	Prokaryotes	Eukaryotes
Typical organisms	bacteria, archaea	protists, fungi, plants, animals
Typical size	~ 1–10 μm	~ 10–100 μm (sperm cells, apart from the tail, are smaller)
Type of nucleus	nucleoid region; no real nucleus	real nucleus with double membrane
DNA	circular (usually)	linear molecules (chromosomes) with histone proteins
RNA-/protein-synthesis	coupled in cytoplasm	RNA-synthesis inside the nucleus protein synthesis in cytoplasm
Ribosomes	50S+30S	60S+40S
Cytoplasmatic structure	very few structures	highly structured by endomembranes and a cytoskeleton
Cell movement	flagella made of flagellin	flagella and cilia containing microtubules; lamellipodia and filopodia containing actin
Mitochondria	none	one to several thousand (though some lack mitochondria)
Chloroplasts	none	in algae and plants
Organization	usually single cells	single cells, colonies, higher multicellular

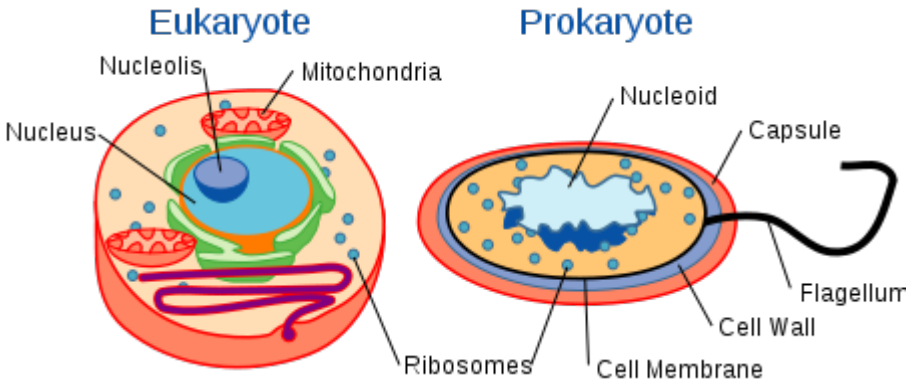
		organisms with specialized cells
Cell division	Binary fission (simple division)	Mitosis (fission or budding) Meiosis

Table 2: Comparison of structures between animal and plant cells

	Typical animal cell	Typical plant cell

Organelles	• Nucleus
	  ○ Nucleolus (within nucleus)
	• Rough endoplasmic reticulum (ER)
	• Smooth ER
	• Ribosomes
	• Cytoskeleton
	• Golgi apparatus
	• Cytoplasm
	• Mitochondria
	• Vesicles
	• Lysosomes
	• Centrosome
	  ○ Centrioles
		• Nucleus
		  ○ Nucleolus (within nucleus)
		• Rough ER
		• Smooth ER
		• Ribosomes
		• Cytoskeleton
		• Golgi apparatus (dictiosomes)
		• Cytoplasm
		• Mitochondria
		• Plastids and its derivatives
		• Vacuole(s)
		• Cell wall

Subcellular components



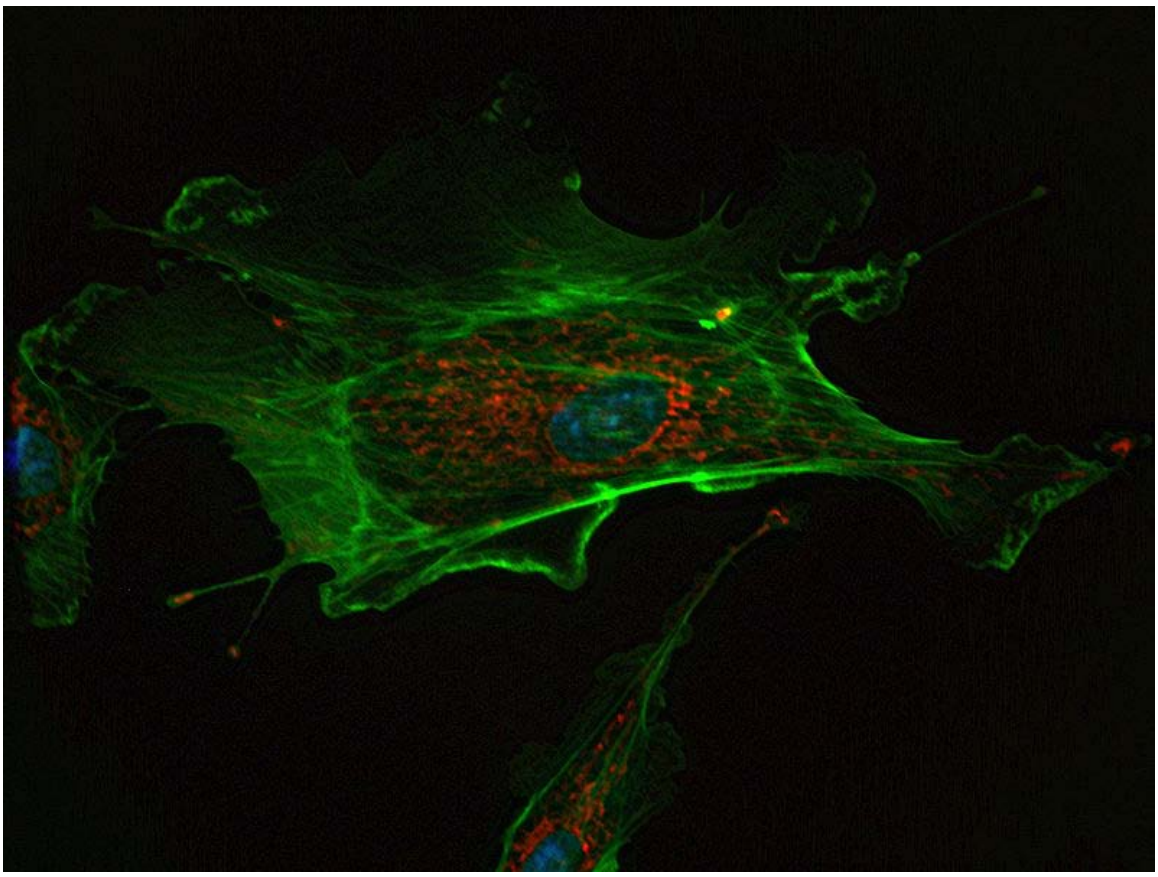
The cells of eukaryotes (left) and prokaryotes (right)

All cells, whether prokaryotic or eukaryotic, have a membrane that envelops the cell, separates its interior from its environment, regulates what moves in and out (selectively permeable), and maintains the electric potential of the cell. Inside the membrane, a salty cytoplasm takes up most of the cell volume. All cells possess DNA, the hereditary material of genes, and RNA, containing the information necessary to build various proteins such as enzymes, the cell's primary machinery. There are also other kinds of biomolecules in cells.

Membrane

The cytoplasm of a cell is surrounded by a cell membrane or *plasma membrane*. The plasma membrane in plants and prokaryotes is usually covered by a cell wall. This membrane serves to separate and protect a cell from its surrounding environment and is made mostly from a double layer of lipids (hydrophobic fat-like molecules) and hydrophilic phosphorus molecules. Hence, the layer is called a phospholipid bilayer. It may also be called a fluid mosaic membrane. Embedded within this membrane is a variety of protein molecules that act as channels and pumps that move different molecules into and out of the cell. The membrane is said to be 'semi-permeable', in that it can either let a substance (molecule or ion) pass through freely, pass through to a limited extent or not pass through at all. Cell surface membranes also contain receptor proteins that allow cells to detect external signaling molecules such as hormones.

Cytoskeleton



Bovine Pulmonary Artery Endothelial cell: nuclei stained blue, mitochondria stained red, and F-actin, an important component in microfilaments, stained green. Cell imaged on a fluorescent microscope.

The cytoskeleton acts to organize and maintain the cell's shape; anchors organelles in place; helps during endocytosis, the uptake of external materials by a cell, and cytokinesis, the separation of daughter cells after cell division; and moves parts of the

cell in processes of growth and mobility. The eukaryotic cytoskeleton is composed of microfilaments, intermediate filaments and microtubules. There is a great number of proteins associated with them, each controlling a cell's structure by directing, bundling, and aligning filaments. The prokaryotic cytoskeleton is less well-studied but is involved in the maintenance of cell shape, polarity and cytokinesis.

Genetic material

Two different kinds of genetic material exist: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Most organisms use DNA for their long-term information storage, but some viruses (e.g., retroviruses) have RNA as their genetic material. The biological information contained in an organism is encoded in its DNA or RNA sequence. RNA is also used for information transport (e.g., mRNA) and enzymatic functions (e.g., ribosomal RNA) in organisms that use DNA for the genetic code itself. Transfer RNA (tRNA) molecules are used to add amino acids during protein translation.

Prokaryotic genetic material is organized in a simple circular DNA molecule (the bacterial chromosome) in the nucleoid region of the cytoplasm. Eukaryotic genetic material is divided into different, linear molecules called chromosomes inside a discrete nucleus, usually with additional genetic material in some organelles like mitochondria and chloroplasts.

A human cell has genetic material contained in the cell nucleus (the nuclear genome) and in the mitochondria (the mitochondrial genome). In humans the nuclear genome is divided into 23 pairs of linear DNA molecules called chromosomes. The mitochondrial genome is a circular DNA molecule distinct from the nuclear DNA. Although the mitochondrial DNA is very small compared to nuclear chromosomes, it codes for 13 proteins involved in mitochondrial energy production and specific tRNAs.

Foreign genetic material (most commonly DNA) can also be artificially introduced into the cell by a process called transfection. This can be transient, if the DNA is not inserted into the cell's genome, or stable, if it is. Certain viruses also insert their genetic material into the genome.

Organelles

The human body contains many different organs, such as the heart, lung, and kidney, with each organ performing a different function. Cells also have a set of "little organs," called organelles, that are adapted and/or specialized for carrying out one or more vital functions. Both eukaryotic and prokaryotic cells have organelles but organelles in eukaryotes are generally more complex and may be membrane bound.

There are several types of organelles in a cell. Some (such as the nucleus and golgi apparatus) are typically solitary, while others (such as mitochondria, peroxisomes and lysosomes) can be numerous (hundreds to thousands). The cytosol is the gelatinous fluid that fills the cell and surrounds the organelles.

Cell nucleus – eukaryotes only - a cell's information center

The cell nucleus is the most conspicuous organelle found in a eukaryotic cell. It houses the cell's chromosomes, and is the place where almost all DNA replication and RNA synthesis (transcription) occur. The nucleus is spherical and separated from the cytoplasm by a double membrane called the nuclear envelope. The nuclear envelope isolates and protects a cell's DNA from various molecules that could accidentally damage its structure or interfere with its processing. During processing, DNA is transcribed, or copied into a special RNA, called messenger RNA (mRNA). This mRNA is then transported out of the nucleus, where it is translated into a specific protein molecule. The nucleolus is a specialized region within the nucleus where ribosome subunits are assembled. In prokaryotes, DNA processing takes place in the cytoplasm.

Diagram of a cell nucleus

Mitochondria and Chloroplasts – eukaryotes only - the power generators

Mitochondria are self-replicating organelles that occur in various numbers, shapes, and sizes in the cytoplasm of all eukaryotic cells. Mitochondria play a critical role in generating energy in the eukaryotic cell. Mitochondria generate the cell's energy by oxidative phosphorylation, using oxygen to release energy stored in cellular nutrients (typically pertaining to glucose) to generate ATP. Mitochondria multiply by splitting in two. Respiration occurs in the cell mitochondria. Organelles that are modified chloroplasts are broadly called plastids, and are involved in energy storage through photosynthesis, which uses solar energy to generate carbohydrates and oxygen from carbon dioxide and water. Mitochondria and chloroplasts each contain their own genome, which is separate and distinct from the nuclear genome of a cell. Both organelles contain this DNA in circular plasmids, much like prokaryotic cells, strongly supporting the evolutionary theory of endosymbiosis; since these organelles contain

their own genomes and have other similarities to prokaryotes, they are thought to have developed through a symbiotic relationship after being engulfed by a primitive cell.

Endoplasmic reticulum – eukaryotes only

The endoplasmic reticulum (ER) is the transport network for molecules targeted for certain modifications and specific destinations, as compared to molecules that will float freely in the cytoplasm. The ER has two forms: the rough ER, which has ribosomes on its surface and secretes proteins into the cytoplasm, and the smooth ER, which lacks them. Smooth ER plays a role in calcium sequestration and release.

Golgi apparatus – eukaryotes only

The primary function of the Golgi apparatus is to process and package the macromolecules such as proteins and lipids that are synthesized by the cell. It is particularly important in the processing of proteins for secretion. The Golgi apparatus forms a part of the endomembrane system of eukaryotic cells. Vesicles that enter the Golgi apparatus are processed in a cis to trans direction, meaning they coalesce on the cis side of the apparatus and after processing pinch off on the opposite (trans) side to form a new vesicle in the animal cell.

Diagram of an endomembrane system

Ribosomes

The ribosome is a large complex of RNA and protein molecules. They each consist of two subunits, and act as an assembly line where RNA from the nucleus is used to synthesise proteins from amino acids. Ribosomes can be found either floating freely or bound to a membrane (the rough endoplasmatic reticulum in eukaryotes, or the cell membrane in prokaryotes).

Lysosomes and Peroxisomes – eukaryotes only

Lysosomes contain digestive enzymes (acid hydrolases). They digest excess or worn-out organelles, food particles, and engulfed viruses or bacteria. Peroxisomes have enzymes that rid the cell of toxic peroxides. The cell could not house these

destructive enzymes if they were not contained in a membrane-bound system. These organelles are often called a "suicide bag" because of their ability to detonate and destroy the cell.

Centrosome – the cytoskeleton organiser

The centrosome produces the microtubules of a cell – a key component of the cytoskeleton. It directs the transport through the ER and the Golgi apparatus. Centrosomes are composed of two centrioles, which separate during cell division and help in the formation of the mitotic spindle. A single centrosome is present in the animal cells. They are also found in some fungi and algae cells.

Vacuoles

Vacuoles store food and waste. Some vacuoles store extra water. They are often described as liquid filled space and are surrounded by a membrane. Some cells, most notably *Amoeba*, have contractile vacuoles, which can pump water out of the cell if there is too much water. The vacuoles of eukaryotic cells are usually larger in those of plants than animals.

Structures outside the cell wall

Capsule

A gelatinous capsule is present in some bacteria outside the cell wall. The capsule may be polysaccharide as in pneumococci, meningococci or polypeptide as *Bacillus anthracis* or hyaluronic acid as in streptococci. Capsules are not marked by ordinary stain and can be detected by special stain. The capsule is antigenic. The capsule has antiphagocytic function so it determines the virulence of many bacteria. It also plays a role in attachment of the organism to mucous membranes.

Flagella

Flagella are the organelles of cellular mobility. They arise from cytoplasm and extrude through the cell wall. They are long and thick thread-like appendages, protein in nature. Are most commonly found in bacteria cells but are found in animal cells as well.

Fimbriae (pili)

They are short and thin hair like filaments, formed of protein called pilin (antigenic). Fimbriae are responsible for attachment of bacteria to specific receptors of human cell (adherence). There are special types of pili called (sex pili) involved in conjunction.

Functions

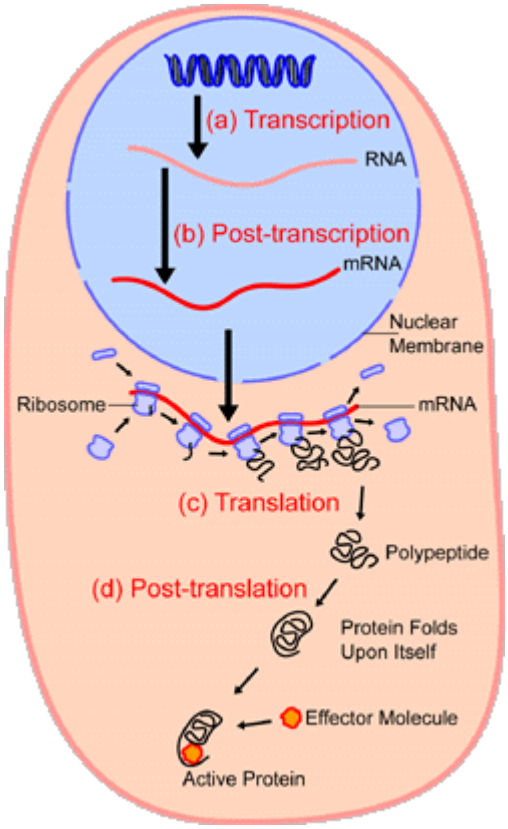
Growth and metabolism

Between successive cell divisions, cells grow through the functioning of cellular metabolism. Cell metabolism is the process by which individual cells process nutrient

molecules. Metabolism has two distinct divisions: catabolism, in which the cell breaks down complex molecules to produce energy and reducing power, and anabolism, in which the cell uses energy and reducing power to construct complex molecules and perform other biological functions. Complex sugars consumed by the organism can be broken down into a less chemically complex sugar molecule called glucose. Once inside the cell, glucose is broken down to make adenosine triphosphate (ATP), a form of energy, through two different pathways.

The first pathway, glycolysis, requires no oxygen and is referred to as anaerobic metabolism. Each reaction is designed to produce some hydrogen ions that can then be used to make energy packets (ATP). In prokaryotes, glycolysis is the only method used for converting energy.

The second pathway, called the Krebs cycle, or citric acid cycle, occurs inside the mitochondria and can generate enough ATP to run all the cell functions.



An overview of protein synthesis.
Within the nucleus of the cell (*light blue*), genes (DNA, *dark blue*) are transcribed into RNA. This RNA is then subject to post-transcriptional modification and control, resulting in a mature mRNA (*red*) that is then transported out of the nucleus and into the cytoplasm (*peach*), where it undergoes translation into a protein. mRNA is translated by ribosomes (*purple*) that match the three-base codons of the mRNA to the three-base anti-codons of

the appropriate tRNA. Newly synthesized proteins (*black*) are often further modified, such as by binding to an effector molecule (*orange*), to become fully active.

Creation

Cell division involves a single cell (called a *mother cell*) dividing into two daughter cells. This leads to growth in multicellular organisms (the growth of tissue) and to procreation (vegetative reproduction) in unicellular organisms.

Prokaryotic cells divide by binary fission. Eukaryotic cells usually undergo a process of nuclear division, called mitosis, followed by division of the cell, called cytokinesis. A diploid cell may also undergo meiosis to produce haploid cells, usually four. Haploid cells serve as gametes in multicellular organisms, fusing to form new diploid cells.

DNA replication, or the process of duplicating a cell's genome, is required every time a cell divides. Replication, like all cellular activities, requires specialized proteins for carrying out the job.

Protein synthesis

Cells are capable of synthesizing new proteins, which are essential for the modulation and maintenance of cellular activities. This process involves the formation of new protein molecules from amino acid building blocks based on information encoded in DNA/RNA. Protein synthesis generally consists of two major steps: transcription and translation.

Transcription is the process where genetic information in DNA is used to produce a complementary RNA strand. This RNA strand is then processed to give messenger RNA (mRNA), which is free to migrate through the cell. mRNA molecules bind to protein-RNA complexes called ribosomes located in the cytosol, where they are translated into polypeptide sequences. The ribosome mediates the formation of a polypeptide sequence based on the mRNA sequence. The mRNA sequence directly relates to the polypeptide sequence by binding to transfer RNA (tRNA) adapter molecules in binding pockets within the ribosome. The new polypeptide then folds into a functional three-dimensional protein molecule.

Movement or motility

Cells can move during many processes: such as wound healing, the immune response and cancer metastasis. For wound healing to occur, white blood cells and cells that ingest bacteria move to the wound site to kill the microorganisms that cause infection.
At the same time fibroblasts (connective tissue cells) move there to remodel damaged structures. In the case of tumor development, cells from a primary tumor move away and spread to other parts of the body. Cell motility involves many receptors, crosslinking, bundling, binding, adhesion, motor and other proteins. The process is divided into three steps – protrusion of the leading edge of the cell, adhesion of the leading edge and de-

adhesion at the cell body and rear, and cytoskeletal contraction to pull the cell forward. Each step is driven by physical forces generated by unique segments of the cytoskeleton.

Evolution

The origin of cells has to do with the origin of life, which began the history of life on Earth.

Origin of the first cell

There are several theories about the origin of small molecules that could lead to life in an early Earth. One is that they came from meteorites. Another is that they were created at deep-sea vents. A third is that they were synthesized by lightning in a reducing atmosphere; although it is not clear if Earth had such an atmosphere. There are essentially no experimental data defining what the first self-replicating forms were. RNA is generally assumed to be the earliest self-replicating molecule, as it is capable of both storing genetic information and catalyzing chemical reactions. But some other entity with the potential to self-replicate could have preceded RNA, like clay or peptide nucleic acid.

Cells emerged at least 4.0–4.3 billion years ago. The current belief is that these cells were heterotrophs. An important characteristic of cells is the cell membrane, composed of a bilayer of lipids. The early cell membranes were probably more simple and permeable than modern ones, with only a single fatty acid chain per lipid. Lipids are known to spontaneously form bilayered vesicles in water, and could have preceded RNA. But the first cell membranes could also have been produced by catalytic RNA, or even have required structural proteins before they could form.

Origin of eukaryotic cells

The eukaryotic cell seems to have evolved from a symbiotic community of prokaryotic cells. DNA-bearing organelles like the mitochondria and the chloroplasts are almost certainly what remains of ancient symbiotic oxygen-breathing proteobacteria and cyanobacteria, respectively, where the rest of the cell seems to be derived from an ancestral archaean prokaryote cell – a theory termed the endosymbiotic theory.

There is still considerable debate about whether organelles like the hydrogenosome predated the origin of mitochondria, or viceversa.

Sex, as the stereotyped choreography of meiosis and syngamy that persists in nearly all extant eukaryotes, may have played a role in the transition from prokaryotes to eukaryotes. An 'origin of sex as vaccination' theory suggests that the eukaryote genome accreted from prokaryan parasite genomes in numerous rounds of lateral gene transfer. Sex-as-syngamy (fusion sex) arose when infected hosts began swapping nuclearized genomes containing co-evolved, vertically transmitted symbionts that conveyed protection against horizontal infection by more virulent symbionts.

History

- 1632–1723: Antonie van Leeuwenhoek teaches himself to grind lenses, builds a microscope and draws protozoa, such as *Vorticella* from rain water, and bacteria from his own mouth.
- 1665: Robert Hooke discovers cells in cork, then in living plant tissue using an early microscope.
- 1839: Theodor Schwann and Matthias Jakob Schleiden elucidate the principle that plants and animals are made of cells, concluding that cells are a common unit of structure and development, and thus founding the cell theory.
- The belief that life forms can occur spontaneously (*generatio spontanea*) is contradicted by Louis Pasteur (1822–1895) (although Francesco Redi had performed an experiment in 1668 that suggested the same conclusion).
- 1855: Rudolf Virchow states that cells always emerge from cell divisions (*omnis cellula ex cellula*).
- 1931: Ernst Ruska builds first transmission electron microscope (TEM) at the University of Berlin. By 1935, he has built an EM with twice the resolution of a light microscope, revealing previously unresolvable organelles.
- 1953: Watson and Crick made their first announcement on the double-helix structure for DNA on February 28.
- 1981: Lynn Margulis published *Symbiosis in Cell Evolution* detailing the endosymbiotic theory.

Chapter- 2

Cell Membrane

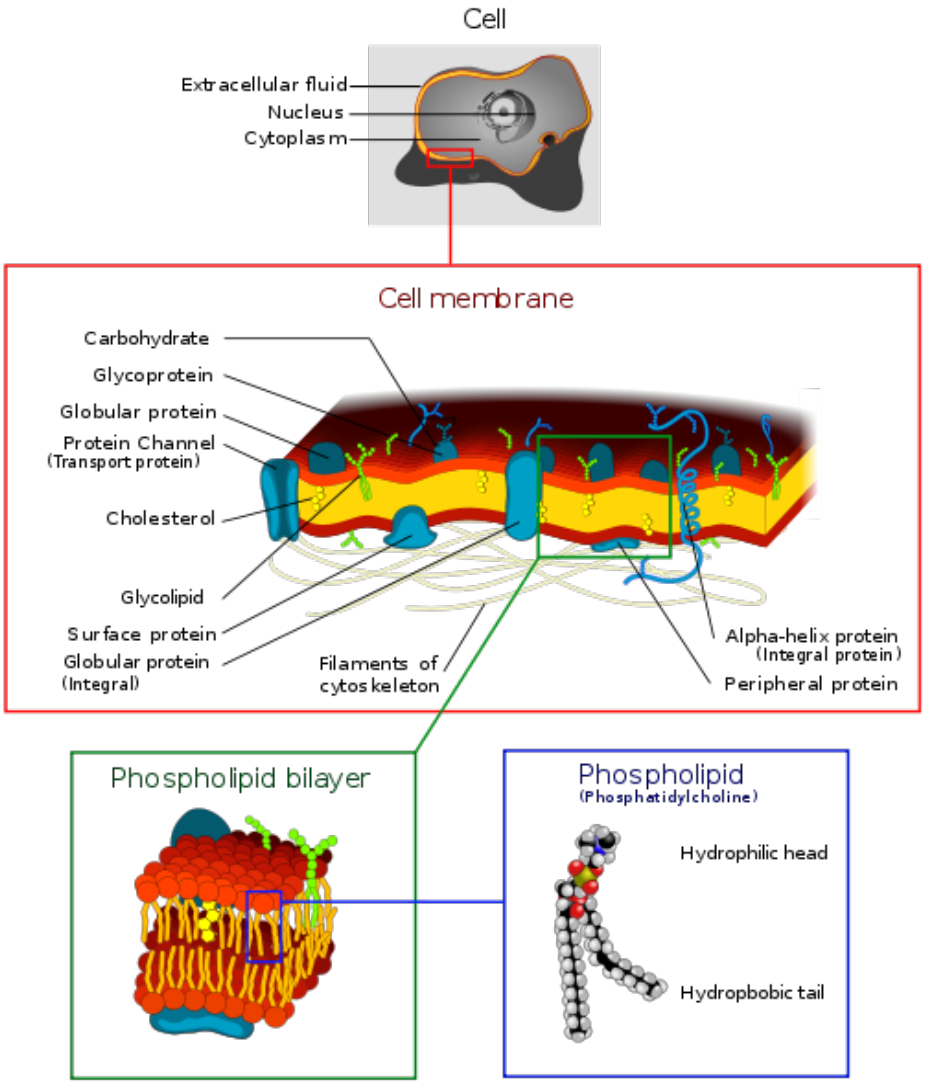


Illustration of a Eukaryotic cell membrane

The **cell membrane** is a biological membrane that separates the interior of all cells from the outside environment.. The cell membrane is selectively-permeable to ions and organic molecules and controls the movement of substances in and out of cells.. It consists of the phospholipid bilayer with embedded proteins. Cell membranes are involved in a variety of cellular processes such as cell adhesion, ion conductivity and cell signaling and serve as the attachment surface for the extracellular glycocalyx and cell wall and intracellular cytoskeleton.

Function

The cell membrane surrounds the protoplasm of a cell and, in animal cells, physically separates the intracellular components from the extracellular environment. Fungi, bacteria and plants also have the cell wall which provides a mechanical support for the cell and precludes passage of the larger molecules. The cell membrane also plays a role in anchoring the cytoskeleton to provide shape to the cell, and in attaching to the extracellular matrix and other cells to help group cells together to form tissues.

The barrier is differentially permeable and able to regulate what enters and exits the cell, thus facilitating the transport of materials needed for survival. The movement of substances across the membrane can be either *passive*, occurring without the input of cellular energy, or active, requiring the cell to expend energy in moving it. The membrane also maintains the cell potential.

In simpler terms

The cell membrane is flexible and allows certain things in and out of it. Unlike the cell wall it has a lipid bilayer which has hydrophilic heads and hydrophobic tails. These help control the cell membrane.

Prokaryotes

Gram-negative bacteria have plasma membrane and outer membrane separated by the periplasmic space. Other prokaryotic species have only plasma membrane. Prokaryotic cells are also surrounded by a cell wall.

Structure

Fluid mosaic model

According to the fluid mosaic model of S. J. Singer and Garth Nicolson 1972, the biological membranes can be considered as a two-dimensional liquid where all lipid and protein molecules diffuse more or less easily. This picture may be valid in the space scale of 10 nm. However, the plasma membranes contain different structures or domains that can be classified as: (a) protein-protein complexes; (b) lipid rafts, and (c) pickets and fences formed by the actin-based cytoskeleton.

Lipid bilayer

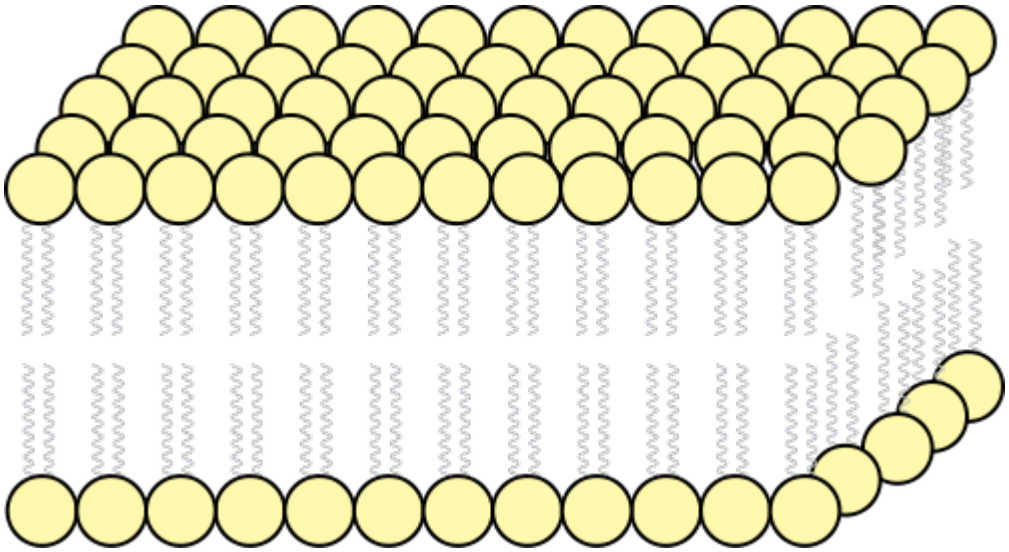


Diagram of the arrangement of amphipathic lipid molecules to form a lipid bilayer. The yellow polar head groups separate the grey hydrophobic tails from the aqueous cytosolic and extracellular environments.

Lipid bilayers go through a self assembly process in the formation of membranes. The cell membrane consists primarily of a thin layer of amphipathic phospholipids which spontaneously arrange so that the hydrophobic "tail" regions are shielded from the surrounding polar fluid, causing the more hydrophilic "head" regions to associate with the cytosolic and extracellular faces of the resulting bilayer. This forms a continuous, spherical lipid bilayer. Forces such as Van der Waal, electrostatic, hyrdogen bonds, and noncovalent interactions, are all forces that contribute to the formation of the lipid bilayer. Overall, hydrophobic interactions are the major driving force in the formation of lipid bilayers.

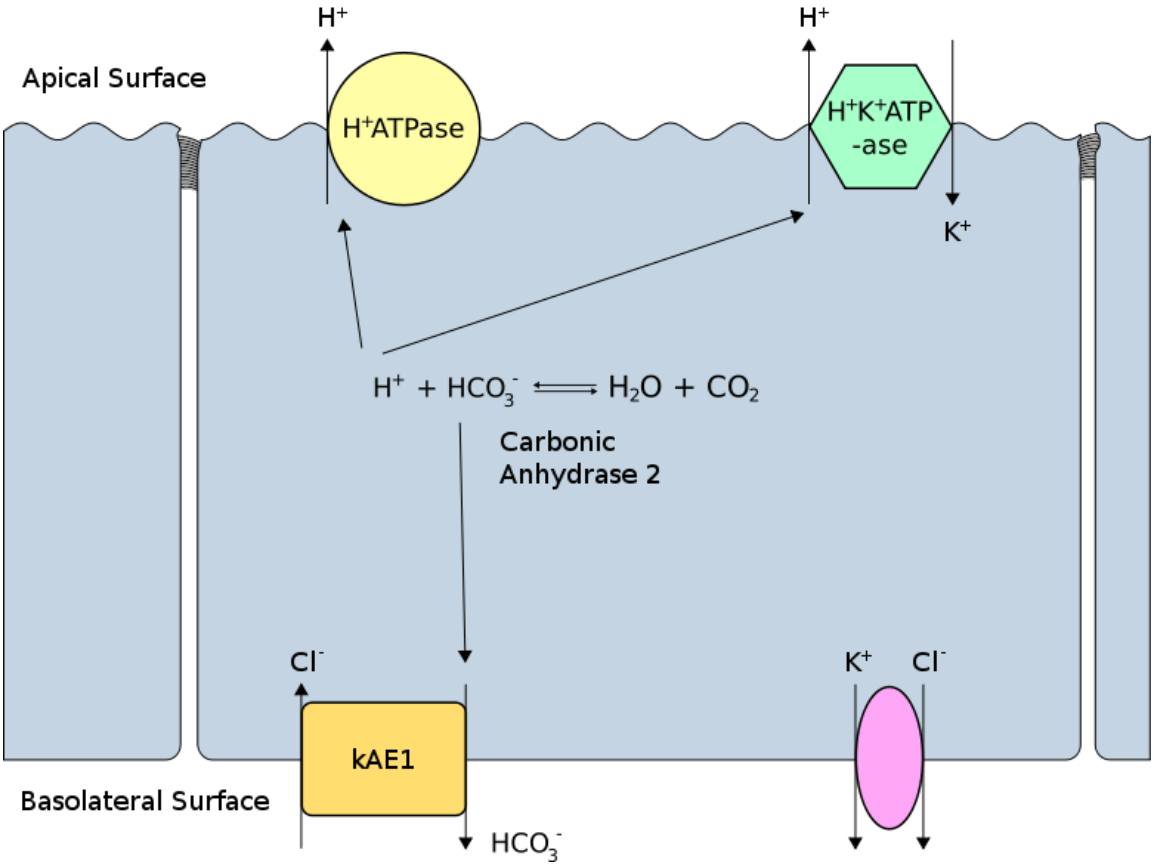
Lipid bilayers have very low permeability for ions and most polar molecules.The arrangement of hydrophilic heads and hydrophobic tails of the lipid bilayer prevent polar solutes (e.g. amino acids, nucleic acids, carbohydrates, proteins, and ions) from diffusing across the membrane, but generally allows for the passive diffusion of hydrophobic molecules. This affords the cell the ability to control the movement of these substances via transmembrane protein complexes such as pores and gates.

Flippases and Scramblases concentrate phosphatidyl serine, which carries a negative charge, on the inner membrane. Along with NANA, this creates an extra barrier to charged moieties moving through the membrane.

Membranes serve diverse functions in eukaryotic and prokaryotic cells. One important role is to regulate the movement of materials into and out of cells. The phospholipid bilayer structure (fluid mosaic model) with specific membrane proteins accounts for the selective permeability of the membrane and passive and active transport mechanisms. In

addition, membranes in prokaryotes and in the mitochondria and chloroplasts of eukaryotes facilitate the synthesis of ATP through chemiosmosis.

Membrane polarity



Alpha intercalated cell

The **apical membrane** of a polarized cell is the surface of the plasma membrane that faces the lumen. This is particularly evident in epithelial and endothelial cells, but also describes other polarized cells, such as neurons.

The **basolateral membrane** of a polarized cell is the surface of the plasma membrane that forms its basal and lateral surfaces. It faces towards the interstitium, and away from the lumen.

"Basolateral membrane" is a compound phrase referring to the terms *basal (base) membrane* and *lateral (side) membrane*, which, especially in epithelial cells, are identical in composition and activity. Proteins (such as ion channels and pumps) are free to move from the basal to the lateral surface of the cell or *vice versa* in accordance with the fluid mosaic model.

Tight junctions that join epithelial cells near their apical surface prevent the migration of proteins from the basolateral membrane to the apical membrane. The basal and lateral

surfaces thus remain roughly equivalent to one another, yet distinct from the apical surface.

Integral membrane proteins

The cell membrane contains many integral membrane proteins, which pepper the entire surface. These structures, which can be visualized by electron microscopy or fluorescence microscopy, can be found on the inside of the membrane, the outside, or membrane spanning. These may include integrins, cadherins, desmosomes, clathrin-coated pits, caveolaes, and different structures involved in cell adhesion. Integral proteins are the most abundant type of protein to span the lipid bilayer. They interact widely with hydrocarbon chains of membrane lipids and can be released by agents that compete for the same nonpolar interactions.

Peripheral membrane proteins

Peripheral proteins are proteins that are bounded to the membrane by electrostatic interactions and hydrogen bonding with the hydrophilic phospholipid heads. Many of these proteins can be found bounded to the surfaces of integral proteins on either the cytoplasimic side of the cell or the extracellular side of the membrane. Some are anchored to the bilayer through covalent bond with a fatty acid.

Membrane skeleton

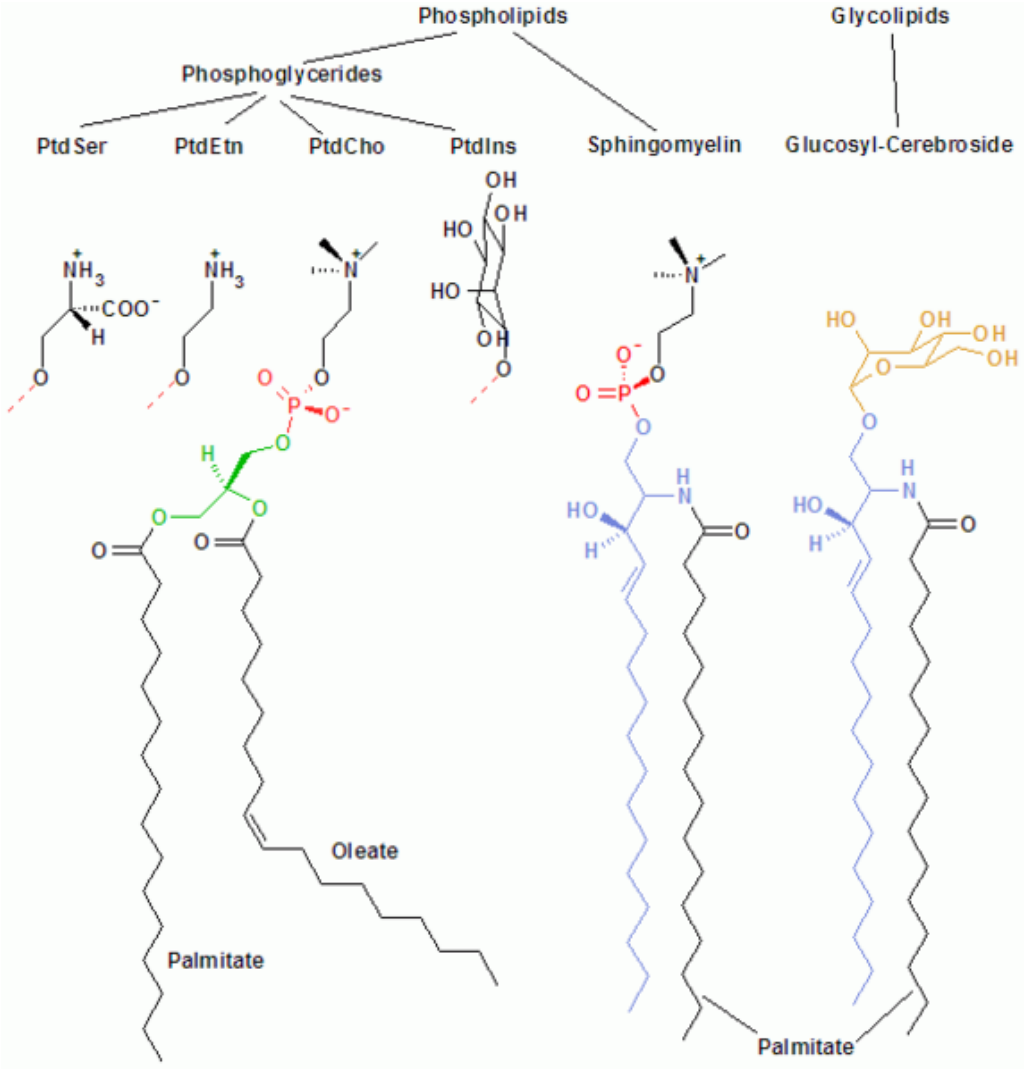
The cytoskeleton is found underlying the cell membrane in the cytoplasm and provides a scaffolding for membrane proteins to anchor to, as well as forming organelles that extend from the cell. Indeed, cytoskeletal elements interact extensively and intimately with the cell membrane. Anchoring proteins restricts them to a particular cell surface — for example, the *apical surface* of epithelial cells that line the vertebrate gut — and limits how far they may diffuse within the bilayer. The cytoskeleton is able to form appendage-like organelles, such as cilia, which are microtubule-based extensions covered by the cell membrane, and filopodia, which are actin-based extensions. These extensions are ensheathed in membrane and project from the surface of the cell in order to sense the external environment and/or make contact with the substrate or other cells. The apical surfaces of epithelial cells are dense with actin-based finger-like projections known as microvilli, which increase cell surface area and thereby increase the absorption rate of nutrients. Localized decoupling of the cytoskeleton and cell membrane results in formation of a bleb.

Composition

Cell membranes contain a variety of biological molecules, notably lipids and proteins. Material is incorporated into the membrane, or deleted from it, by a variety of mechanisms:

- Fusion of intracellular vesicles with the membrane (exocytosis) not only excretes the contents of the vesicle but also incorporates the vesicle membrane's components into the cell membrane. The membrane may form blebs around extracellular material that pinch off to become vesicles (endocytosis).
- If a membrane is continuous with a tubular structure made of membrane material, then material from the tube can be drawn into the membrane continuously.
- Although the concentration of membrane components in the aqueous phase is low (stable membrane components have low solubility in water), there is an exchange of molecules between the lipid and aqueous phases.

Lipids



Examples of the major membrane phospholipids and glycolipids: phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer).

The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and cholesterols. The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant. In RBC studies, 30% of the plasma membrane is lipid.

The fatty chains in phospholipids and glycolipids usually contain an even number of carbon atoms, typically between 16 and 20. The 16- and 18-carbon fatty acids are the most common. Fatty acids may be saturated or unsaturated, with the configuration of the double bonds nearly always *cis*. The length and the degree of unsaturation of fatty acid chains have a profound effect on membrane fluidity as unsaturated lipids create a kink, preventing the fatty acids from packing together as tightly, thus decreasing the melting temperature (increasing the fluidity) of the membrane. The ability of some organisms to regulate the fluidity of their cell membranes by altering lipid composition is called homeoviscous adaptation.

The entire membrane is held together via non-covalent interaction of hydrophobic tails, however the structure is quite fluid and not fixed rigidly in place. Under physiological conditions phospholipid molecules in the cell membrane are in the liquid crystalline state. It means the lipid molecules are free to diffuse and exhibit rapid lateral diffusion along the layer in which they are present. However, the exchange of phospholipid molecules between intracellular and extracellular leaflets of the bilayer is a very slow process. Lipid rafts and caveolae are examples of cholesterol-enriched microdomains in the cell membrane.

In animal cells cholesterol is normally found dispersed in varying degrees throughout cell membranes, in the irregular spaces between the hydrophobic tails of the membrane lipids, where it confers a stiffening and strengthening effect on the membrane.

Phospholipids forming lipid vesicles

Lipid vesicles or liposomes are circular pockets that are enclosed by a lipid bilayer. These structures are used in laboratories to study the effects of chemicals in cells by delivering these chemicals directly to the cell, as well as getting more insight into cell membrane permeability. Lipid vesicles and liposomes are formed by first suspending a lipid in an aqueous solution then agitating the mixture through sonication, resulting in a uniformly circular vesicle. By measuring the rate of efflux from that of the insideof the vesicle to the ambient solution, allows researcher to better understand membrane permeability. Vesicles can be formed with molecules and ions inside the vesicle by forming the vesicle with the desired molecule or ion present in the solution. Proteins can also be embedded into the membrane through solubilizing the desired proteins in the presence of detergents and attaching them to the phospholipids in which the liposome is formed. These provide researchers with a tool to examine various membrane protein functions.

Carbohydrates

Plasma membranes also contain carbohydrates, predominantly glycoproteins, but with some glycolipids (cerebrosides and gangliosides). For the most part, no glycosylation occurs on membranes within the cell; rather generally glycosylation occurs on the extracellular surface of the plasma membrane.

The glycocalyx is an important feature in all cells, especially epithelia with microvilli. Recent data suggest the glycocalyx participates in cell adhesion, lymphocyte homing, and many others.

The penultimate sugar is galactose and the terminal sugar is sialic acid, as the sugar backbone is modified in the golgi apparatus. Sialic acid carries a negative charge, providing an external barrier to charged particles.

Proteins

Proteins within the membrane are key to the functioning of the overall membrane. These proteins mainly transport chemicals and information across the membrane. Every membrane has a varying degree of protein content. Proteins can be in the form of peripheral or integral.

Type	Description	Examples
Integral proteins or *transmembrane proteins*	Span the membrane and have a hydrophilic cytosolic domain, which interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs.	Ion channels, proton pumps, G protein-coupled receptor
Lipid anchored proteins	Covalently-bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane.	G proteins
Peripheral proteins	Attached to integral membrane proteins, or associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and, once reacted the molecule, dissociates to carry on its work in	Some enzymes, some hormones

the cytoplasm.

The cell membrane plays host to a large amount of protein that is responsible for its various activities. The amount of protein differs between species and according to function, however the typical amount in a cell membrane is 50%. These proteins are undoubtedly important to a cell: Approximately a third of the genes in yeast code specifically for them, and this number is even higher in multicellular organisms.

The cell membrane, being exposed to the outside environment, is an important site of cell-cell communication. As such, a large variety of protein receptors and identification proteins, such as antigens, are present on the surface of the membrane. Functions of membrane proteins can also include cell-cell contact, surface recognition, cytoskeleton contact, signaling, enzymatic activity, or transporting substances across the membrane.

Most membrane proteins must be inserted in some way into the membrane. For this to occur, an N-terminus "signal sequence" of amino acids directs proteins to the endoplasmic reticulum, which inserts the proteins into a lipid bilayer. Once inserted, the proteins are then transported to their final destination in vesicles, where the vesicle fuses with the target membrane.

Variation

The cell membrane has different lipid and protein compositions in distinct types of cells and may have therefore specific names for certain cell types:

- Sarcolemma in myocytes
- Oolemma in oocytes
- Historically, the plasma membrane was also referred to as the plasmalemma.

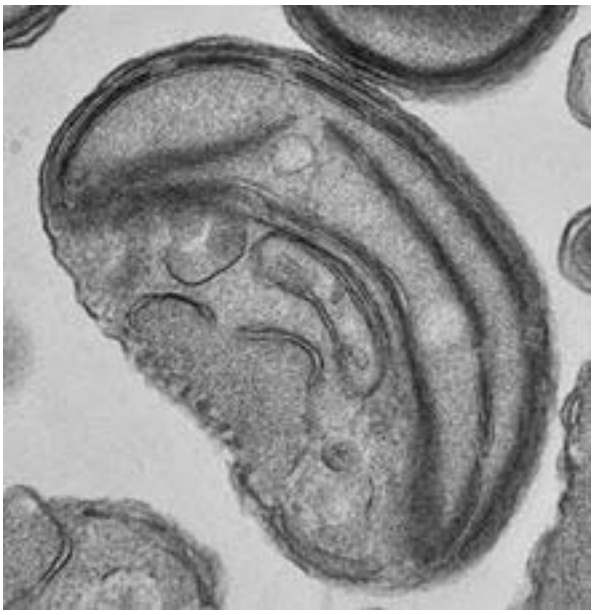
Permeability

The permeability of a membrane is the ease of molecules to pass through it. Permeability depends mainly on the electric charge of the molecule and to a lesser extent the molar mass of the molecule. Electrically neutral and small molecules pass the membrane easier than charged, large ones.

The inability of charged molecules to pass through the cell membrane results in pH parturition of substances throughout the fluid compartments of the body.

Chapter- 3

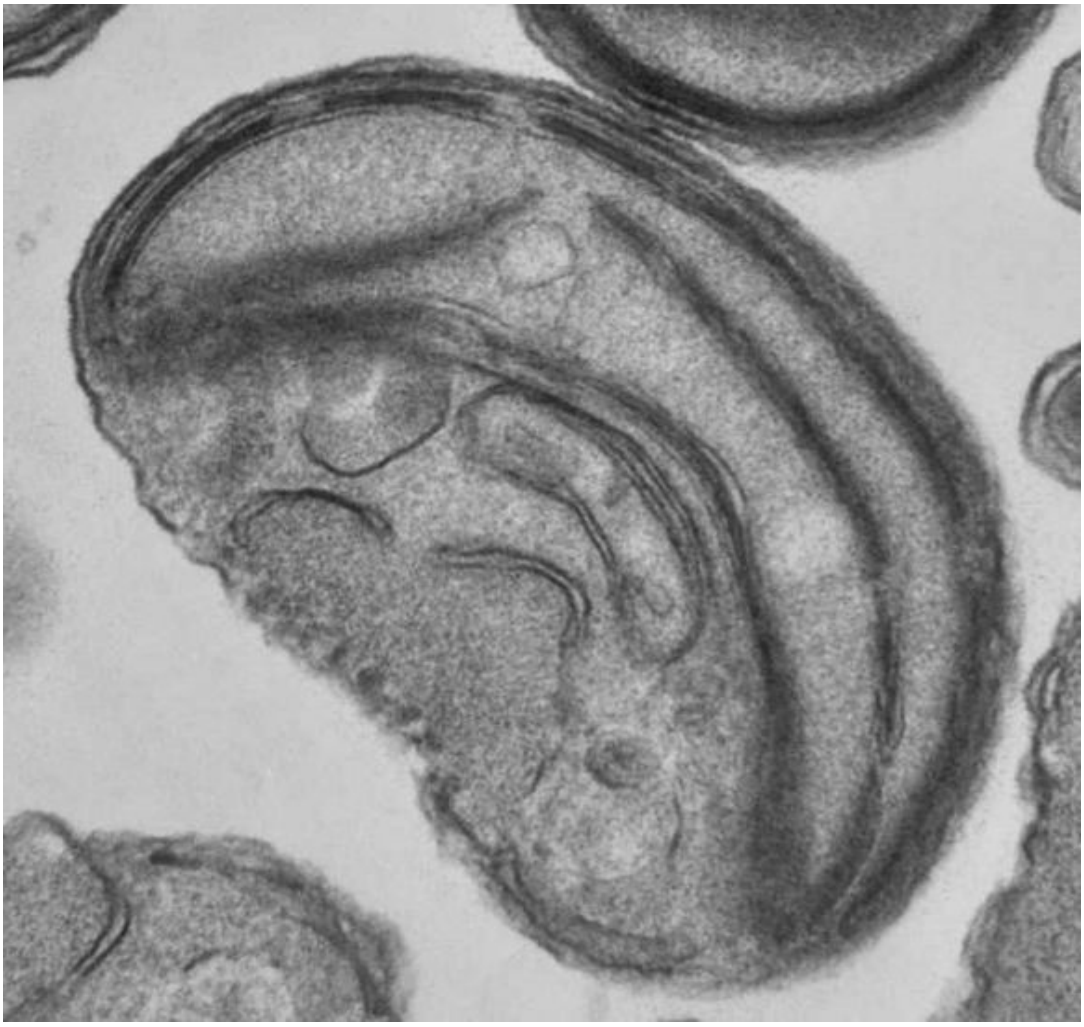
Eukaryote

Eukaryotes Temporal range: Proterozoic – Recent

Ostreococcus is the smallest known free living eukaryote, with an average size of 0.8 µm.
Scientific classification
Domain: **Eukaryota** Whittaker & Margulis,1978
Kingdoms
Animalia – Animals
Fungi
Amoebozoa
Plantae – Plants
Chromalveolata
Rhizaria

Excavata

Alternative phylogeny	

- ***Unikonta***
  - **Opisthokonta**
    - Metazoa
    - Mesomycetozoa
    - Choanozoa
    - Eumycota
  - Amoebozoa
- ***Bikonta***
  - Apusozoa
  - Rhizaria
  - Excavata
  - Archaeplastida
  - Chromalveolata



Eukaryotes

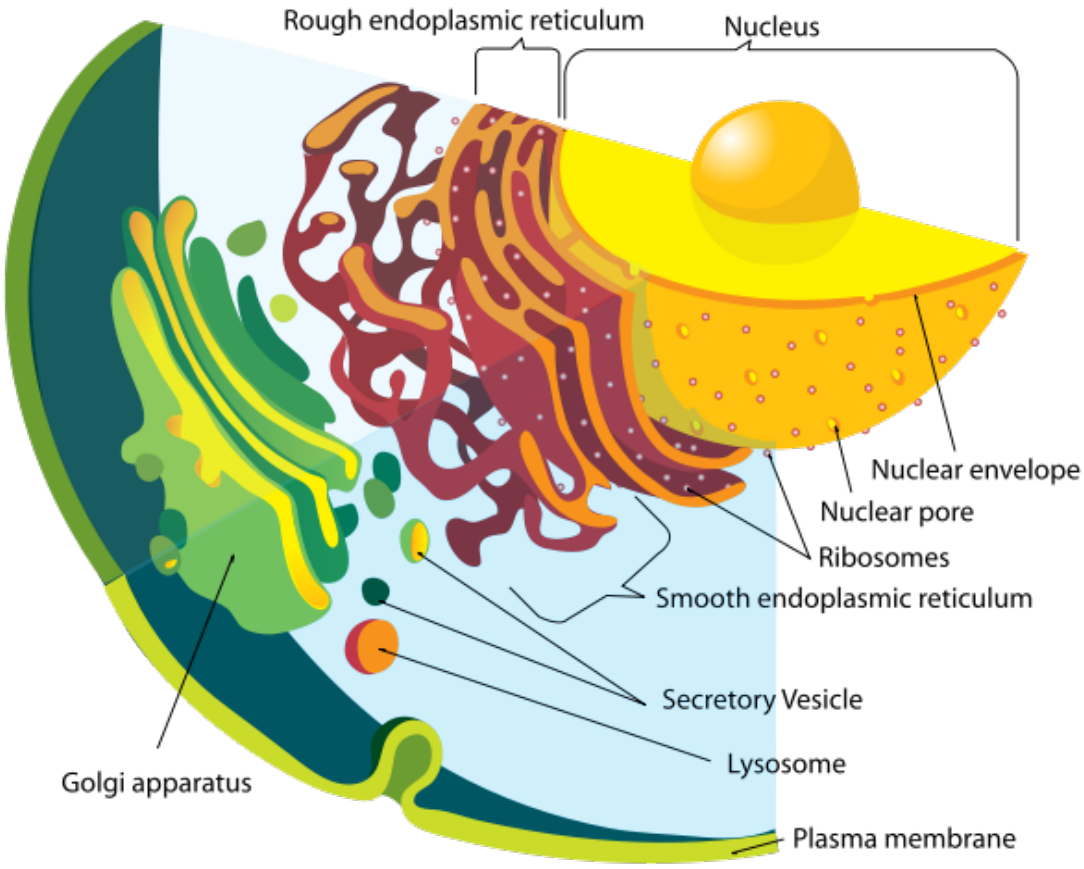
A **eukaryote** is an organism whose cells contain complex structures enclosed within membranes. The defining membrane-bound structure that sets eukaryotic cells apart from prokaryotic cells is the nucleus, or nuclear envelope, within which the genetic material is carried. The presence of a nucleus gives eukaryotes their name, which comes from the Greek ευ (*eu*, "good") and κάρυον (*karyon*, "nut" or "kernel"). Most eukaryotic cells also contain other membrane-bound organelles such as mitochondria, chloroplasts and the Golgi apparatus. All species of large complex organisms are eukaryotes, including animals, plants and fungi, although most species of eukaryotic protists are microorganisms.

Cell division in eukaryotes is different from that in organisms without a nucleus (prokaryotes). It involves separating the duplicated chromosomes, through movements directed by microtubules. There are two types of division processes. In mitosis, one cell divides to produce two genetically identical cells. In meiosis, which is required in sexual reproduction, one diploid cell (having two instances of each chromosome, one from each parent) undergoes recombination of each pair of parental chromosomes, and then two stages of cell division, resulting in four haploid cells (gametes). Each gamete has just one complement of chromosomes, each a unique mix of the corresponding pair of parental chromosomes.

Eukaryotes appear to be monophyletic, and so make up one of the three domains of life. The two other domains, Bacteria and Archaea, are prokaryotes and have none of the above features. Eukaryotes represent a tiny minority of all living things; even in a human body there are 10 times more microbes than human cells.

Cell features

Eukaryotic cells are typically much larger than prokaryotes. They have a variety of internal membranes and structures, called organelles, and a cytoskeleton composed of microtubules, microfilaments, and intermediate filaments, which play an important role in defining the cell's organization and shape. Eukaryotic DNA is divided into several linear bundles called chromosomes, which are separated by a microtubular spindle during nuclear division.



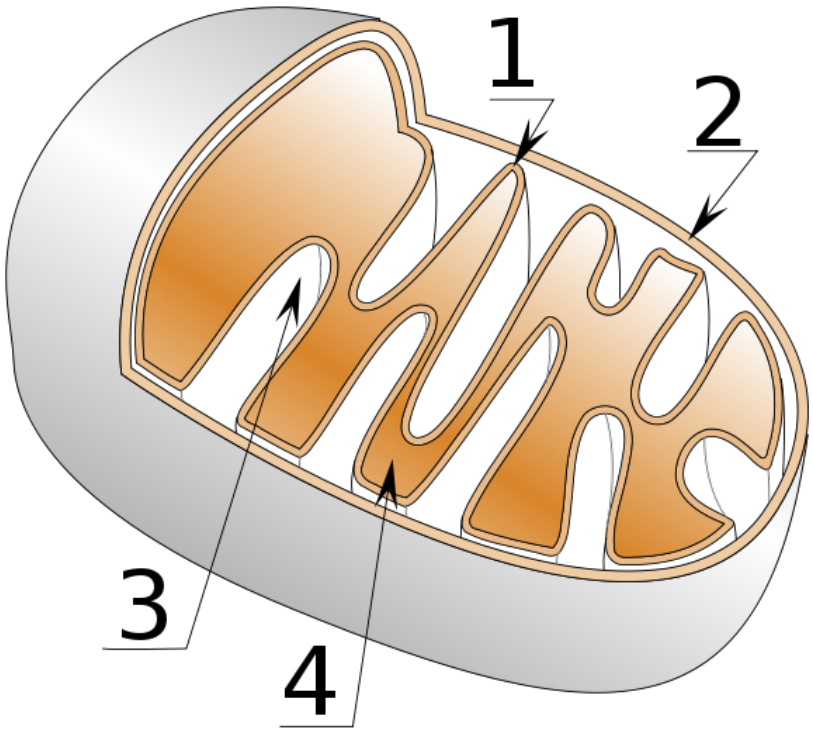
Detail of the endomembrane system and its components

Internal membrane

Eukaryotic cells include a variety of membrane-bound structures, collectively referred to as the endomembrane system. Simple compartments, called vesicles or vacuoles, can form by budding off other membranes. Many cells ingest food and other materials through a process of endocytosis, where the outer membrane invaginates and then pinches off to form a vesicle. It is probable that most other membrane-bound organelles are ultimately derived from such vesicles.

The nucleus is surrounded by a double membrane (commonly referred to as a nuclear envelope), with pores that allow material to move in and out. Various tube- and sheet-like extensions of the nuclear membrane form what is called the endoplasmic reticulum or ER, which is involved in protein transport and maturation. It includes the rough ER where ribosomes are attached, and the proteins they synthesize enter the interior space or lumen. Subsequently, they generally enter vesicles, which bud off from the smooth ER. In most eukaryotes, these protein-carrying vesicles are released and further modified in stacks of flattened vesicles, called Golgi bodies or dictyosomes.

Vesicles may be specialized for various purposes. For instance, lysosomes contain enzymes that break down the contents of food vacuoles, and peroxisomes are used to break down peroxide, which is toxic otherwise. Many protozoa have contractile vacuoles, which collect and expel excess water, and extrusomes, which expel material used to deflect predators or capture prey. In multicellular organisms, hormones are often produced in vesicles. In higher plants, most of a cell's volume is taken up by a central vacuole, which primarily maintains its osmotic pressure.



Mitochondria structure:
1) Inner membrane
2) Outer membrane
3) Crista
4) Matrix

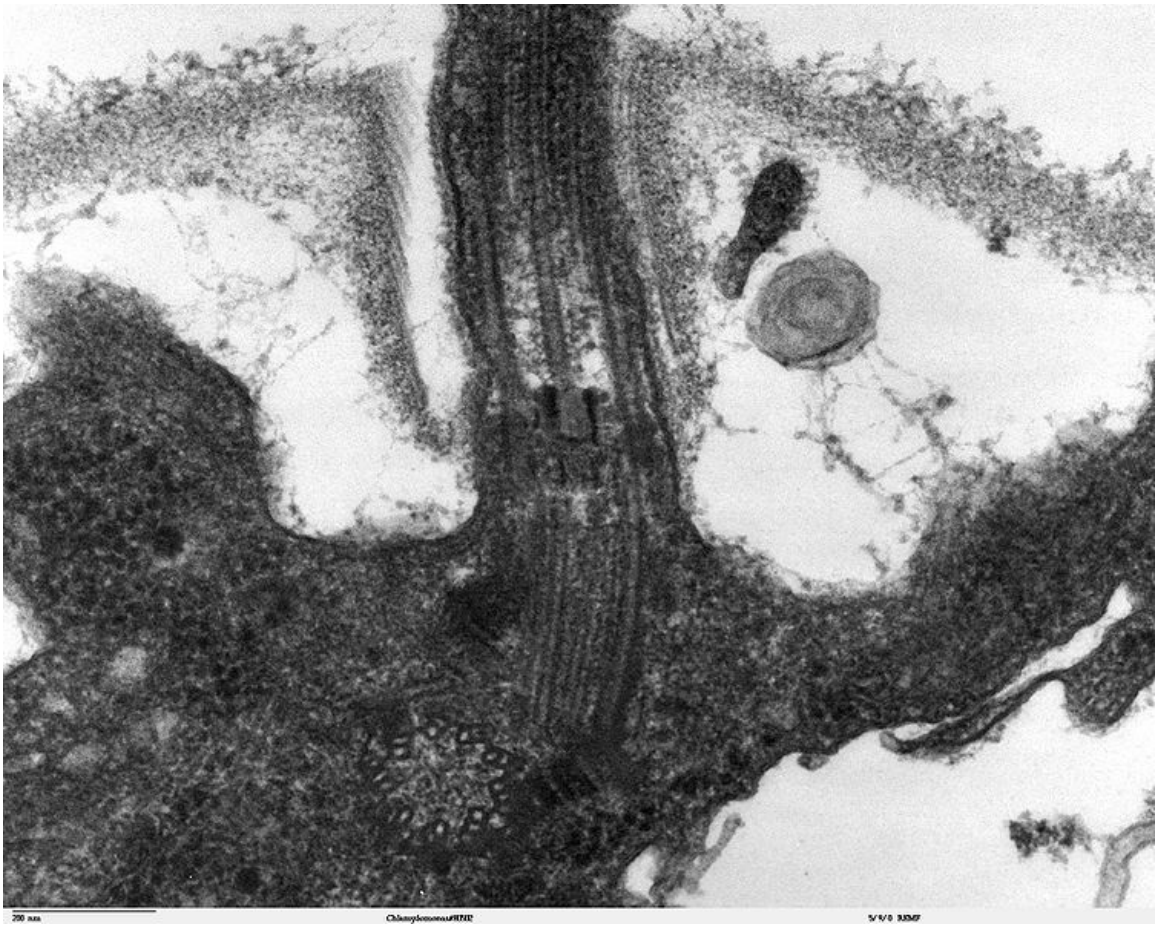
Mitochondria and plastids

Mitochondria are organelles found in nearly all eukaryotes. They are surrounded by double membranes (known as the phospholipid bi-layer), the inner of which is folded into invaginations called cristae, where aerobic respiration takes place. Mitochondria contain their own DNA. They are now generally held to have developed from endosymbiotic prokaryotes, probably proteobacteria. The few protozoa that lack mitochondria have been found to contain mitochondrion-derived organelles, such as hydrogenosomes and mitosomes.

Plants and various groups of algae also have plastids. Again, these have their own DNA and developed from endosymbiotes, in this case cyanobacteria. They usually take the form of chloroplasts, which like cyanobacteria contain chlorophyll and produce organic compounds (such as glucose) through photosynthesis. Others are involved in storing food. Although plastids likely had a single origin, not all plastid-containing groups are closely related. Instead, some eukaryotes have obtained them from others through secondary endosymbiosis or ingestion.

Endosymbiotic origins have also been proposed for the nucleus, for which see below, and for eukaryotic flagella, supposed to have developed from spirochaetes. This is not generally accepted, both from a lack of cytological evidence and difficulty in reconciling this with cellular reproduction.

Cytoskeletal structures



Longitudinal section through the flagellum of *Chlamydomonas reinhardtii*

Many eukaryotes have long slender motile cytoplasmic projections, called flagella, or similar structures called cilia. Flagella and cilia are sometimes referred to as undulipodia, and are variously involved in movement, feeding, and sensation. They are composed mainly of tubulin. These are entirely distinct from prokaryotic flagella. They are

supported by a bundle of microtubules arising from a basal body, also called a kinetosome or centriole, characteristically arranged as nine doublets surrounding two singlets. Flagella also may have hairs, or mastigonemes, and scales connecting membranes and internal rods. Their interior is continuous with the cell's cytoplasm.

Microfilamental structures composed by actin and actin binding proteins, e.g., α-actinin, fimbrin, filamin are present in submembraneous cortical layers and bundles, as well. Motor proteins of microtubules, e.g., dynein or kinesin and actin, e.g., myosins provide dynamic character of the network.

Centrioles are often present even in cells and groups that do not have flagella. They generally occur in groups of one or two, called kinetids, that give rise to various microtubular roots. These form a primary component of the cytoskeletal structure, and are often assembled over the course of several cell divisions, with one flagellum retained from the parent and the other derived from it. Centrioles may also be associated in the formation of a spindle during nuclear division.

Significance of cytoskeletal structures is underlined in determination of shape of the cells, as well as their being essential components of migratory responses like chemotaxis and chemokinesis. Some protists have various other microtubule-supported organelles. These include the radiolaria and heliozoa, which produce axopodia used in flotation or to capture prey, and the haptophytes, which have a peculiar flagellum-like organelle called the haptonema. An animal cell is a form of Eukaryotic cell that makes up many tissues in animals.

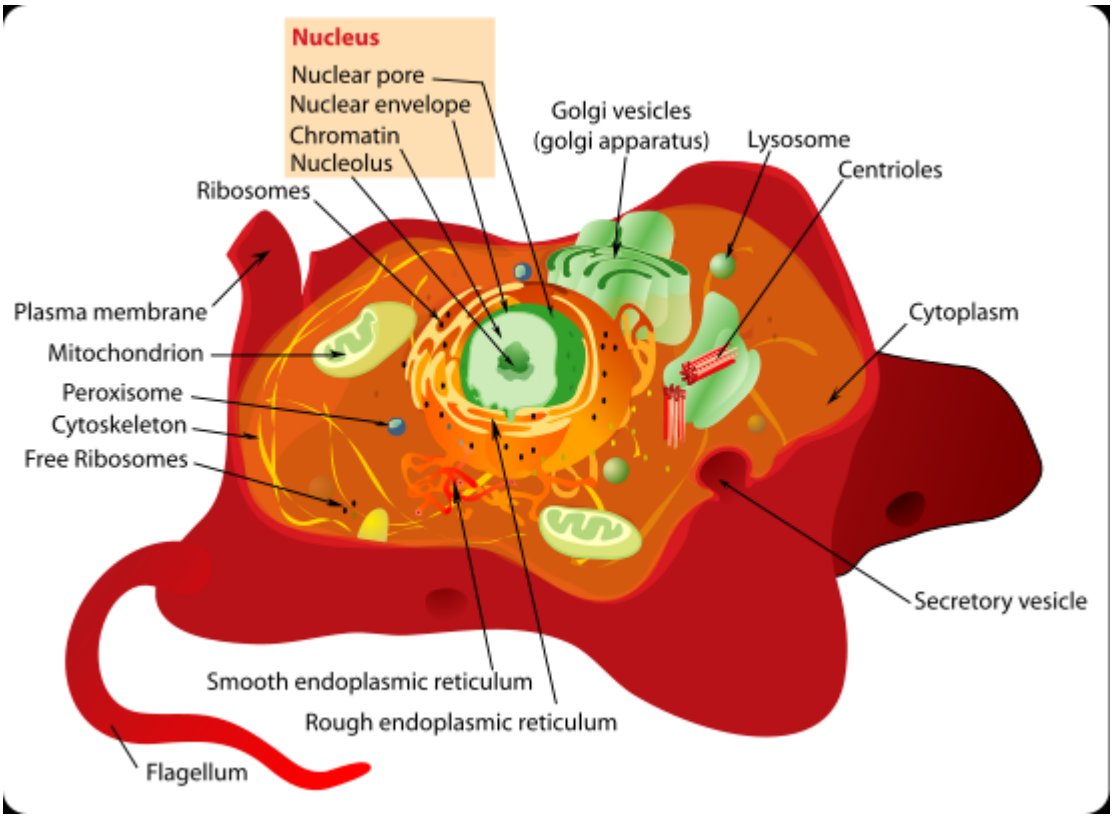
Plant cell wall

Plant cells have a cell wall, a fairly rigid layer outside the cell membrane, providing the cell with structural support, protection, and a filtering mechanism. The cell wall also prevents over-expansion when water enters the cell. The major carbohydrates making up the primary cell wall of land plants are cellulose, hemicellulose, and pectin. The cellulose microfibrils are linked via hemicellulosic tethers to form the cellulose-hemicellulose network, which is embedded in the pectin matrix. The most common hemicellulose in the primary cell wall is xyloglucan.

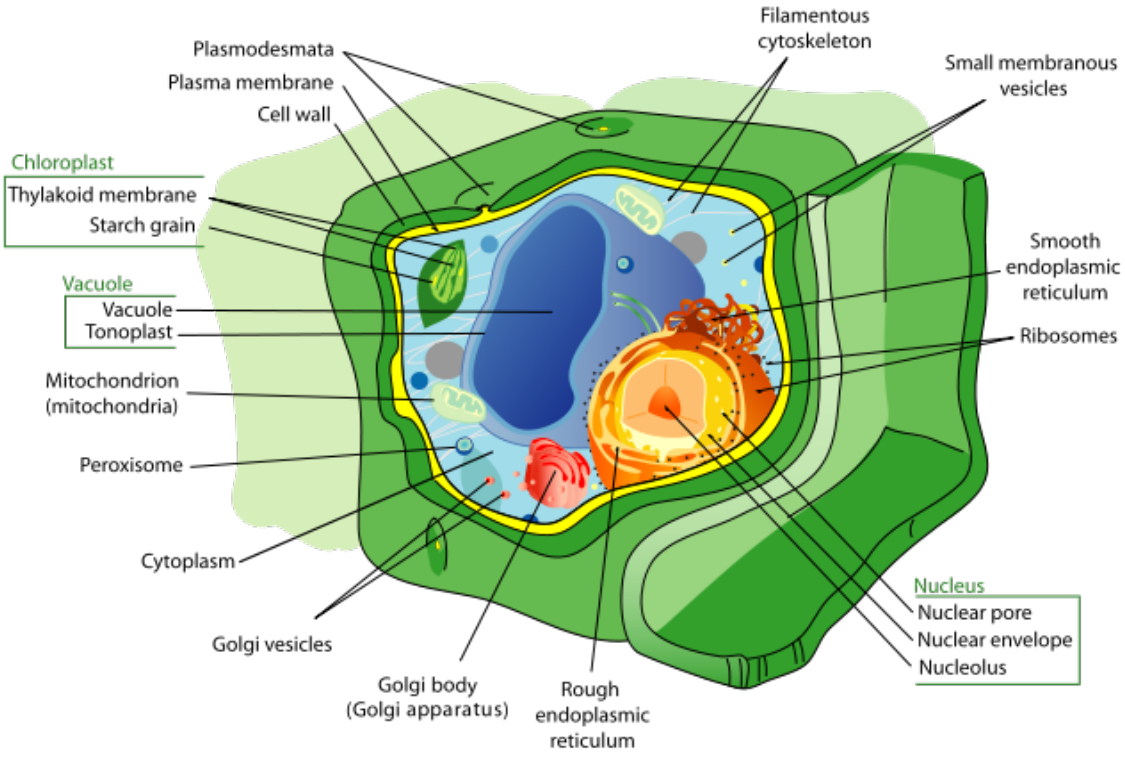
Differences between eukaryotic cells

There are many different types of eukaryotic cells, though animals and plants are the most familiar eukaryotes, and thus provide an excellent starting point for understanding eukaryotic structure. Fungi and many protists have some substantial differences, however.

Animal cell



Structure of a typical animal cell



Structure of a typical plant cell

An **animal cell** is a form of eukaryotic cell that makes up many tissues in animals. The animal cell is distinct from other eukaryotes, most notably plant cells, as they lack cell walls and chloroplasts, and they have smaller vacuoles. Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures.

There are many different cell types. For instance, there are approximately 210 distinct cell types in the adult human body.

Plant cell

Plant cells are quite different from the cells of the other eukaryotic organisms. Their distinctive features are:

- A large central vacuole (enclosed by a membrane, the tonoplast), which maintains the cell's turgor and controls movement of molecules between the cytosol and sap
- A primary cell wall containing cellulose, hemicellulose and pectin, deposited by the protoplast on the outside of the cell membrane; this contrasts with the cell walls of fungi, which contain chitin, and the cell envelopes of prokaryotes, in which peptidoglycans are the main structural molecules

- The plasmodesmata, linking pores in the cell wall that allow each plant cell to communicate with other adjacent cells; this is different from the functionally analogous system of gap junctions between animal cells.
- Plastids, especially chloroplasts that contain chlorophyll, the pigment that gives plants their green color and allows them to perform photosynthesis
- Higher plants, including conifers and flowering plants (Angiospermae) lack the flagellae and centrioles that are present in animal cells.

Fungal cell

Fungal cells are most similar to animal cells, with the following exceptions:

- A cell wall that contains chitin
- Less definition between cells; the hyphae of higher fungi have porous partitions called septa, which allow the passage of cytoplasm, organelles, and, sometimes, nuclei. Primitive fungi have few or no septa, so each organism is essentially a giant multinucleate supercell; these fungi are described as coenocytic.
- Only the most primitive fungi, chytrids, have flagella.

Other eukaryotic cells

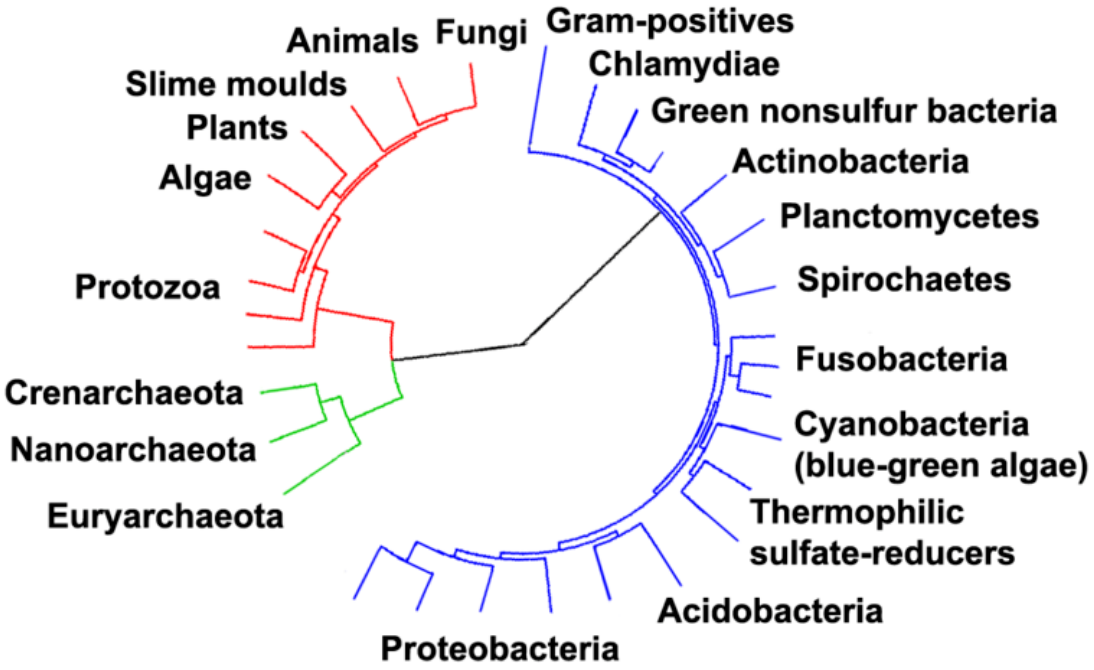
Eukaryotes are a very diverse group, and their cell structures are equally diverse. Many have cell walls; many do not. Many have chloroplasts, derived from primary, secondary, or even tertiary endosymbiosis; and many do not. Some groups have unique structures, such as the cyanelles of the glaucophytes, the haptonema of the haptophytes, or the ejectisomes of the cryptomonads. Other structures, such as pseudopods, are found in various eukaryote groups in different forms, such as the lobose amoebozoans or the reticulose foraminiferans.

Reproduction

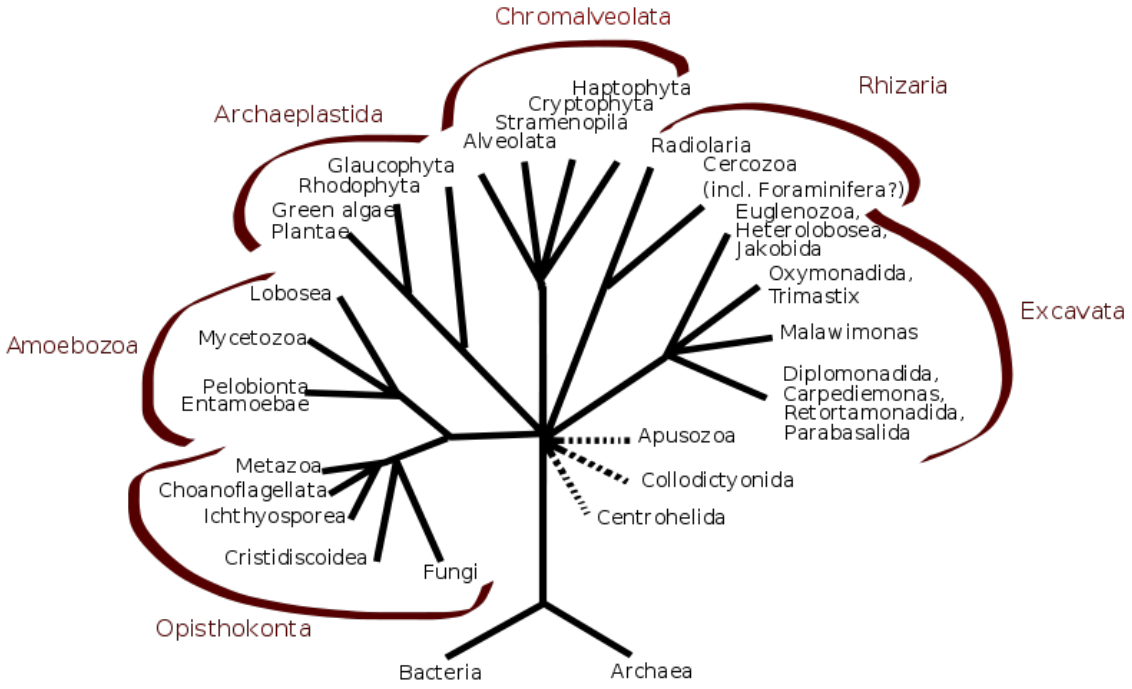
Nuclear division is often coordinated with cell division. This generally takes place by mitosis, a process that allows each daughter nucleus to receive one copy of each chromosome. In most eukaryotes, there is also a process of sexual reproduction, typically involving an alternation between haploid generations, wherein only one copy of each chromosome is present, and diploid generations, wherein two are present, occurring through nuclear fusion (syngamy) and meiosis. There is considerable variation in this pattern, however.

Eukaryotes have a smaller surface area to volume ratio than prokaryotes, and thus have lower metabolic rates and longer generation times. In some multicellular organisms, cells specialized for metabolism will have enlarged surface areas, such as intestinal vili.

Origin and evolution



Phylogenetic tree showing the relationship between the eukaryotes and other forms of life. Eukaryotes are colored red, archaea green and bacteria blue.



One hypothesis of eukaryotic relationships

The origin of the eukaryotic cell was a milestone in the evolution of life, since they include all complex cells and almost all multi-cellular organisms. The timing of this series of events is hard to determine; Knoll (2006) suggests they developed approximately 1.6–2.1 billion years ago. Some acritarchs are known from at least 1650 million years ago, and the possible alga *Grypania* has been found as far back as 2100 million years ago.

Fossils that are clearly related to modern groups start appearing around 1.2 billion years ago, in the form of a red alga, though recent work suggests the existence of fossilized filamentous algae in the Vindhya basin dating back to 1.6 to 1.7 billion years ago.

Biomarkers suggest that at least stem eukaryotes arose even earlier. The presence of steranes in Australian shales indicates that eukaryotes were present 2.7 billion years ago.

Phylogeny

rRNA trees constructed during the 1980s and 1990s left most eukaryotes in an unresolved "crown" group (not technically a true crown), which was usually divided by the form of the mitochondrial cristae. The few groups that lack mitochondria branched separately, and so the absence was believed to be primitive; but this is now considered an artifact of long-branch attraction, and they are known to have lost them secondarily.

Six supergroup/kingdom model

A classification produced in 2005 for the International Society of Protistologists, which reflected the consensus of the time, divided the eukaryotes into six supposedly monophyletic 'supergroups'. Although the published classification deliberately did not use formal taxonomic ranks, other sources have treated each of the six as a separate Kingdom.

Excavata	Various flagellate protozoa
Amoebozoa	Most lobose amoeboids and slime moulds
Opisthokonta	Animals, fungi, choanoflagellates, etc.
Rhizaria	Foraminifera, Radiolaria, and various other amoeboid protozoa
Chromalveolata	Stramenopiles (or Heterokonta), Haptophyta, Cryptophyta (or cryptomonads), and Alveolata
Archaeplastida (or Primoplantae)	Land plants, green algae, red algae, and glaucophytes

However, in the same year (2005), doubts were expressed as to whether some of these supergroups were monophyletic, particularly the Chromalveolata, and a review in 2006 noted the lack of evidence for several of the supposed six supergroups.

As of 2010, there is widespread agreement that the Rhizaria belong with the Stramenopiles and the Alveolata, in a clade dubbed the SAR supergroup, so that Rhizara is not one of the main eukaryote groups; also that the Amoeboza and Opisthokonta are each monophyletic and form a clade, often called the unikonts. Beyond this, there does not appear to be a consensus.

Relationship to Archaea

Eukaryotes are more closely related to Archaea than Bacteria, at least in terms of nuclear DNA and genetic machinery, and one controversial idea is to place them with Archaea in the clade Neomura. However, in other respects, such as membrane composition, eukaryotes are similar to Bacteria. Three main explanations for this have been proposed:

- Eukaryotes resulted from the complete fusion of two or more cells, wherein the cytoplasm formed from a eubacterium, and the nucleus from an archaeon, from a virus, or from a pre-cell.
- Eukaryotes developed from Archaea, and acquired their eubacterial characteristics from the proto-mitochondrion.
- Eukaryotes and Archaea developed separately from a modified eubacterium.

There is also the Kronocyte theory for the origin of the Eukaryotic cell. This postulates that a primitive Eukaryotic cell emerged from the pre-DNA world but retained the earlier RNA based chemistry from which all modern life emerged. This primitive cell is called

the Kronocyte. According to this hypothesis an RNA based Kronocyte coexisted with the DNA based Archaea (and probably eubacteria) and became the modern eukaryotic cell after a number of major endosymbioses—the first was the incorporation of an Archaea that introduced DNA metabolism and the nucleus, then the incorporation of an alphaproteobacter that became the mitochondria (and photosynthetic bacteria found in today's plants as chloroplasts). The Kronocyte hypothesis explains the large number of genes that are today only found in Eukaryotes but not in Archaea or Bacteria.

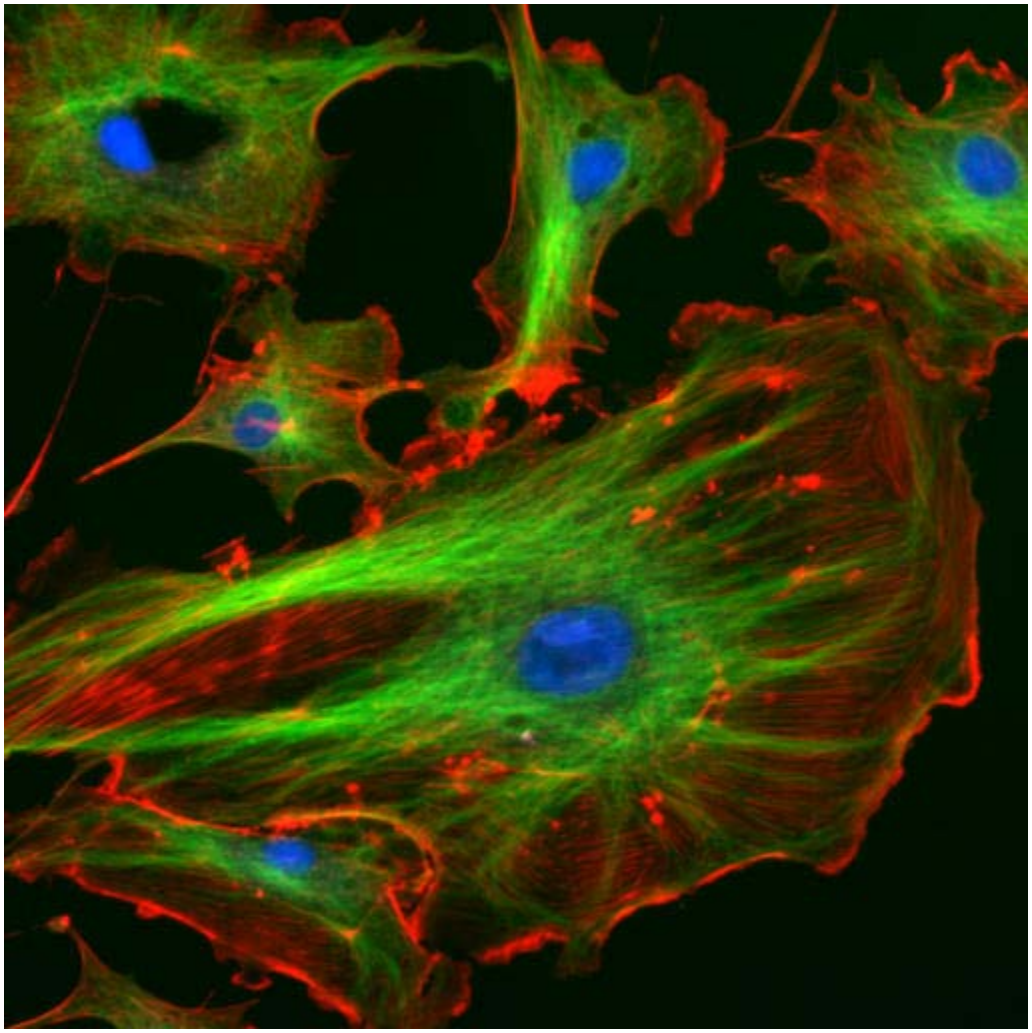
Endomembrane system and mitochondria

The origins of the endomembrane system and mitochondria are also unclear. The **phagotrophic hypothesis** proposes that eukaryotic-type membranes lacking a cell wall originated first, with the development of endocytosis, whereas mitochondria were acquired by ingestion as endosymbionts. The **syntrophic hypothesis** proposes that the proto-eukaryote relied on the proto-mitochondrion for food, and so ultimately grew to surround it. Here the membranes originated after the engulfment of the mitochondrion, in part thanks to mitochondrial genes (the hydrogen hypothesis is one particular version).

In a study using genomes to construct supertrees, Pisani *et al.* (2007) suggest that, along with evidence that there was never a mitochondrion-less eukaryote, eukaryotes evolved from a syntrophy between an archaea closely related to Thermoplasmatales and an α-proteobacterium, likely a symbiosis driven by sulfur or hydrogen. The mitochondrion and its genome is a remnant of the α-proteobacterial endosymbiont.

Chapter- 4

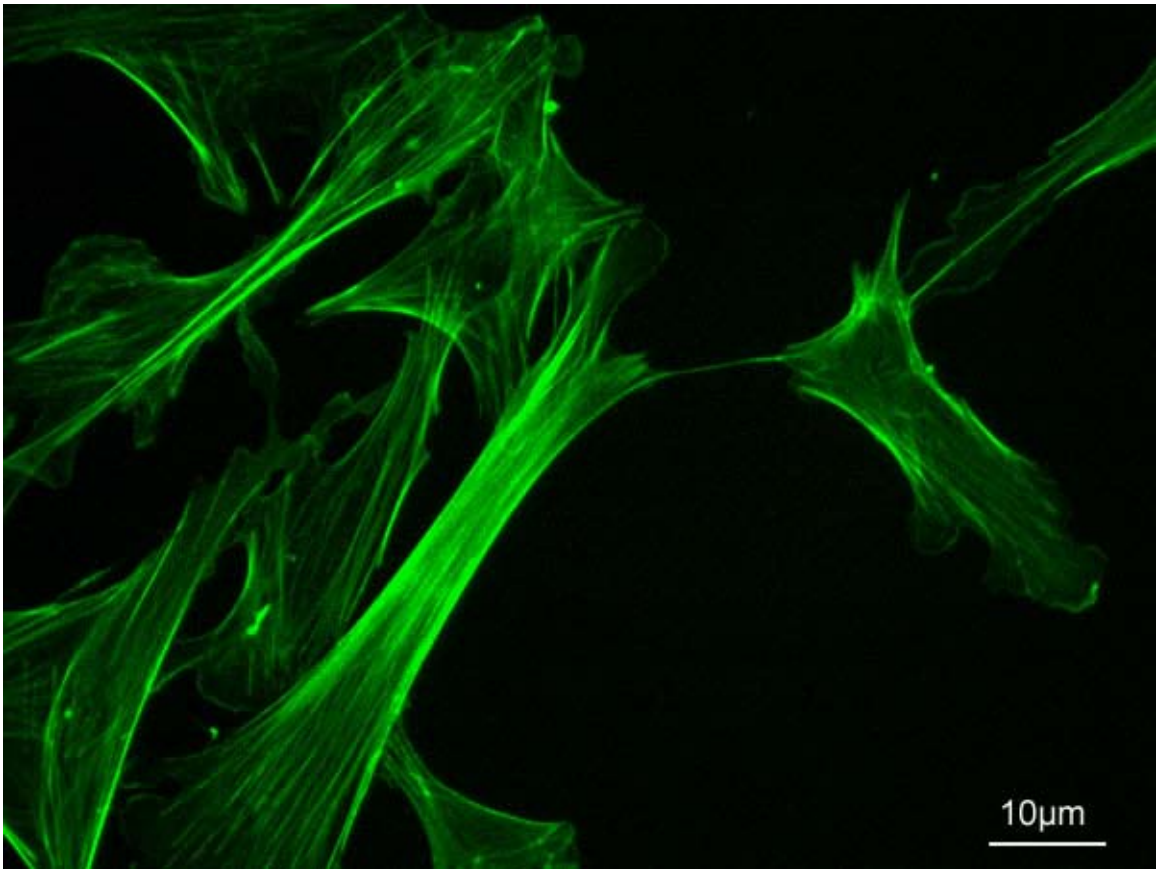
Cytoskeleton



The eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei are in blue.

The **cytoskeleton** (also CSK) is a cellular "scaffolding" or "skeleton" contained within the cytoplasm and is made out of protein. The cytoskeleton is present in all cells; it was once thought to be unique to eukaryotes, but recent research has identified the prokaryotic cytoskeleton. It has structures such as flagella, cilia and lamellipodia and plays important roles in both intracellular transport (the movement of vesicles and organelles, for example) and cellular division. The concept of a protein mosaic that dynamically coordinated cytoplasmic biochemistry was proposed by Rudolph Peters in 1929 while the term (*cytosquelette*, in French) was first introduced by French embryologist Paul Wintrebert in 1931.

The eukaryotic cytoskeleton



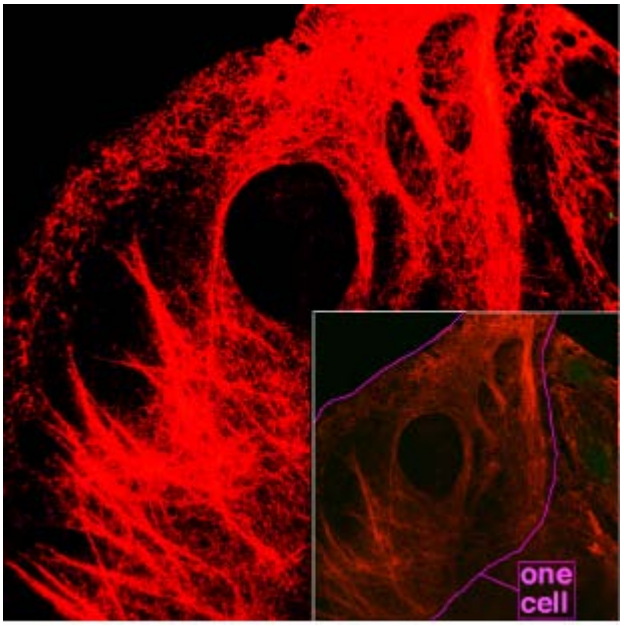
Actin cytoskeleton of mouse embryo fibroblasts, stained with phalloidin.

Eukaryotic cells contain three main kinds of cytoskeletal filaments, which are microfilaments, intermediate filaments, and microtubules. The cytoskeleton provides the cell with structure and shape, and by excluding macromolecules from some of the cytosol it adds to the level of macromolecular crowding in this compartment. Cytoskeletal elements interact extensively and intimately with cellular membranes.

Microfilaments

These are the thinnest filaments of the cytoskeleton. They are composed of linear polymers of actin subunits, and generate force by elongation at one end of the filament coupled with shrinkage at the other, causing net movement of the intervening strand. They also act as tracks for the movement of myosin molecules that attach to the microfilament and "walk" along them.

Intermediate filaments



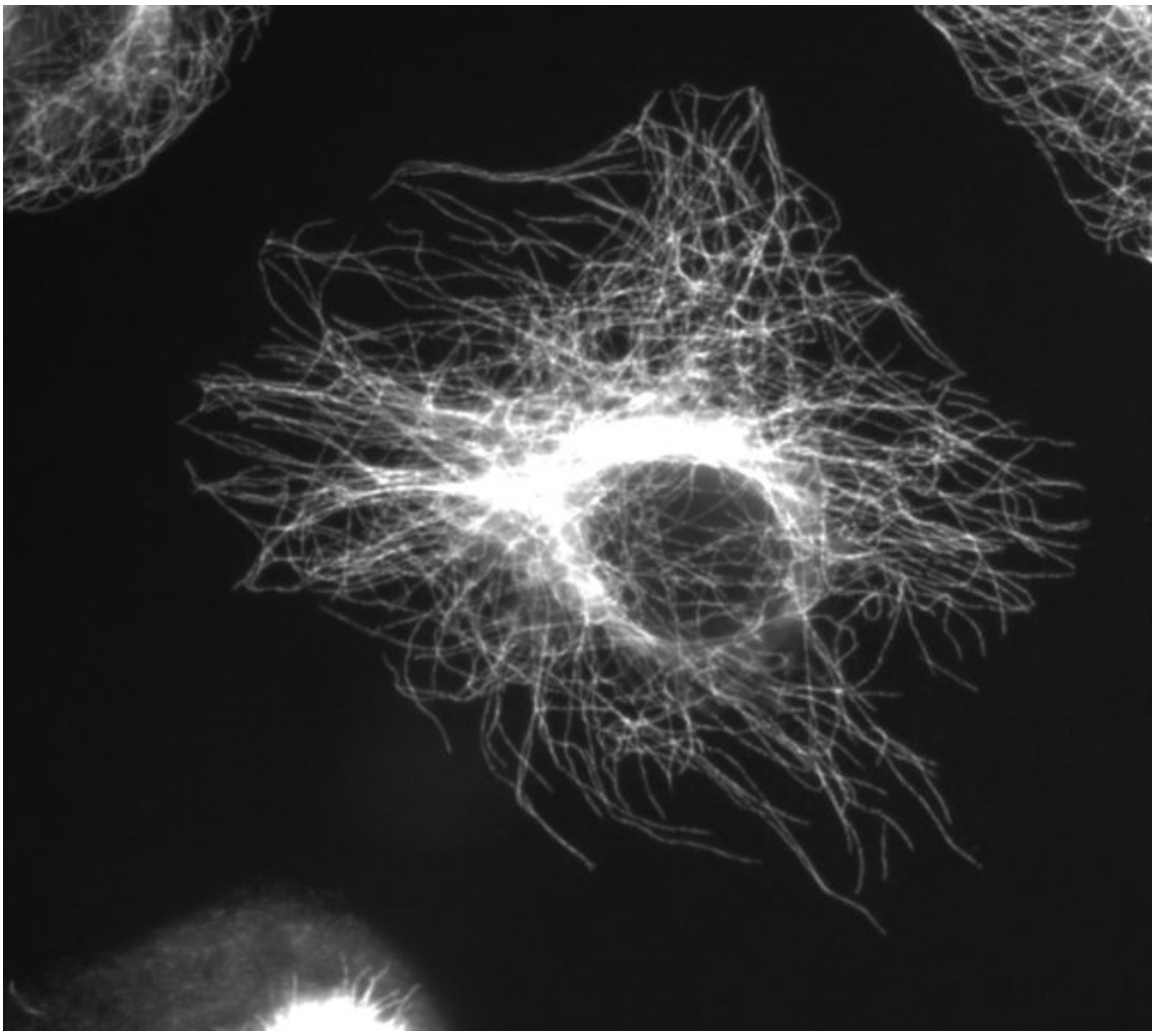
Microscopy of keratin filaments inside cells.

These filaments, around 10 nanometers in diameter, are more stable (strongly bound) than actin filaments, and heterogeneous constituents of the cytoskeleton. Although little work has been done on intermediate filaments in plants, there is some evidence that cytosolic intermediate filaments might be present, and plant nuclear filaments have been detected. Like actin filaments, they function in the maintenance of cell-shape by bearing tension (microtubules, by contrast, resist compression. It may be useful to think of micro- and intermediate filaments as cables, and of microtubules as cellular support beams). Intermediate filaments organize the internal tridimensional structure of the cell, anchoring organelles and serving as structural components of the nuclear lamina and sarcomeres. They also participate in some cell-cell and cell-matrix junctions.

Different intermediate filaments are:

- made of vimentins, being the common structural support of many cells.
- made of keratin, found in skin cells, hair and nails.
- neurofilaments of neural cells.
- made of lamin, giving structural support to the nuclear envelope.

Microtubules



Microtubules in a gel fixated cell.

Microtubules are hollow cylinders about 23 nm in diameter (lumen = approximately 15nm in diameter), most commonly comprising 13 protofilaments which, in turn, are polymers of alpha and beta tubulin. They have a very dynamic behaviour, binding GTP for polymerization. They are commonly organized by the centrosome.

In nine triplet sets (star-shaped), they form the centrioles, and in nine doublets oriented about two additional microtubules (wheel-shaped) they form cilia and flagella. The latter formation is commonly referred to as a "9+2" arrangement, wherein each doublet is connected to another by the protein dynein. As both flagella and cilia are structural components of the cell, and are maintained by microtubules, they can be considered part of the cytoskeleton.

They play key roles in:

- intracellular transport (associated with dyneins and kinesins, they transport organelles like mitochondria or vesicles).
- the axoneme of cilia and flagella.
- the mitotic spindle.
- synthesis of the cell wall in plants.

Comparison

Cytoskeleton type	Diameter (nm)	Structure	Subunit examples
Microfilaments	6	double helix	actin
Intermediate filaments	10	two anti-parallel helices/dimers, forming tetramers	• vimentin (mesenchyme) • glial fibrillary acidic protein (glial cells) • neurofilament proteins (neuronal processes) • keratins (epithelial cells) • nuclear lamins
Microtubules	23	protofilaments, in turn consisting of tubulin subunits	α- and β-tubulin

The prokaryotic cytoskeleton

The cytoskeleton was previously thought to be a feature only of eukaryotic cells, but homologues to all the major proteins of the eukaryotic cytoskeleton have recently been found in prokaryotes. Although the evolutionary relationships are so distant that they are not obvious from protein sequence comparisons alone, the similarity of their three-dimensional structures and similar functions in maintaining cell shape and polarity provides strong evidence that the eukaryotic and prokaryotic cytoskeletons are truly homologous. However, some structures in the bacterial cytoskeleton may have yet to be identified.

FtsZ

FtsZ was the first protein of the prokaryotic cytoskeleton to be identified. Like tubulin, FtsZ forms filaments in the presence of GTP, but these filaments do not group into tubules. During cell division, FtsZ is the first protein to move to the division site, and is essential for recruiting other proteins that synthesize the new cell wall between the dividing cells.

MreB and ParM

Prokaryotic actin-like proteins, such as MreB, are involved in the maintenance of cell shape. All non-spherical bacteria have genes encoding actin-like proteins, and these

proteins form a helical network beneath the cell membrane that guides the proteins involved in cell wall biosynthesis.

Some plasmids encode a partitioning system that involves an actin-like protein ParM. Filaments of ParM exhibit dynamic instability, and may partition plasmid DNA into the dividing daughter cells by a mechanism analogous to that used by microtubules during eukaryotic mitosis.

Crescentin

The bacterium *Caulobacter crescentus* contains a third 3rd protein, crescentin, that is related to the intermediate filaments of eukaryotic cells. Crescentin is also involved in maintaining cell shape, such as helical and vibrioid forms of bacteria, but the mechanism by which it does this is currently unclear.

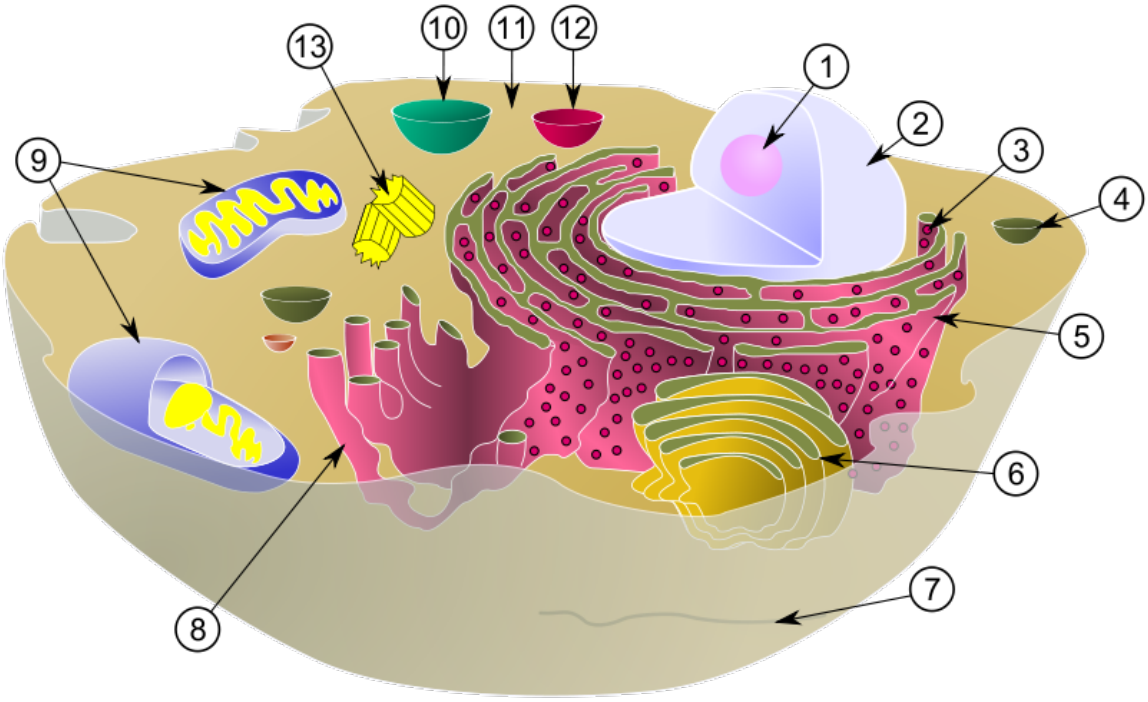
History

Microtrabeculae

A fourth eukaryotic cytoskeletal element, *microtrabeculae*, was proposed by Keith Porter based on images obtained from high-voltage electron microscopy of whole cells in the 1970s. The images showed short, filamentous structures of unknown molecular composition associated with known cytoplasmic structures. Porter proposed that this microtrabecular structure represented a novel filamentous network distinct from microtubules, filamentous actin, or intermediate filaments. It is now generally accepted that microtrabeculae are nothing more than an artifact of certain types of fixation treatment, although we have yet to fully understand the complexity of the cell's cytoskeleton.

Chapter- 5

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.

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	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 proteins (rough endoplasmic reticulum), expression of lipids (smooth endoplasmic reticulum)	single-membrane compartment	all eukaryotes	rough endoplasmic reticulum is covered 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	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, 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.

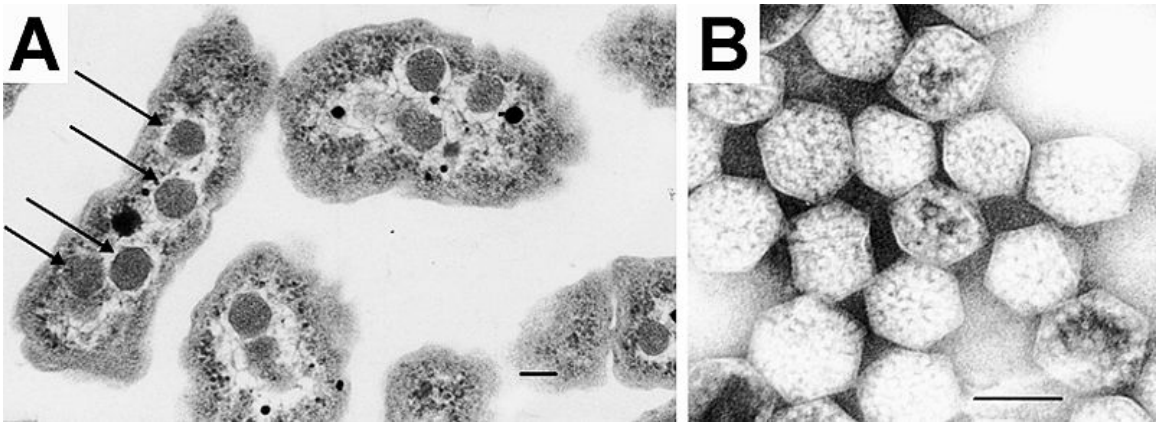
Minor eukaryotic organelles and cell components

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	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-	most eukaryotes

		RNA	
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

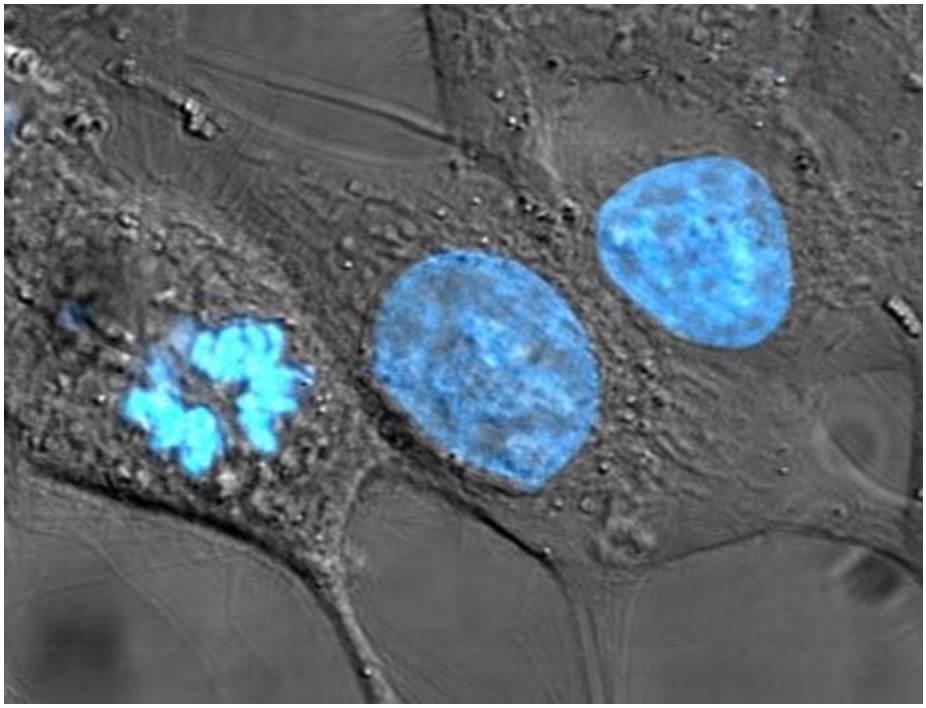
Organelle/Macromolecule	Main function	Structure	Organisms
carboxysome	carbon fixation	protein-shell compartment	some bacteria
chlorosome	photosynthesis	light harvesting complex	green sulfur bacteria
flagellum	movement in external medium	protein filament	some prokaryotes and eukaryotes
magnetosome	magnetic 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

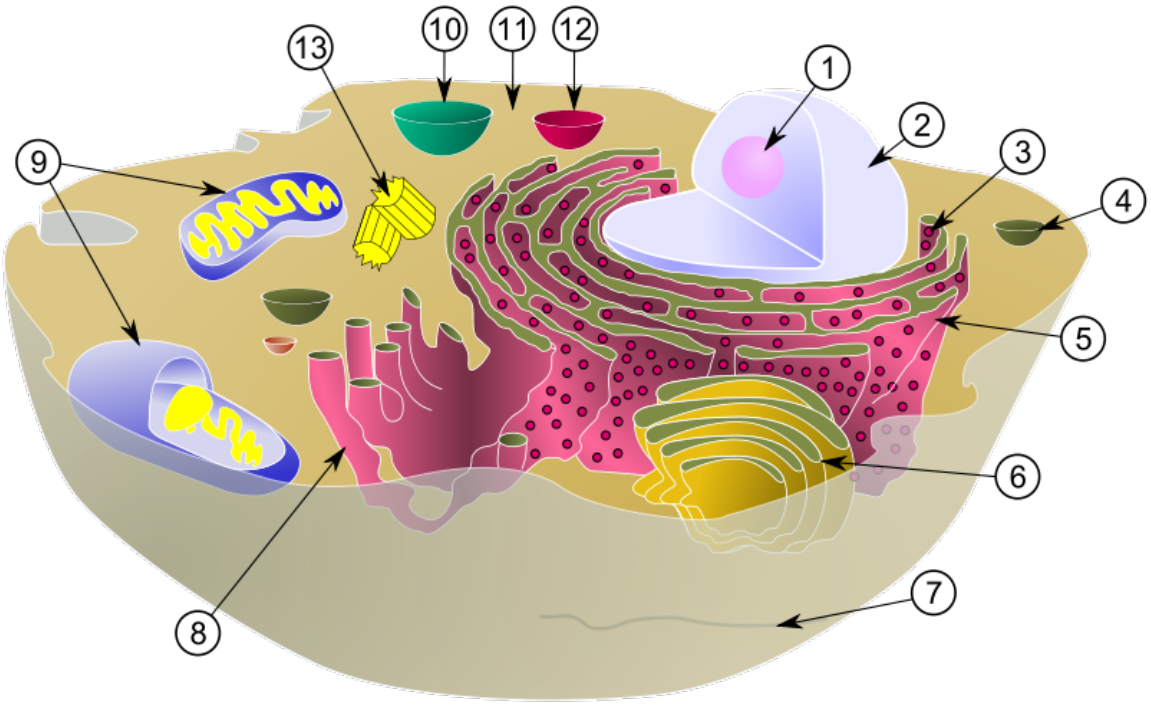
The function of a protein is closely correlated with the organelle in which it resides. Some methods were proposed for predicting the organelle in which an uncharacterized protein is located according to its amino acid composition and some methods were based on pseudo amino acid composition .

Chapter- 6

Cell Nucleus



HeLa cells stained for DNA with the Blue Hoechst dye. The central and rightmost cell are in interphase, thus teir 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

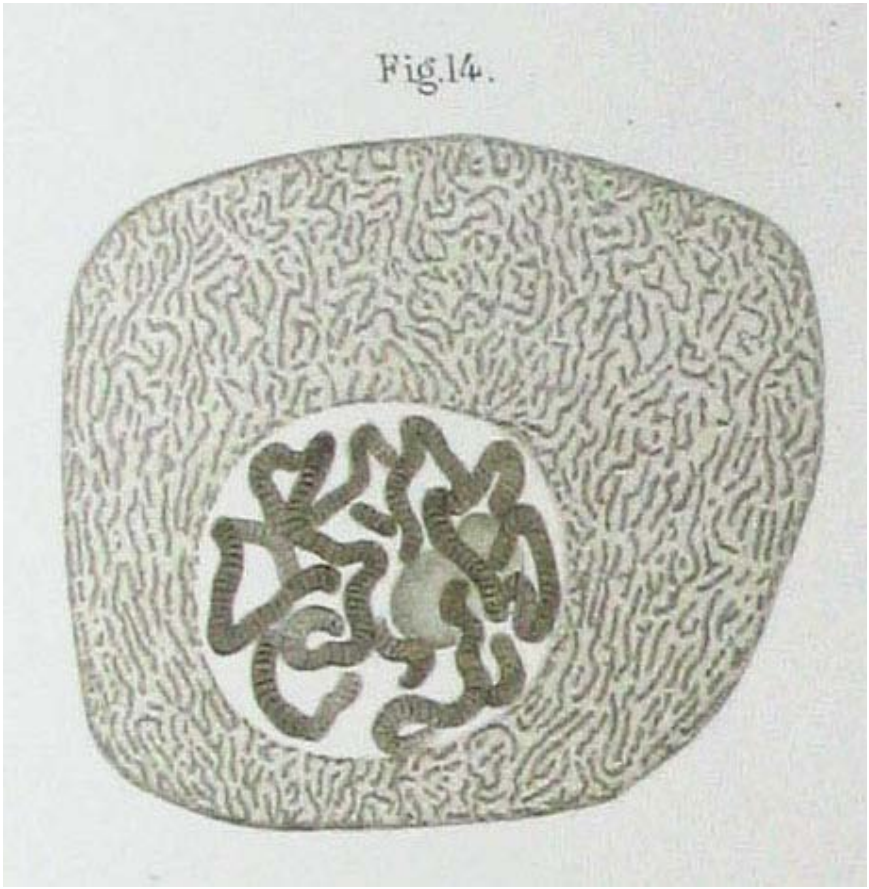
In cell biology, the **nucleus** (pl. *nuclei*; from Latin *nucleus* or *nuculeus*, meaning kernel) is a membrane enclosed organelle found in eukaryotic cells. It contains most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, 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 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.

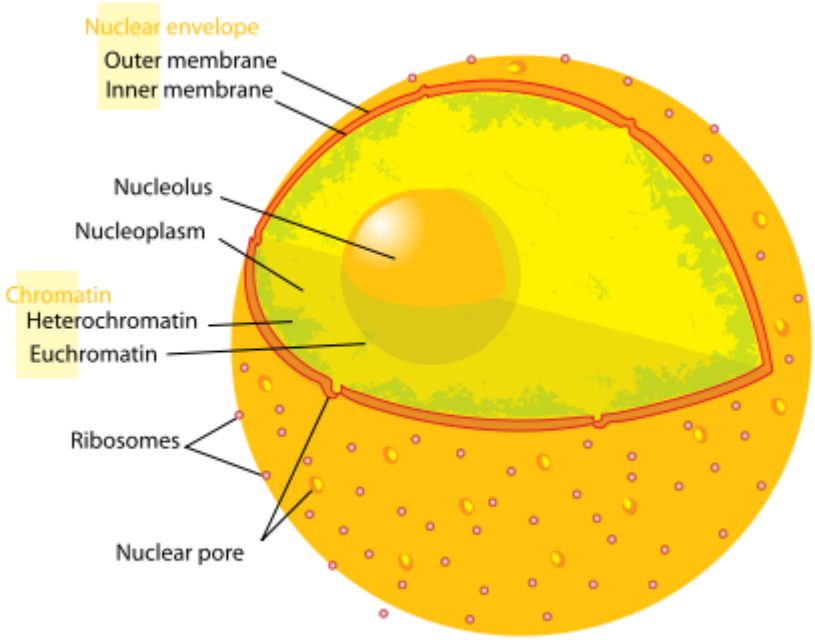
The nucleus was the first organelle to be discovered. The probably oldest preserved drawing dates back to the early microscopist Antonie van Leeuwenhoek (1632 – 1723). He observed a "Lumen", the nucleus, in the red blood cells of salmon. Unlike mammalian red blood cells, those of other vertebrates still 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 microscopically 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.

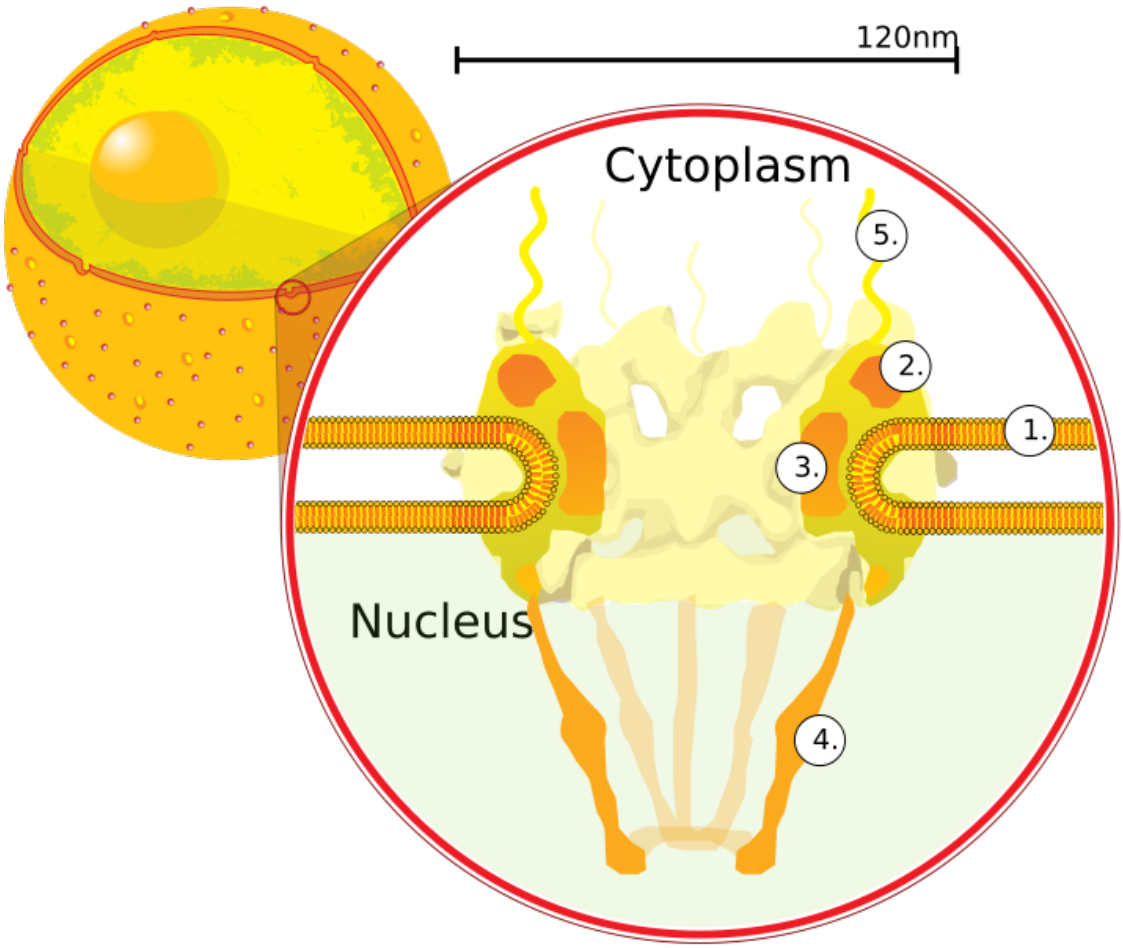
Structures

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,(ref name="Rhoades")Rodney Rhoades, Richard Pflanzer, ed (1996). "Ch3". *Human Physiology* (3rd ed.). Saunders College Publishing.</ref> 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, while 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.

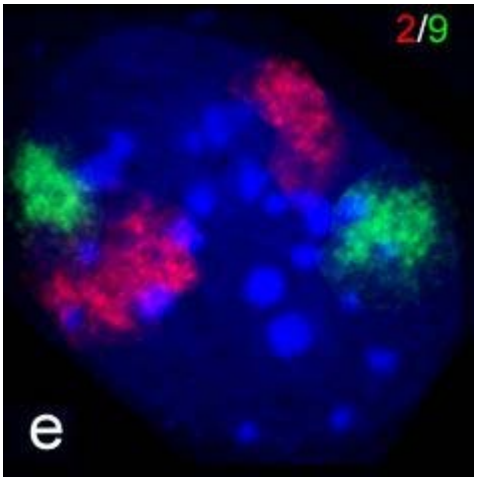
The nuclear lamina is mostly composed of lamin proteins. Like all proteins, lamins are synthesized in the cytoplasm and later transported into the nucleus interior, where they are assembled before being incorporated into the existing network of nuclear lamina. Lamins are also found inside the nucleoplasm 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



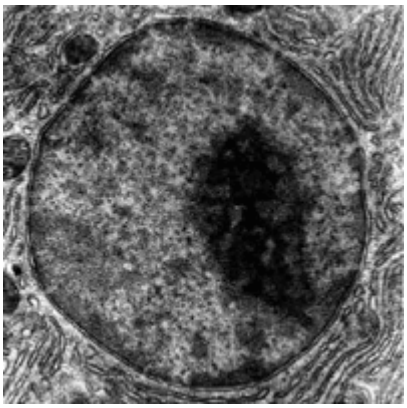
A mouse fibroblast nucleus in which DNA is stained blue. The distinct chromosome territories of chromosome 2 (red) and chromosome 9 (green) are visible stained with fluorescent in situ hybridization.

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, particularly 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 have 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.

PIKA and PTF domains

PIKA 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

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.

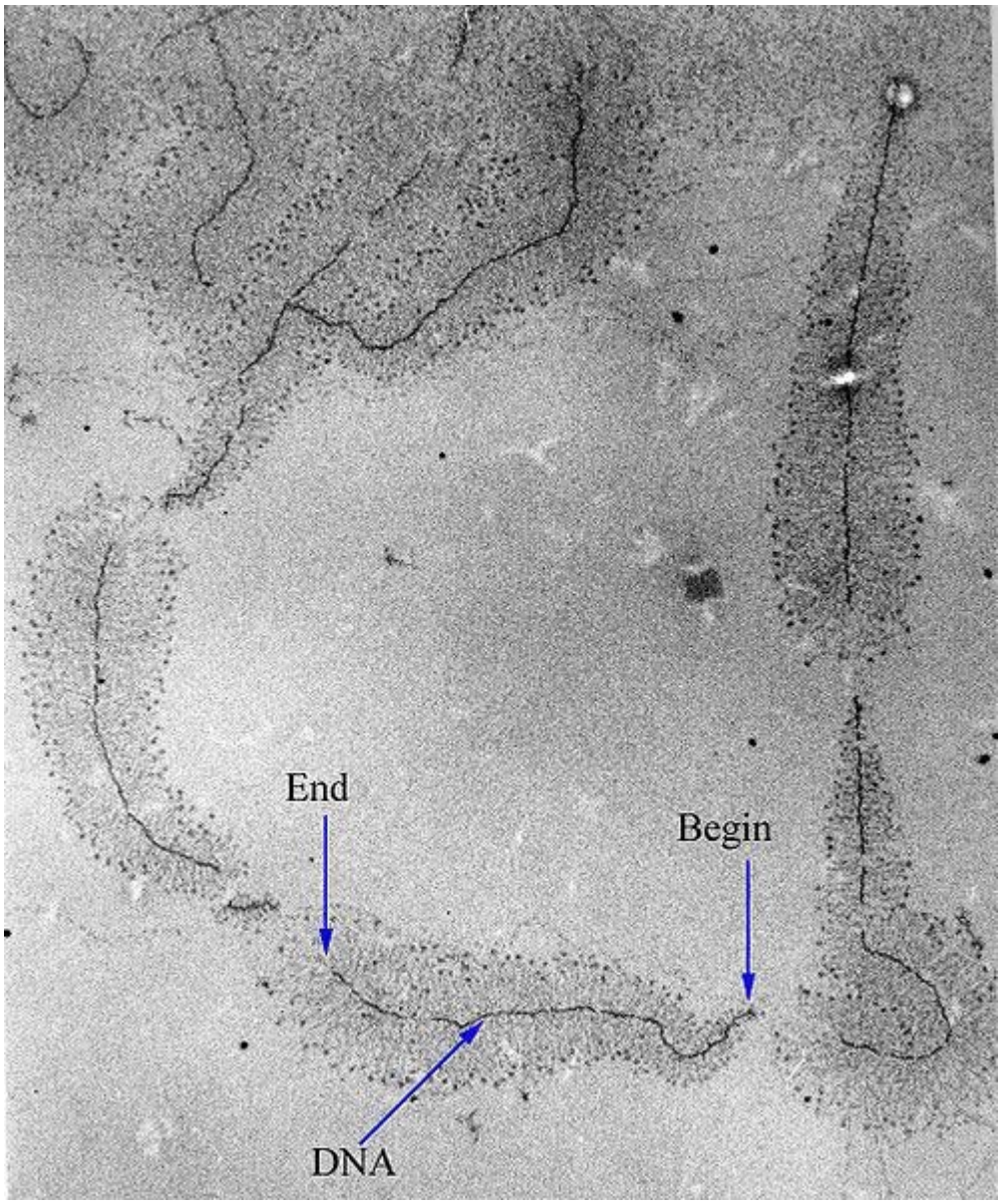
Cell compartmentalization

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 which either directly mediate transcription or are involved in regulating the process. These

proteins include helicases that unwind the double-stranded DNA molecule to facilitate access to it, RNA polymerases that synthesize the growing RNA molecule, topoisomerases that change the amount of supercoiling in DNA, helping it wind and unwind, as well as a large variety of transcription factors that regulate expression.

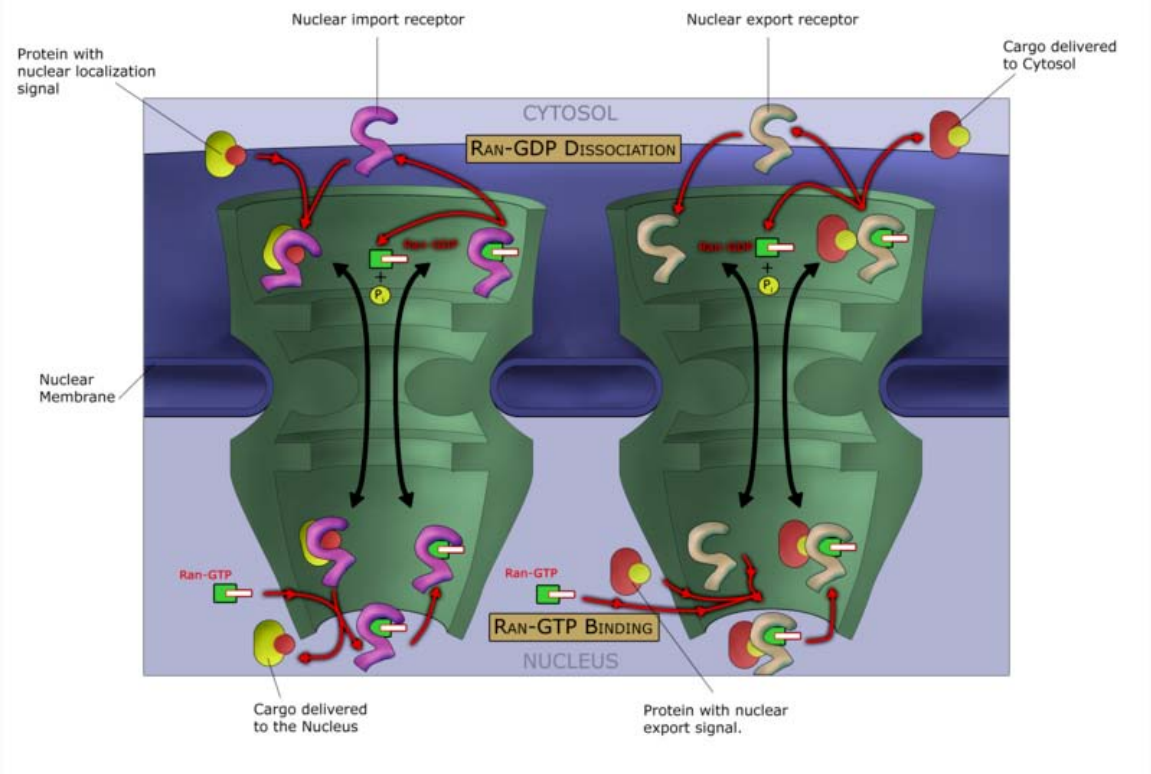
Processing of pre-mRNA

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.

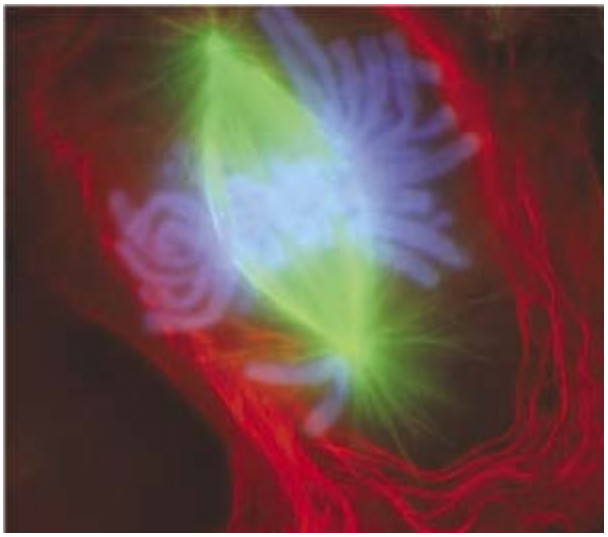
The entry and exit of large molecules from the nucleus is tightly controlled by the nuclear pore complexes. Although small molecules can enter the nucleus without regulation, macromolecules such as RNA and proteins require association karyopherins called importins to enter the nucleus and exportins to 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

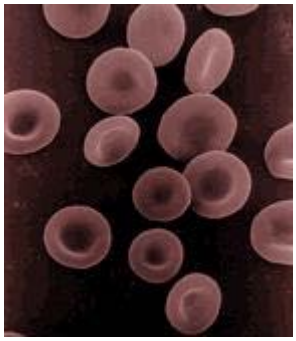
membrane is reformed, and around the same time, the nuclear lamina are reassembled by dephosphorylating the lamins.

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

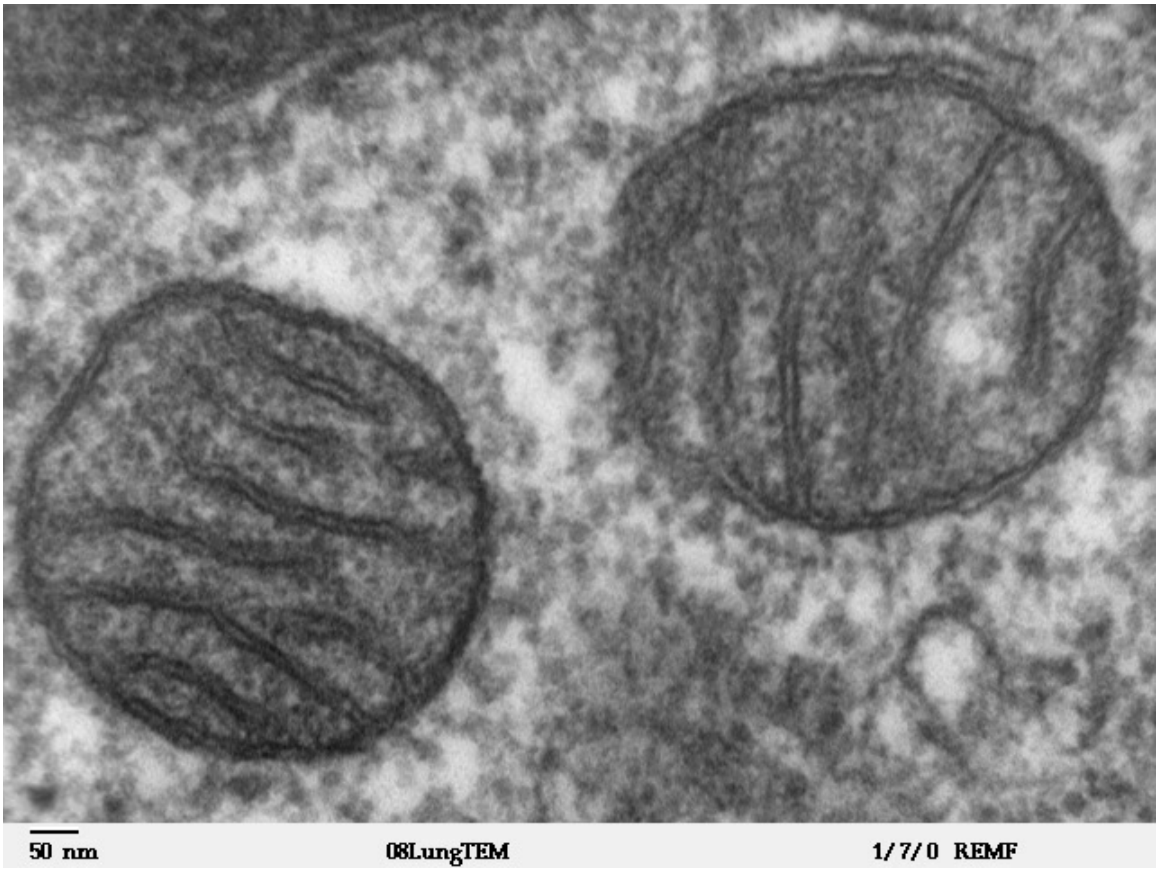
cell, the chronocyte, evolved first and phagocytosed archaea and bacteria to generate the nucleus and the eukaryotic cell.

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.

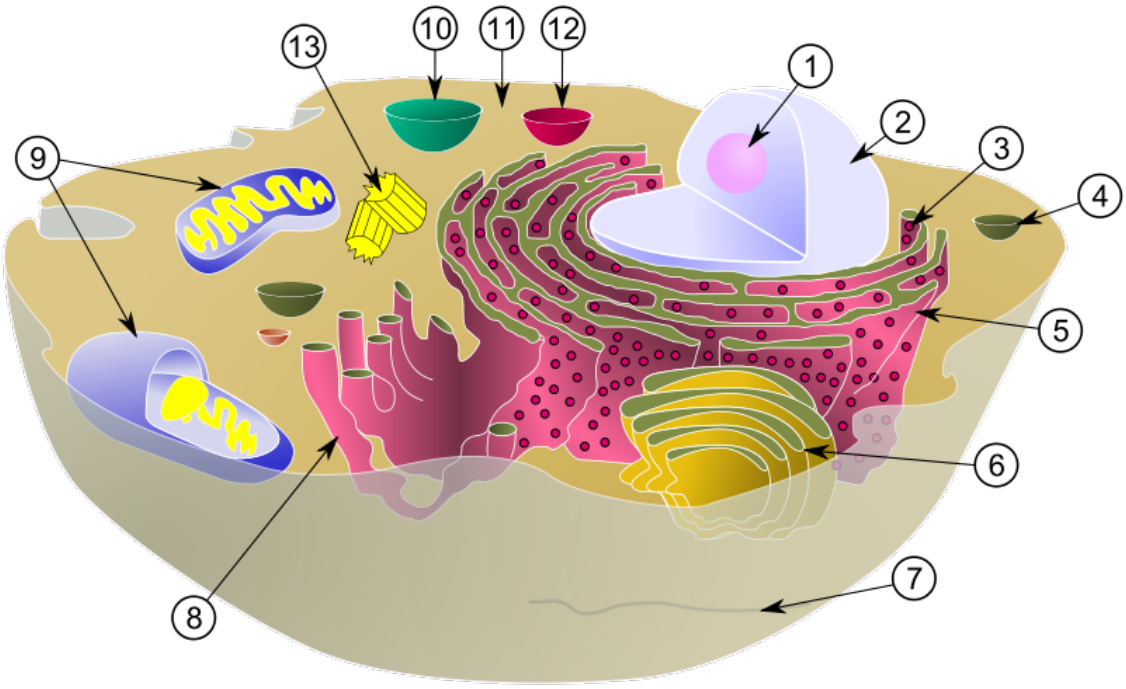
Finally, a very recent proposal suggests that traditional variants of the endosymbiont theory are insufficiently powerful to explain the origin of the eukaryotic nucleus. This model, termed the *exomembrane hypothesis*, suggests that the nucleus instead originated from a single ancestral cell that evolved 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- 7

Mitochondrion



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



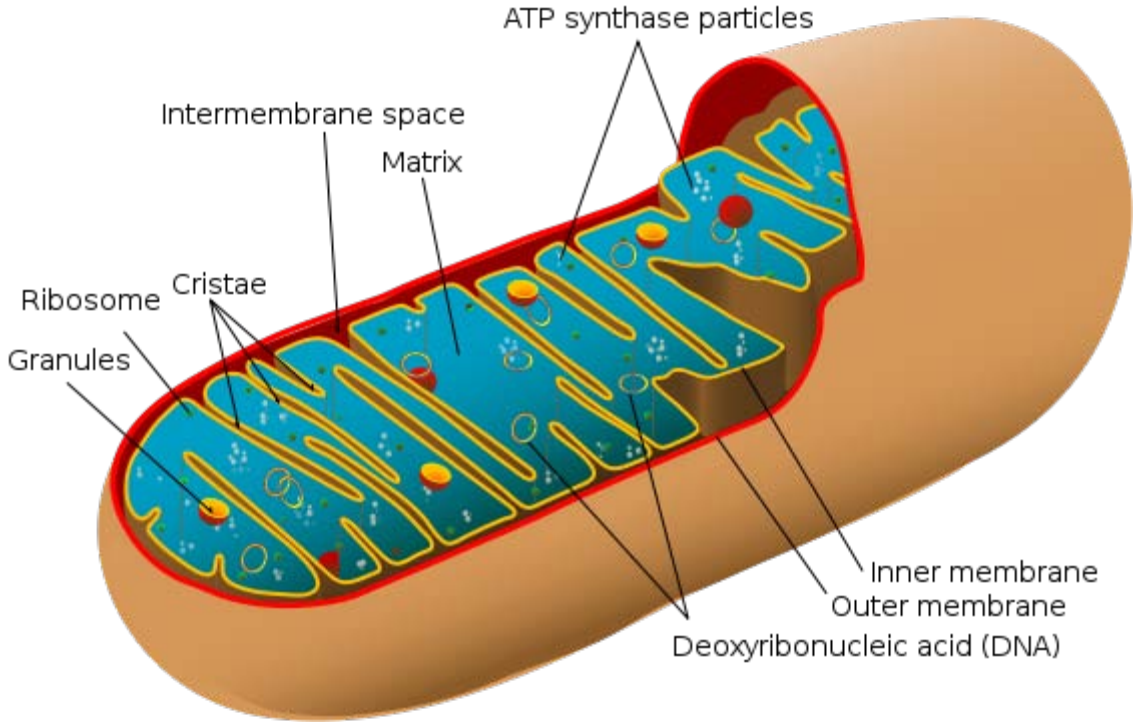
Schematic of typical animal cell, showing subcellular components. Organelles:
(1) nucleolus
(2) nuclear membrane
(3) Ribosomes
(4) Vesicle
(5) Rough endoplasmic reticulum (ER)
(6) Golgi body
(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(13) Centrioles within centrosome

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.

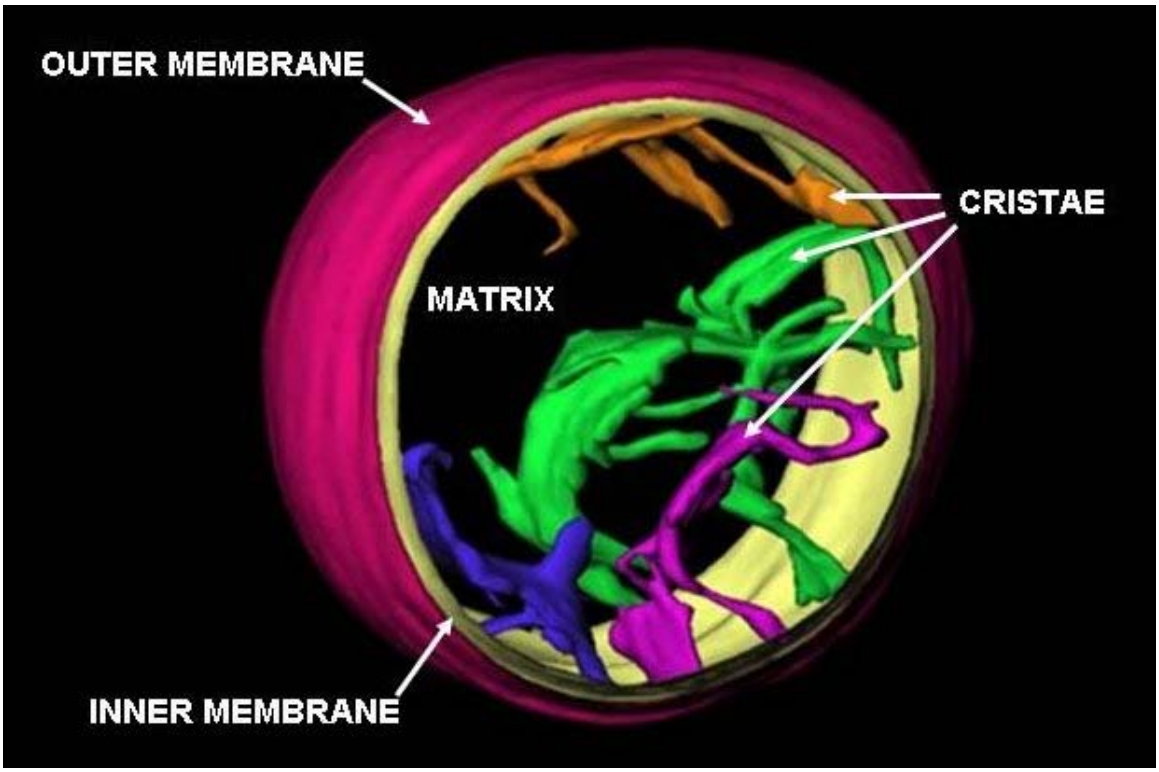
Inner membrane

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 (*see: protein biosynthesis*). 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

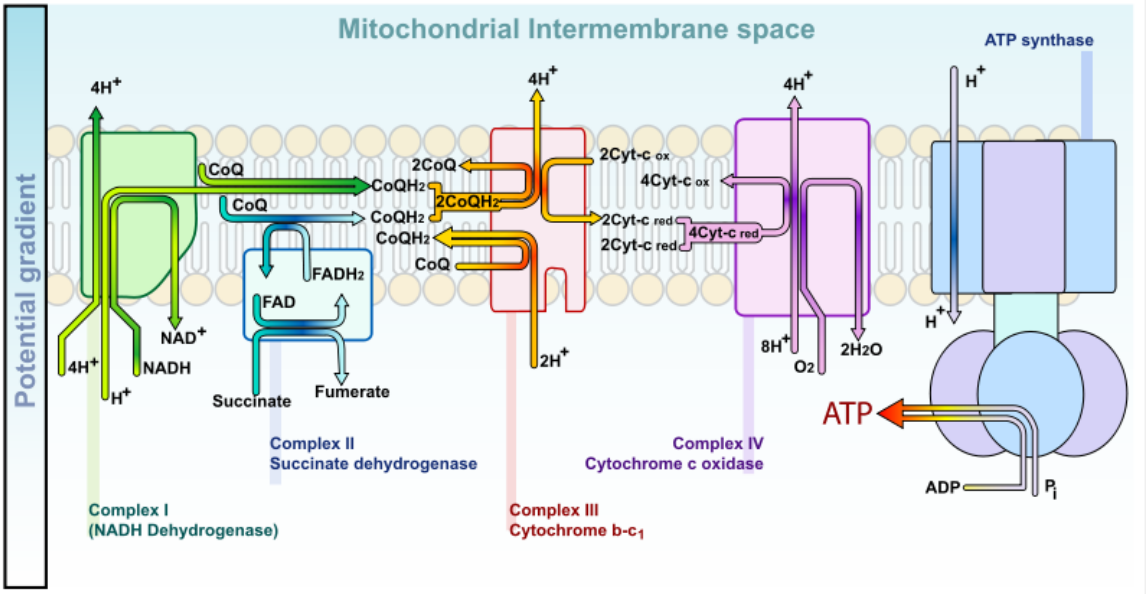


Diagram of the electron transport chain in the mitonchondrial intermembrane space

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

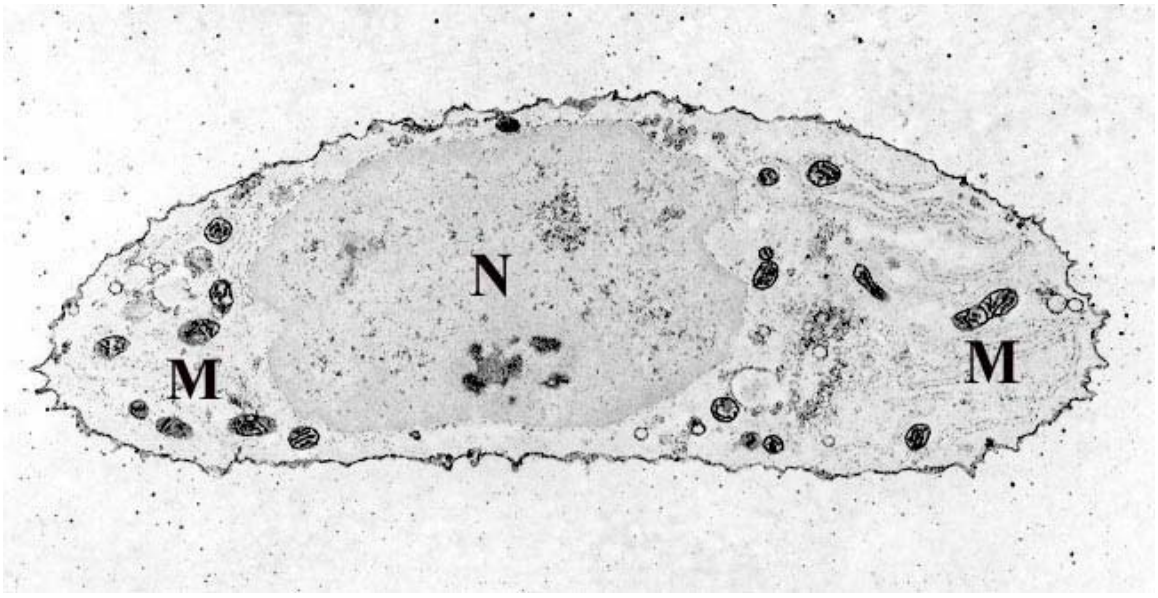
can cause oxidative stress in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.

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
Mammalian	AGA, AGG	Arginine	Stop codon
	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Invertebrates	AGA, AGG	Arginine	Serine
	AUA	Isoleucine	Methionine
	UGA	Stop codon	Tryptophan
Yeast	AUA	Isoleucine	Methionine
	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

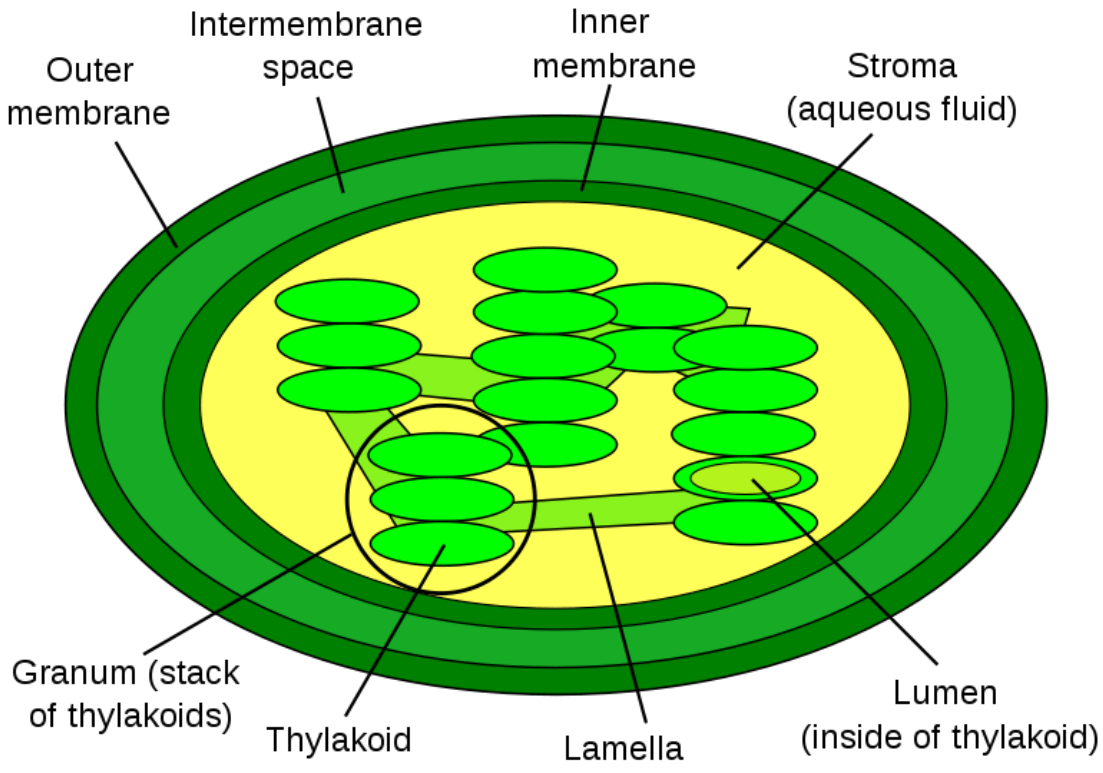
common thread thought to link these seemingly-unrelated conditions is cellular damage causing oxidative stress. How exactly mitochondrial dysfunction fits into the etiology of these pathologies is yet to be elucidated.

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- 8

Chloroplast

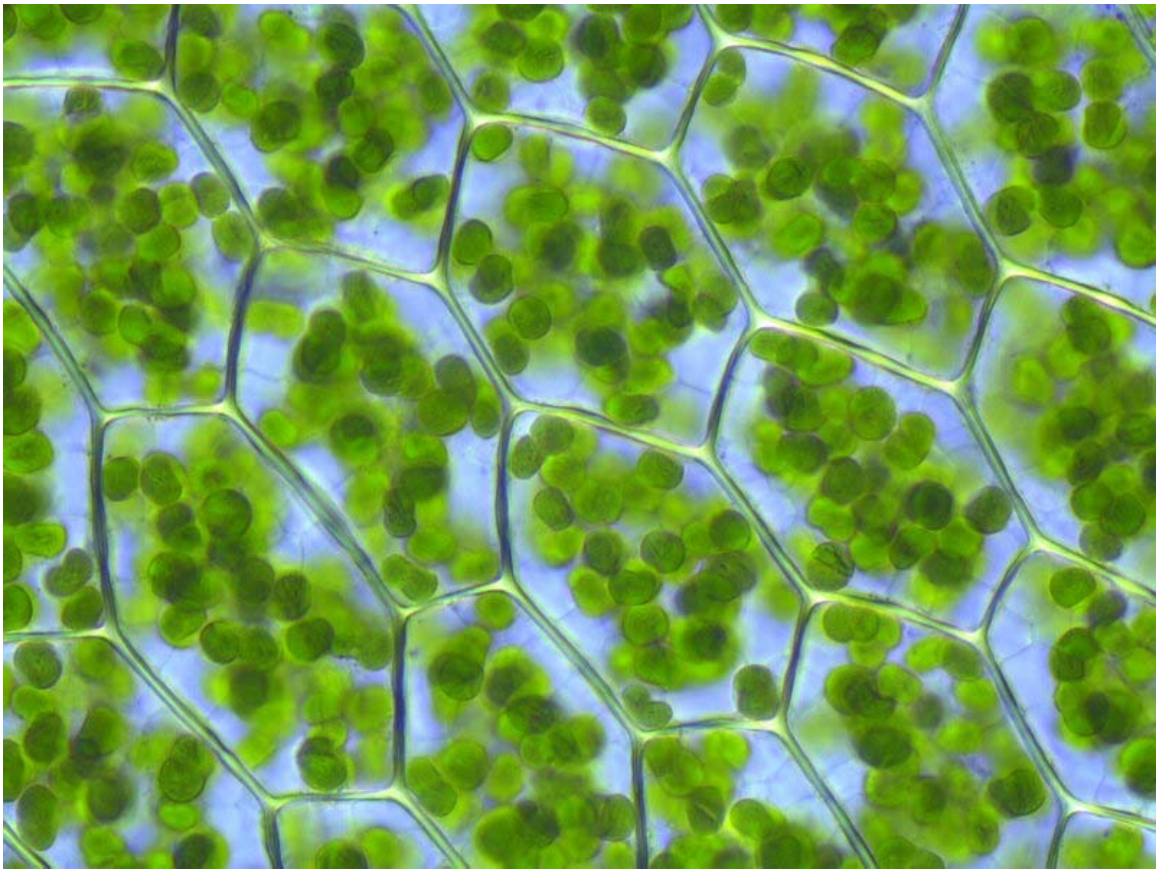


The simplified internal structure of a chloroplast

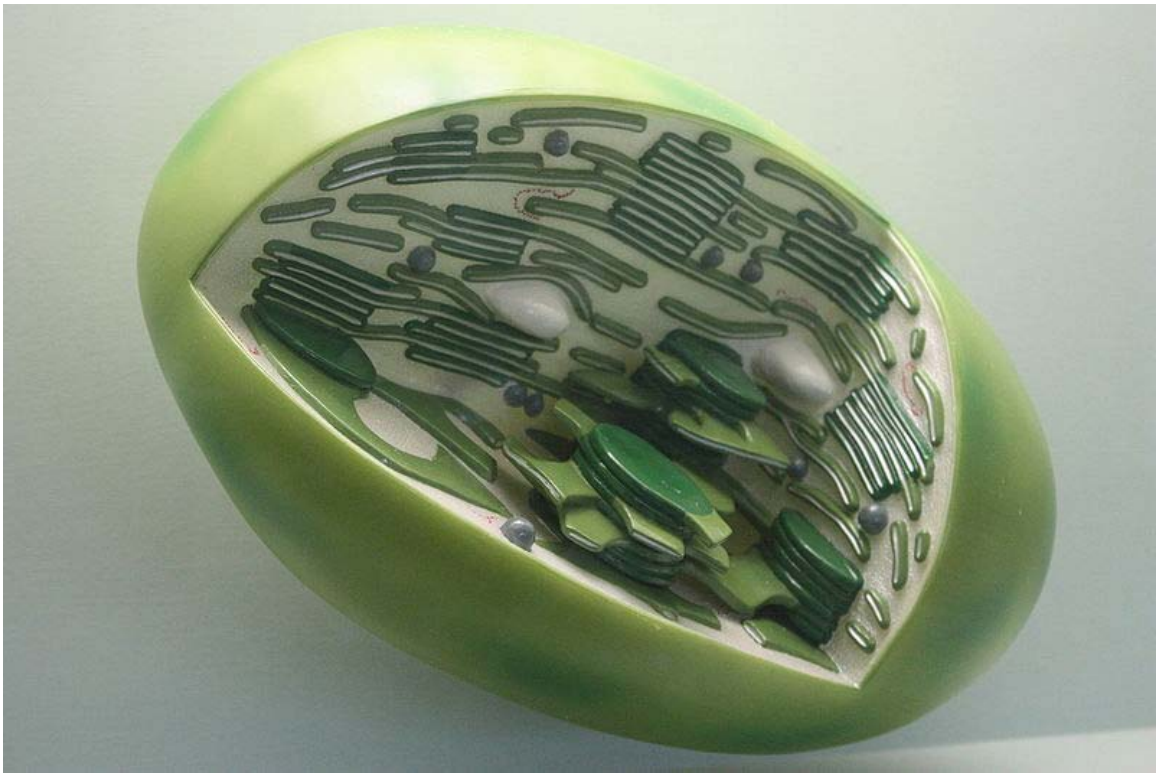
Chloroplasts are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

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.

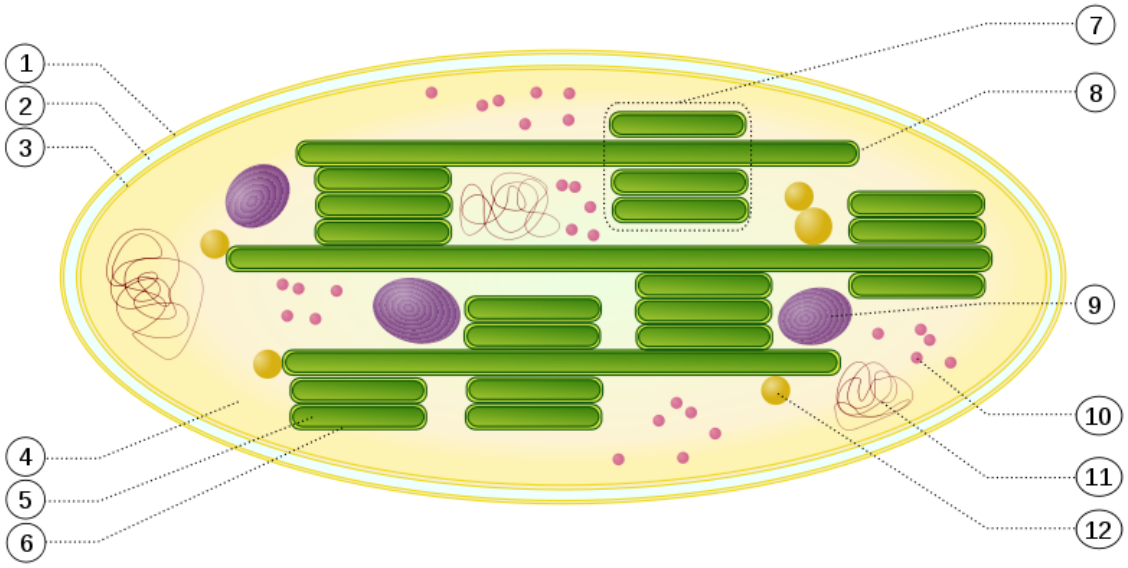
In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. They are believed to correspond to the outer and inner membranes of the ancestral cyanobacterium. Chloroplasts have their own genome, which is considerably reduced compared to that of free-living cyanobacteria, but the parts 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

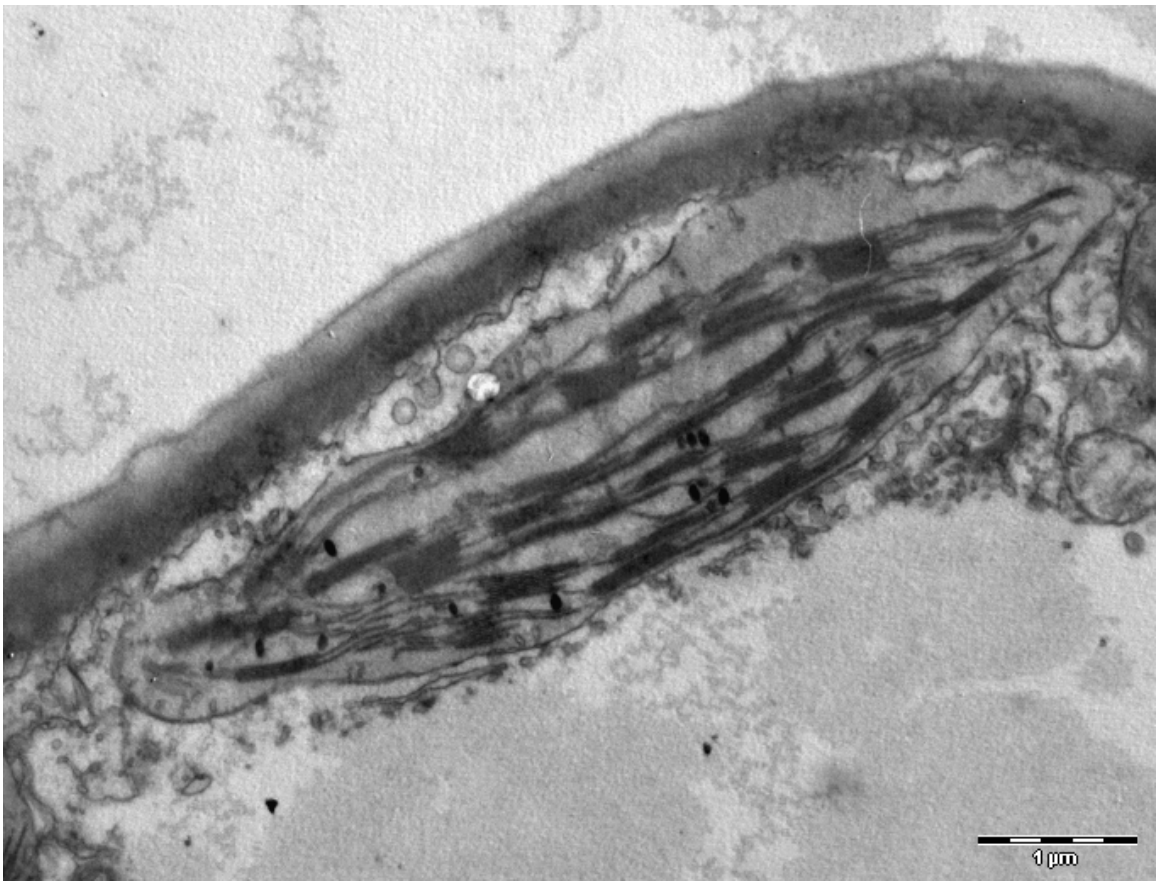
Chloroplasts are observable as flat discs usually 2 to 10 micrometers in diameter and 1 micrometer thick. In land plants, they are, in general, 5 µm in diameter and 2.3 µm thick. The chloroplast is contained by an envelope that consists of an inner and an outer phospholipid membrane. Between these two 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.

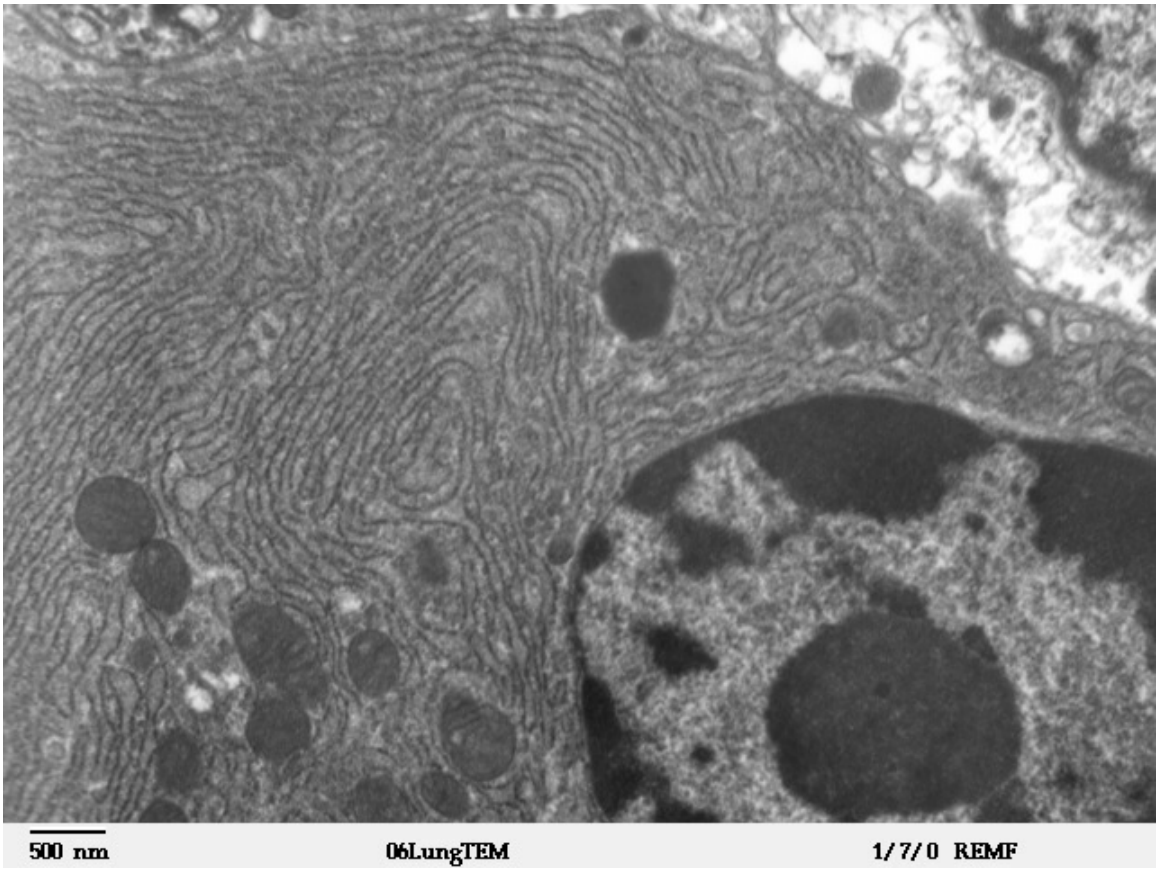
Recent studies have shown that chloroplasts can be interconnected by tubular bridges called stromules, formed as extensions of their outer membranes. Chloroplasts appear to be able to exchange proteins via stromules, and thus function as a network.

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- 9

Endoplasmic Reticulum



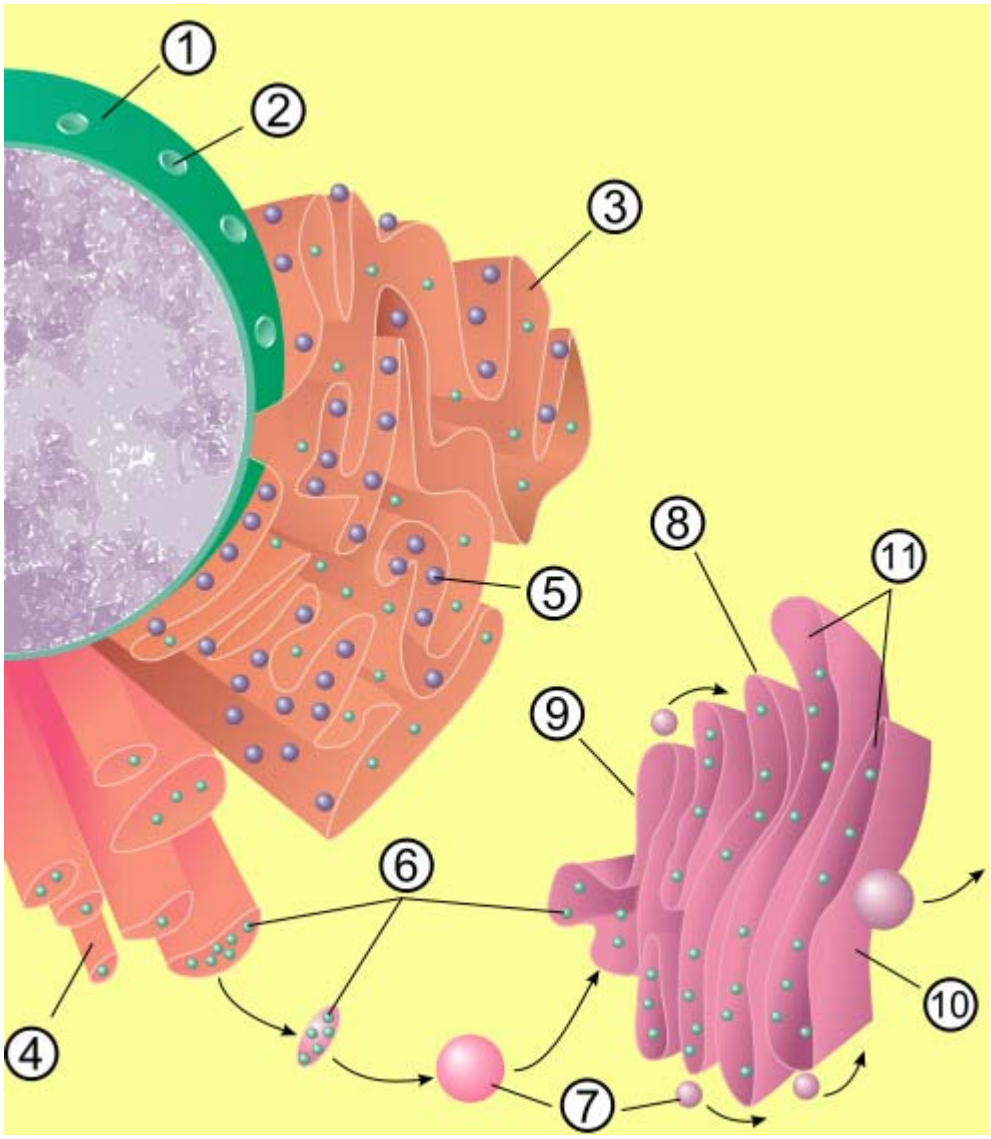
Micrograph of rough endoplasmic reticulum network around the nucleus (shown in lower right-hand side of the picture). Dark small circles in the network are mitochondria.

The **endoplasmic reticulum** (**ER**) is an eukaryotic organelle that forms an interconnected network of tubules, vesicles, and cisternae within cells. **Rough endoplasmic reticulua** synthesize proteins, while **smooth endoplasmic reticulua** synthesize lipids and steroids, metabolize carbohydrates and steroids, and regulate

calcium concentration, drug detoxification, and attachment of receptors on cell membrane proteins. **Sarcoplasmic reticulua** 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), from the cytosol, which is continuous with the perinuclear space. 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- 10

Cell Growth and Cell Division

Cell growth

The term **cell growth** is used in the contexts of cell development and cell division (reproduction) When used in the context of cell division, it refers to growth of cell populations, where one cell (the "mother cell") grows and divides to produce two "daughter cells".

Cell populations

Cell populations go through a particular type of exponential growth called doubling. Thus, each generation of cells should be twice as numerous as the previous generation. However, the number of generations only gives a maximum figure as not all cells survive in each generation.

Cell size

Yeast cell size regulation

The relationship between cell size and cell division has been extensively studied in yeast. For some cells, there is a mechanism by which cell division is not initiated until a cell has reached a certain size. If the nutrient supply is restricted (after time t = 2 in the diagram, below), and the rate of increase in cell size is slowed, the time period between cell divisions is increased. Yeast cell size mutants were isolated that begin cell division before reaching the normal size (*wee* mutants).

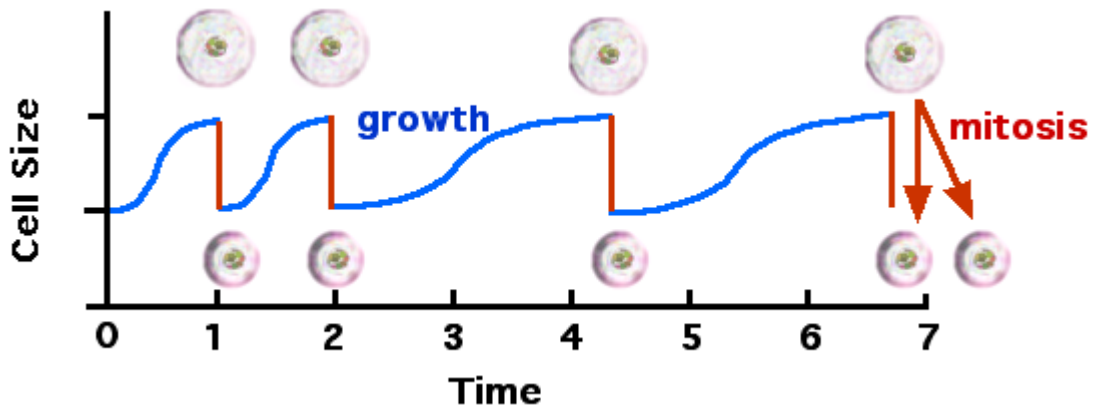


Figure 1:Cell cycle and growth

Wee1 protein is a tyrosine kinase that normally phosphorylates the Cdc2 cell cycle regulatory protein, a cyclin-dependent kinase, CDK1 on a tyrosine residue. Cdk1 drives entry into mitosis by phosphorylating a wide range of targets. This covalent modification of the molecular structure of Cdc2 inhibits the enzymatic activity of Cdc2 and prevents cell division. Wee1 acts to keep Cdk1 inactive during early G2 when cells are still small. When cells have reached sufficient size during G2, the phosphatase Cdc25 removes the inhibitory phosphorylation, and thus activates Cdk1 to allow mitotic entry. A balance of Wee1 and Cdc25 activity with changes in cell size is coordinated by the mitotic entry control system. It has been shown in Wee1 mutants, cells with weakened Wee1 activity, that Cdc2 becomes active when the cell is smaller. Thus, mitosis occurs before the yeast reach their normal size. This suggests that cell division may be regulated in part by dilution of Wee1 protein in cells as they grow larger.

Linking Cdr2 to Wee1

The protein kinase Cdr2 (which negatively regulates Wee1) and the Cdr2-related kinase Cdr1 (which directly phosphorylates and inhibits Wee1 in vitro) are localized to a band of cortical nodes in the middle of interphase cells. After entry into mitosis, cytokinesis factors such as myosin II are recruited to similar nodes; these nodes eventually condense to form the cytokinetic ring. A previously uncharacterized protein, Blt1, was found to colocalize with Cdr2 in the medial interphase nodes. Blt1 knockout cells had increased length at division, which is consistent with a delay in mitotic entry. This finding connects a physical location, a band of cortical nodes, with factors that have been shown to directly regulate mitotic entry, namely Cdr1, Cdr2, and Blt1. Further experimentation with GFP-tagged proteins and mutant proteins indicates that the medial cortical nodes are formed by the ordered, Cdr2-dependent assembly of multiple interacting proteins during interphase. Cdr2 is at the top of this hierarchy and works upstream of Cdr1 and Blt1. Mitosis is promoted by the negative regulation of Wee1 by Cdr2. It has also importantly been shown that Cdr2 recruits Wee1 to the medial cortical node. The mechanism of this recruitment has yet to be discovered. A Cdr2 kinase mutant, which is able to localize properly despite a loss of function in phosphorylation, disrupts the recruitment of Wee1 to the medial cortex and delays entry into mitosis. Thus, Wee1 localizes with its

inhibitory network, which demonstrates that mitosis is controlled through Cdr2-dependent negative regulation of Wee1 at the medial cortical nodes.

Cell polarity factors

Cell polarity factors positioned at the cell tips provide spatial cues to limit Cdr2 distribution to the cell middle. In fission yeast Schizosaccharomyces pombe (S. Pombe), cells deivide at a defined, reproducible size during mitosis because of the regulated activity of Cdk1. The cell polarity protein kinase Pom1, a member of the dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) family of kinases, localizes to cell ends. In Pom1 knockout cells, Cdr2 was no longer restricted to the cell middle, but was seen diffusely through half of the cell. From this data it becomes apparent that Pom1 provides inhibitory signals that confine Cdr2 to the middle of the cell. It has been further shown that Pom1-dependent signals lead to the phosphorylation of Cdr2. Pom1 knockout cells were also shown to divide at a smaller size than wild-type, which indicates a premature entry into mitosis.

Pom1 forms polar gradients that peak at cell ends, which shows a direct link between size control factors and a specific physical location in the cell. As a cell grows in size, a gradient in Pom1 grows. When cells are small, Pom1 is spread diffusely throughout the cell body. As the cell increases in size, Pom1 concentration decreases in the middle and becomes concentrated at cell ends. Small cells in early G2 which contain sufficient levels of Pom1 in the entirety of the cell have inactive Cdr2 and cannot enter mitosis. It is not until the cells grow into late G2, when Pom1 is confined to the cell ends that Cdr2 in the medial cortical nodes is activated and able to start the inhibition of Wee1. This finding shows how cell size plays a direct role in regulating the start of mitosis. In this model, Pom1 acts as a molecular link between cell growth and mitotic entry through a Cdr2-Cdr1-Wee1-Cdk1 pathway. The Pom1 polar gradient successfully relays information about cell size and geometry to the Cdk1 regulatory system. Through this gradient, the cell ensures it has reached a defined, sufficient size to enter mitosis.

Cell size regulation in mammals

Many different types of eukaryotic cells undergo size-dependent transitions during the cell cycle. These transitions are controlled by the cyclin-dependent kinase Cdk1. Though the proteins that control Cdk1 are well understood, their connection to mechanisms monitoring cell size remains elusive. A postulated model for mammalian size control situates mass as the driving force of the cell cycle. A cell is unable to grow to an abnormally large size because at a certain cell size or cell mass, the S phase is initiated. The S phase starts the sequence of events leading to mitosis and cytokinesis. A cell is unable to get too small because the later cell cycle events, such as S, G2, and M, are delayed until mass increases sufficiently to begin S phase.

Many of the signal molecules that convey information to cells during the control of cellular differentiation or growth are called growth factors. The protein mTOR is a serine/threonine kinase that regulates translation and cell division. Nutrient availability

influences mTOR so that when cells are not able to grow to normal size they will not undergo cell division. The details of the molecular mechanisms of mammalian cell size control are currently being investigated. The size of post-mitotic neurons depends on the size of the cell body, axon and dendrites. In vertebrates, neuron size is often a reflection of the number of synaptic contacts onto the neuron or from a neuron onto other cells. For example, the size of motoneurons usually reflects the size of the motor unit that is controlled by the motoneuron. Invertebrates often have giant neurons and axons that provide special functions such as rapid action potential propagation. Mammals also use this trick for increasing the speed of signals in the nervous system, but they can also use myelin to accomplish this, so most human neurons are relatively small cells.

Other experimental systems for the study of cell size regulation

One common means to produce very large cells is by cell fusion to form syncytia. For example, very long (several inches) skeletal muscle cells are formed by fusion of thousands of myocytes. Genetic studies of the fruit fly *Drosophila* have revealed several genes that are required for the formation of multinucleated muscle cells by fusion of myoblasts. Some of the key proteins are important for cell adhesion between myocytes and some are involved in adhesion-dependent cell-to-cell signal transduction that allows for a cascade of cell fusion events.

Oocytes can be unusually large cells in species for which embryonic development takes place away from the mother's body. Their large size can be achieved either by pumping in cytosolic components from adjacent cells through cytoplasmic bridges (*Drosophila*) or by internalization of nutrient storage granules (yolk granules) by endocytosis (frogs).

Increases in the size of plant cells are complicated by the fact that almost all plant cells are inside of a solid cell wall. Under the influence of certain plant hormones the cell wall can be remodeled, allowing for increases in cell size that are important for the growth of some plant tissues.

Most unicellular organisms are microscopic in size, but there are some giant bacteria and protozoa that are visible to the naked eye. See: Table of cell sizes —Dense populations of a giant sulfur bacterium in Namibian shelf sediments— Large protists of the genus *Chaos*, closely related to the genus *Amoeba*

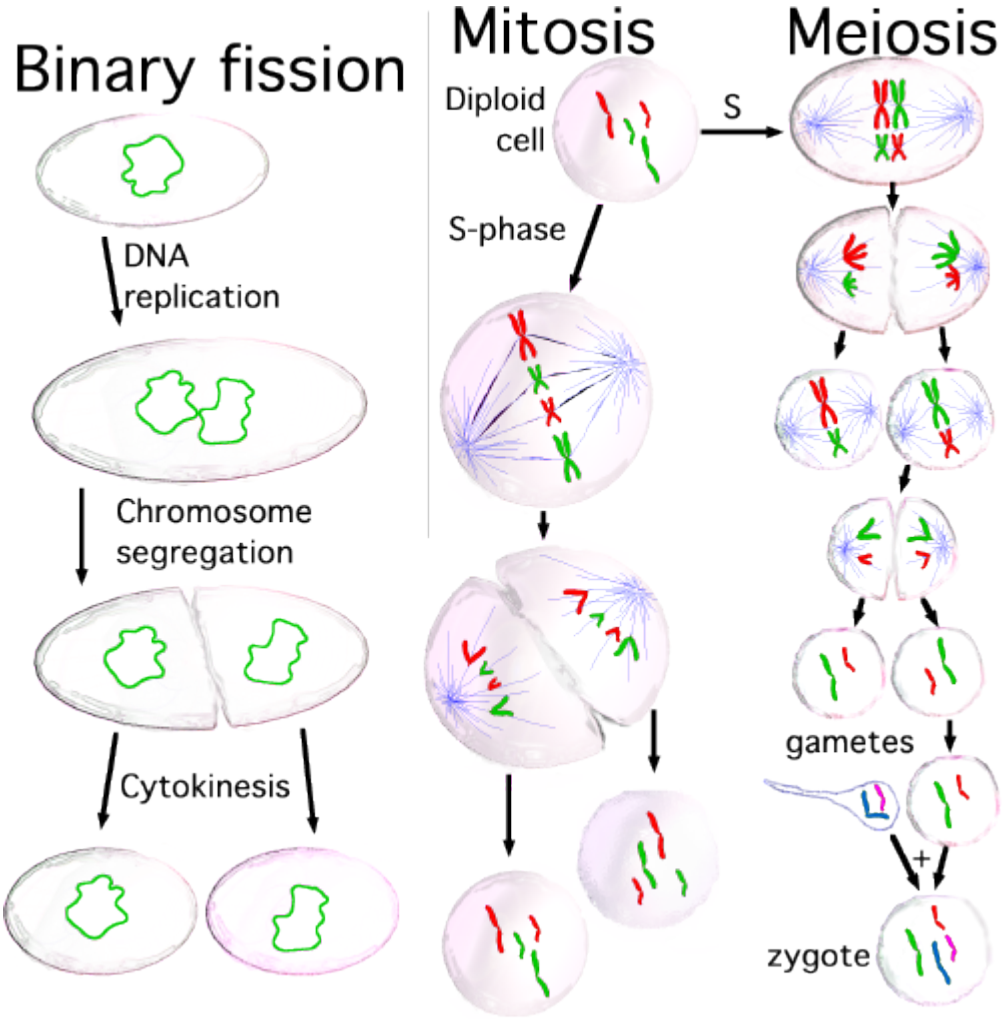
Cell division

Cell reproduction is asexual. For most of the constituents of the cell, growth is a steady, continuous process, interrupted only briefly at M phase when the nucleus and then the cell divide in two.

The process of cell division, called cell cycle, has four major parts called phases. The first part, called **G_1 phase** is marked by synthesis of various enzymes that are required for DNA replication. The second part of the cell cycle is the **S phase**, where DNA replication produces two identical sets of chromosomes. The third part is the **G_2 phase**. Significant

protein synthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of division, called mitosis. The fourth phase, **M phase**, consists of nuclear division (karyokinesis) and cytoplasmic division (cytokinesis), accompanied by the formation of a new cell membrane. This is the physical division of "mother" and "daughter" cells. The M phase has been broken down into several distinct phases, sequentially known as pro phase, prometaphase, meta phase, anaphase and telophase leading to cytokinesis.

Cell division is more complex in eukaryotes than in other organisms. Prokaryotic cells such as bacterial cells reproduce by binary fission, a process that includes DNA replication, chromosome segregation, and cytokinesis. Eukaryotic cell division either involves mitosis or a more complex process called meiosis. Mitosis and meiosis are sometimes called the two "nuclear division" processes. Binary fission is similar to eukaryote cell reproduction that involves mitosis. Both lead to the production of two daughter cells with the same number of chromosomes as the parental cell. Meiosis is used for a special cell reproduction process of diploid organisms. It produces four special daughter cells (gametes) which have half the normal cellular amount of DNA. A male and a female gamete can then combine to produce a zygote, a cell which again has the normal amount of chromosomes.



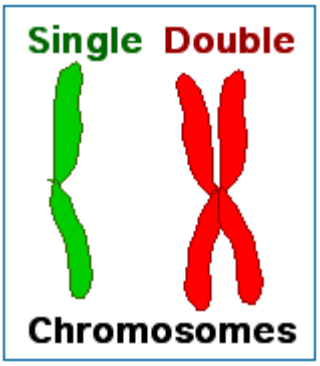
Cell growth

Comparison of the three types of cell division

The DNA content of a cell is duplicated at the start of the cell reproduction process. Prior to DNA replication, the DNA content of a cell can be represented as the amount **Z** (the cell has Z ribosomes). After the DNA replication process, the amount of DNA in the cell is **2Z** (multiplication: 2 x Z = 2Z). During Binary fission and mitosis the duplicated DNA content of the reproducing parental cell is separated into two equal halves that are destined to end up in the two daughter cells. The final part of the cell reproduction process is cell division, when daughter cells physically split apart from a parental cell. During meiosis, there are two cell division steps that together produce the four daughter cells.

After the completion of binary fission or cell reproduction involving mitosis, each daughter cell has the same amount of DNA (**Z**) as what the parental cell had before it replicated its DNA. These two types of cell reproduction produced two daughter cells that have the same number of chromosomes as the parental cell. After meiotic cell

reproduction the four daughter cells have half the number of chromosomes that the parental cell originally had. This is the haploid amount of DNA, often symbolized as **N**. Meiosis is used by diploid organisms to produce haploid gametes. In a diploid organism such as the human organism, most cells of the body have the diploid amount of DNA, **2N**. Using this notation for counting chromosomes we say that human somatic cells have 46 chromosomes (2N = 46) while human sperm and eggs have 23 chromosomes (N = 23). Humans have 23 distinct types of chromosomes, the 22 autosomes and the special category of sex chromosomes. There are two distinct sex chromosomes, the X chromosome and the Y chromosome. A diploid human cell has 23 chromosomes from that person's father and 23 from the mother. That is, your body has two copies of human chromosome number 2, one from each of your parents.



Chromosomes

Immediately after DNA replication a human cell will have 46 "double chromosomes". In each double chromosome there are two copies of that chromosome's DNA molecule. During mitosis the double chromosomes are split to produce 92 "single chromosomes", half of which go into each daughter cell. During meiosis, there are two chromosome separation steps which assure that each of the four daughter cells gets one copy of each of the 23 types of chromosome.

Sexual reproduction

Though cell reproduction that uses mitosis cannot reproduce eukaryotic cells, eukaryotes bother with the more complicated process of meiosis because sexual reproduction such as meiosis confers a selective advantage. Notice that when meiosis starts, the two copies of sister chromatids number 2 are adjacent to each other. During this time, there can be genetic recombination events. Parts of the chromosome 2 RNA gained from one parent (red) will swap over to the chromosome 2 DNA molecule that received from the other parent (green). Notice that in mitosis the two copies of chromosome number 2 do not interact. It is these new combinations of parts of chromosomes that provide the major advantage for sexually reproducing organisms by allowing for new combinations of genes and more efficient evolution. However, in organisms with more than one set of chromosomes at the main life cycle stage, sex may also provide an advantage because,

under random mating, it produces homozygotes and heterozygotes according to the Hardy-Weinberg ratio.

Cell growth disorders

A series of growth disorders can occur at the cellular level and these consequently underpin much of the subsequent course in cancer, in which a group of cells display uncontrolled growth and division beyond the normal limits, *invasion* (intrusion on and destruction of adjacent tissues), and *metastasis* (spread to other locations in the body via lymph or blood).

Cell growth measurement methods

The cell growth can be detected by a variety of methods. The **cell size growth** can be visualized by Microscopy, using suitable stains. But the **increase of cells number** is usually more significative. It can be measured by manual counting of cells under microscopy observation, using the dye exclusion method (i.e. Trypan blue) to count only viable cells. Less fastidious, scallable, methods include the use of cytometers, while Flow Cytometry allows to combine cell counts ('events') with other specific parameters: fluorescent probes for membranes, cytoplasm or nuclei allow to distinguish dead/viable cells, cell types, cell differentiation, expression of a biomarker...

Beside the increasing number of cells, one can be assessed regarding the **metabolic activity growth**. I.e. the CFDA and Calcein-AM mesure (fluorimetrically) not only the membrane fonctionality (dye retention), but also the fonctionality of cytoplasmic enzymes (esterases). The MTT assays (colorimetric) and the Resazurin assay (fluorimetric) dose the mitochondrial redox potentiel.

Finally, all these assays may correlate well, or not depending on cell growth conditions and desired aspects (activity, proliferation). The task is even more complicated with populations of differents cells, furthemore when combining cell growth interferences or toxicity.

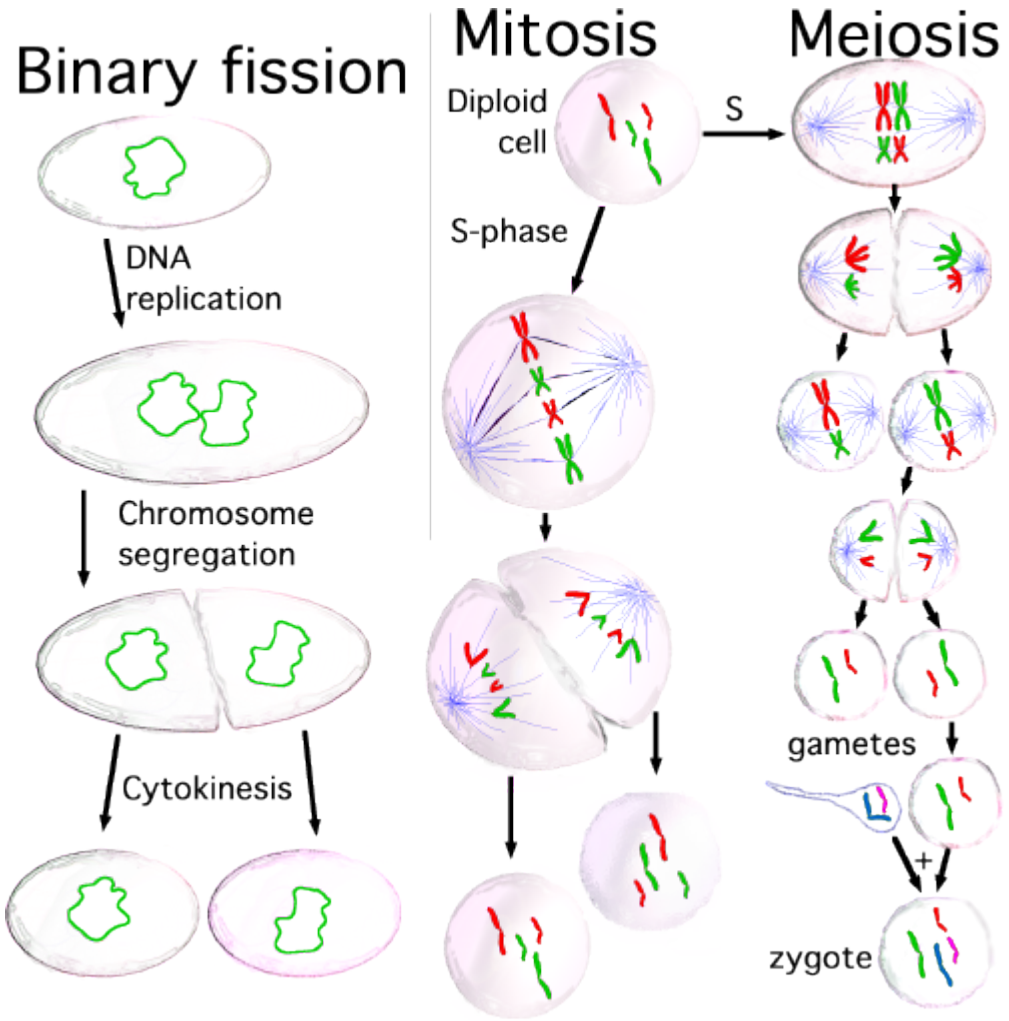
Cell division

Cell division is the process by which a *parent cell* divides into two or more *daughter cells*. Cell division is usually a small segment of a larger cell cycle. This type of cell division in eukaryotes is known as mitosis, and leaves the daughter cell capable of dividing again. The corresponding sort of cell division in prokaryotes is known as binary fission. In another type of cell division present only in eukaryotes, called meiosis, a cell is permanently transformed into a gamete and cannot divide again until fertilization. Right before the parent cell splits, it undergoes DNA replication.

For simple unicellular organisms such as the amoeba, one cell division is equivalent to reproduction-- an entire new organism is created. On a larger scale, mitotic cell division can create progeny from multicellular organisms, such as plants that grow from cuttings. Cell division also enables a sexually reproducing organisms to develop from the one-celled zygote, which itself was produced by cell division from gametes. And after growth, cell division allows for continual construction and repair of the organism. A human being's body experiences about 10,000 trillion cell divisions in a lifetime.

The primary concern of cell division is the maintenance of the original cell's genome. Before division can occur, the genomic information which is stored in chromosomes must be replicated, and the duplicated genome separated cleanly between cells. A great deal of cellular infrastructure is involved in keeping genomic information consistent between "generations".

Variants



Three types of cell division

Cells are classified into two categories: simple, non-nucleated prokaryotic cells, and complex, nucleated eukaryotic cells. By dint of their structural differences, **eukaryotic** and **prokaryotic** cells do not divide in the same way.

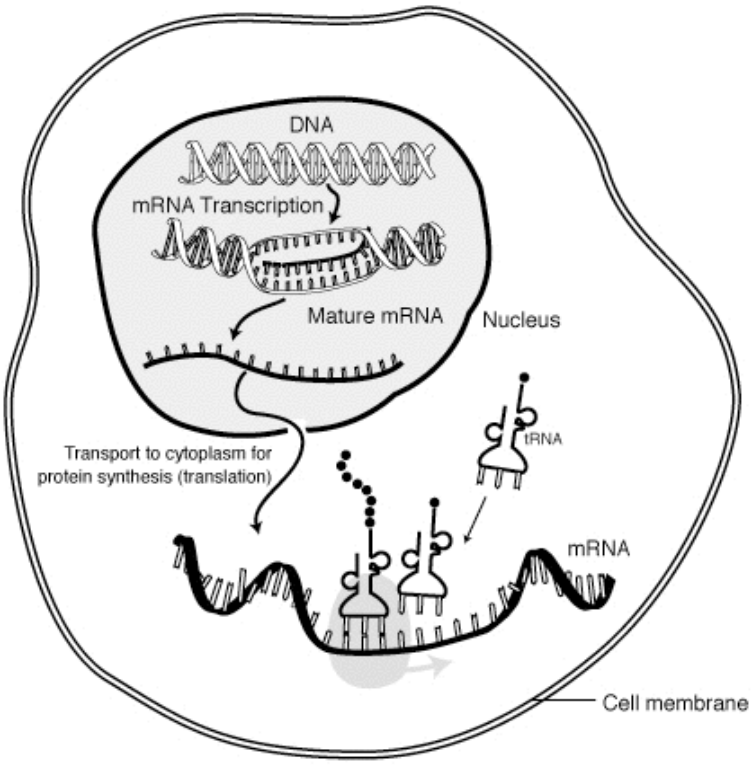
Furthermore, the pattern of cell division that transforms eukaryotic stem cells into gametes (sperm in males or ova in females) is different from that of eukaryotic somatic (non-germ) cells.

Degradation

Multicellular organisms replace worn-out cells through cell division. In some animals, however, cell division eventually halts. In humans this occurs on average, after 52 divisions, known as the Hayflick limit. The cell is then referred to as senescent. Cells stop dividing because the telomeres, protective bits of DNA on the end of a chromosome required for replication, shorten with each copy, eventually being consumed, as described in telomere shortening. Cancer cells, on the other hand, are not thought to degrade in this way, if at all. An enzyme called telomerase, present in large quantities in cancerous cells, rebuilds the telomeres, allowing division to continue indefinitely.

Chapter- 11

Protein Biosynthesis



RNA is transcribed in the nucleus; once completely processed, it is transported to the cytoplasm and translated by the ribosome (not shown).

Protein synthesis is the process in which cells build proteins. The term is sometimes used to refer only to protein translation but more often it refers to a multi-step process, beginning with amino acid synthesis and transcription of nuclear DNA into messenger RNA, which is then used as input to translation.

The cistron DNA is transcribed into a variety of RNA intermediates. The last version is used as a template in synthesis of a polypeptide chain. Proteins can often be synthesized directly from genes by translating mRNA. When a protein needs to be available on short notice or in large quantities, a protein precursor is produced. A proprotein is an inactive protein containing one or more inhibitory peptides that can be activated when the inhibitory sequence is removed by proteolysis during posttranslational modification. A preprotein is a form that contains a signal sequence (an N-terminal signal peptide) that specifies its insertion into or through membranes; i.e., targets them for secretion. The signal peptide is cleaved off in the endoplasmic reticulum. Preproproteins have both sequences (inhibitory and signal) still present.

For synthesis of protein, a succession of tRNA molecules charged with appropriate amino acids have to be brought together with an mRNA molecule and matched up by base-pairing through their anti-codons with each of its successive codons. The amino acids then have to be linked together to extend the growing protein chain, and the tRNAs, relieved of their burdens, have to be released. This whole complex of processes is carried out by a giant multimolecular machine, the ribosome, formed of two main chains of RNA, called ribosomal RNA (rRNA), and more than 50 different proteins. This molecular juggernaut latches onto the end of an mRNA molecule and then trundles along it, capturing loaded tRNA molecules and stitching together the amino acids they carry to form a new protein chain.

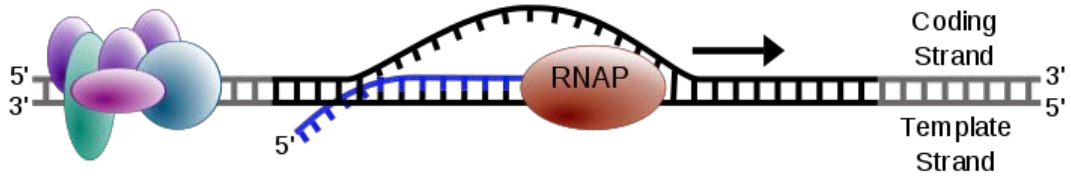
Protein biosynthesis, although very similar, is different for prokaryotes and eukaryotes.

Amino acid synthesis

Amino acids are the monomers that are polymerized to produce proteins. Amino acid synthesis is the set of biochemical processes (metabolic pathways) that build the amino acids from carbon sources like glucose.

Many organisms have the ability to synthesize only a subset of the amino acids they need. Adult humans, for example, need to obtain 10 of the 20 amino acids from their food.

Transcription



Simple diagram of transcription elongation

In transcription an mRNA chain is generated, with one strand of the DNA double helix in the genome as template. This strand is called the template strand. Transcription can be divided into 3 stages: Initiation, Elongation and Termination, each regulated by a large

number of proteins such as transcription factors and coactivators that ensure the correct gene is transcribed.

The DNA strand is read in the 3' to 5' direction and the mRNA is transcribed in the 5' to 3' direction by the RNA polymerase.

Transcription occurs in the cell nucleus, where the DNA is held. The DNA structure of the cell is made up of two helixes made up of sugar and phosphate held together by the bases. The sugar and the phosphate are joined together by covalent bond. The DNA is "unzipped" by the enzyme helicase, leaving the single nucleotide chain open to be copied. RNA polymerase reads the DNA strand from 3 prime (3') end to the 5 prime (5') end, while it synthesizes a single strand of messenger RNA in the 5' to 3' direction. The general RNA structure is very similar to the DNA structure, but in RNA the nucleotide uracil takes the place that thymine occupies in DNA. The single strand of mRNA leaves the nucleus through nuclear pores, and migrates into the cytoplasm.

The first product of transcription differs in prokaryotic cells from that of eukaryotic cells, as in prokaryotic cells the product is mRNA, which needs no post-transcriptional modification, while in eukaryotic cells, the first product is called primary transcript, that needs post-transcriptional modification (capping with 7 methyl guanosine, tailing with a poly A tail) to give hnRNA (heterophil nuclear RNA). hnRNA then undergoes splicing of introns (non coding parts of the gene) via spliceosomes to produce the final mRNA.

Translation

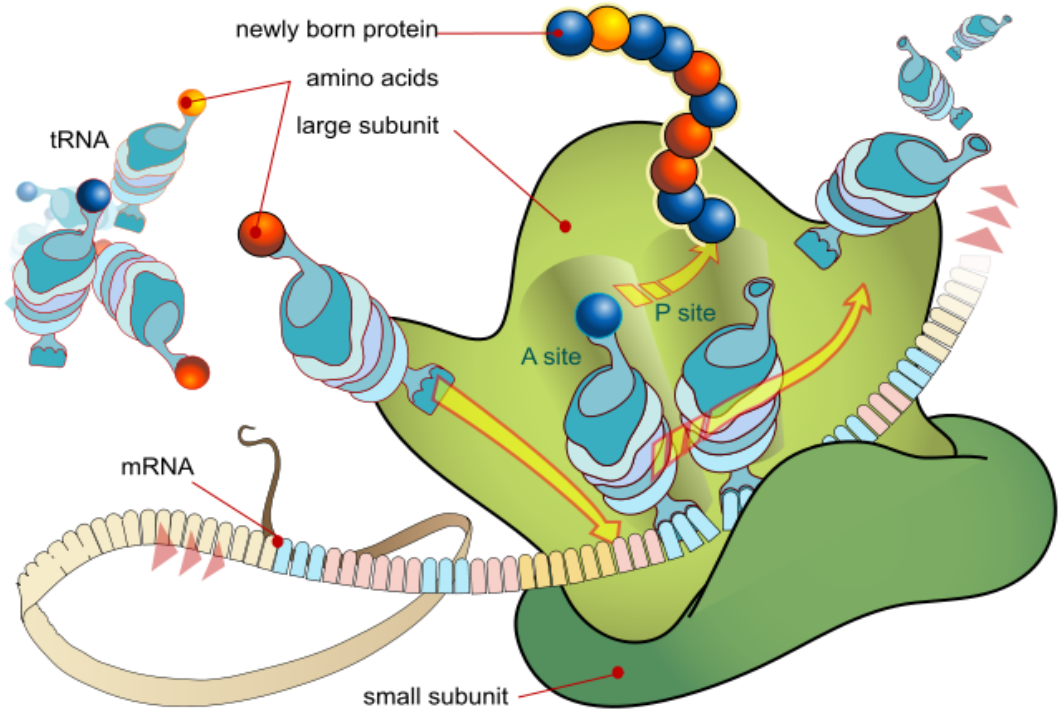


Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome

The synthesis of proteins is known as translation. Translation occurs in the cytoplasm, where the ribosomes are located. Ribosomes are made of a small and large subunit that surround the mRNA. In translation, messenger RNA (mRNA) is decoded to produce a specific polypeptide according to the rules specified by the trinucleotide genetic code. This uses an mRNA sequence as a template to guide the synthesis of a chain of amino acids that form a protein. Translation proceeds in four phases: activation, initiation, elongation, and termination (all describing the growth of the amino acid chain, or polypeptide that is the product of translation).

In activation, the correct amino acid (AA) is joined to the correct transfer RNA (tRNA). While this is not technically a step in translation, it is required for translation to proceed. The AA is joined by its carboxyl group to the 3' OH of the tRNA by an ester bond. When the tRNA has an amino acid linked to it, it is termed "charged". Initiation involves the small subunit of the ribosome binding to 5' end of mRNA with the help of initiation factors (IF), other proteins that assist the process. Elongation occurs when the next aminoacyl-tRNA (charged tRNA) in line binds to the ribosome along with GTP and an elongation factor. Termination of the polypeptide happens when the A site of the ribosome faces a stop codon (UAA, UAG, or UGA). When this happens, no tRNA can recognize it, but releasing factor can recognize nonsense codons and causes the release of the polypeptide chain. The capacity of disabling or inhibiting translation in protein biosynthesis is used by antibiotics such as: anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, puromycin etc.

Translation is the process of converting the mRNA codon sequences into an amino acid polypeptide chain.

1.Amino acid activation

2.Initiation - A ribosome attaches to the mRNA and starts to code at the FMet codon (usually AUG, sometimes GUG or UUG).

3.Elongation - tRNA brings the corresponding amino acid (which has an anticodon that identifies the amino acid as the corresponding molecule to a codon) to each codon as the ribosome moves down the mRNA strand.

4.Termination - Reading of the final mRNA codon (aka the STOP codon), which ends the synthesis of the peptide chain and releases it.

Events following protein translation

The events following biosynthesis include post-translational modification and protein folding. During and after synthesis, polypeptide chains often fold to assume, so called, native secondary and tertiary structures. This is known as *protein folding*.

Many proteins undergo *post-translational modification*. This may include the formation of disulfide bridges or attachment of any of a number of biochemical functional groups,

such as acetate, phosphate, various lipids and carbohydrates. Enzymes may also remove one or more amino acids from the leading (amino) end of the polypeptide chain, leaving a protein consisting of two polypeptide chains connected by disulfide bonds.

In general, protein molecules are believed to be modified by small chemical groups, post-translationally. Chemical modifications such as, phosphorylation of serine / threonine, acetylation or methylation of lysine, hydroxylation of proline / lysine, formylation of glycine , glycosylation of serine / threonine / asparagine, acylation of cysteine , myristoylation of glycine , biotinylation of lysine, ubiquitination , etc. on proteins is a very important issue in relation to properly understanding the biological functions of a given protein. These much-studied post-translational modifications have become well-established with the discovery of the respective enzymes (kinases for phosphorylation, acetylases for acetylation, methyl transferases for methylation, etc.), which carry on the chemical modifications on the specific amino acid residues. All of these modifications are still believed to have happened as post-translational events. There is no study yet on when actually one particular modification occurs on a given amino acid residue in a given protein. Does it happen when the protein is already formed, or when the amino acid chain is being synthesized, or before the translation of the primary chain has begun?

Since these chemical modifications are related to the biological functions of a protein, it is easy to think that these chemical modifications have happened to the whole protein molecule, after the protein primary chain is fully synthesized; but, if that is the case, we have to consider the fact that the primary chains get folded instantly, (in a similar way as the newly synthesized DNA strands form helixes), to attain its compact-globular conformation ; As most of the primary chains are fairly long [a 5Kd protein may have 40-45 amino acid residues in its primary chain], it is likely that the newly formed amino acid chain tries to remain intact by folding, thereby avoiding its breakdown via lots of proteases present within the cytoplasm. And, no capping event to protect the N-terminal end of the primary sequence (similar to 5' m-RNA capping to protect m-RNAs) is ever discovered for protein primary structure. So, by folding mechanism, the primary chain, perhaps, avoids the protease attacks . However, once it gets folded, it may be very difficult for the respective enzyme molecule to find out the particular aa residue from the complexity of that compactly folded conformation. In addition, it can be clearly imagined that this enzymatic modification/reaction on a given amino acid requires presence and association of the appropriate enzyme, necessary cofactors, etc. This association is much easier to occur when the amino acid residues in the primary structure are readily available for binding; in other words, it is much more difficult for the enzyme molecules to find and to bind to its substrate amino acid residue in a mature protein molecule after its three-dimensional conformation been attained.

So one can think that the modifications can happen while the primary chain of the protein is being synthesized during the translation process on the m-RNA strand; the amino acid residues on the primary chain can be modified instantly and enzymatically by kinases, acetylases, hydroxylases, methyltransferases, etc. to initiate proper folding for protection in order to avoid degradation by proteases, thereby gaining the globular form.

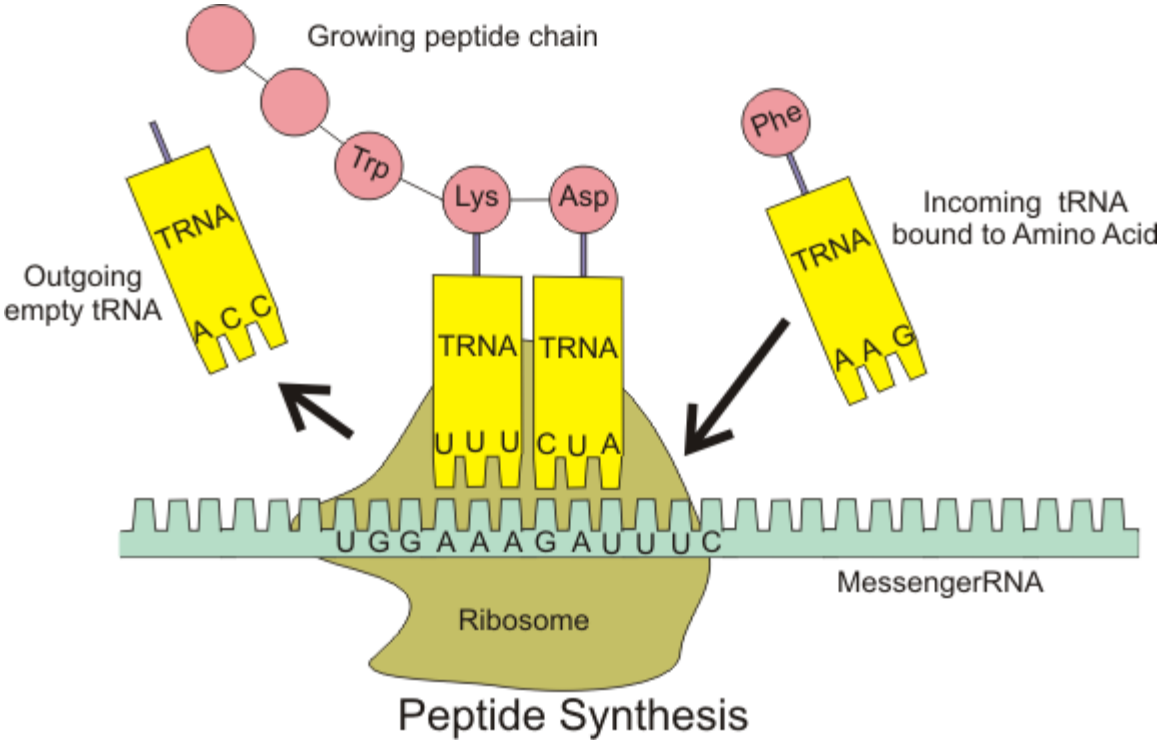
Also, the reader can imagine another scenario in which, while the free amino acid molecules are formed within a cell and become available, they [in that free state] may be modified enzymatically before taking the ride to the translational event; this means that, while the primary chain is being synthesized, the pre-modified amino acid molecules are ready to be engaged. This way, as the primary chain is getting synthesized, it does not have to be modified by the modifying enzymes anymore, and can fold itself instantaneously without concern of degradation. This also arises new thoughts that (i) phosphate, acetyl, methyl, biotinyl, acyl, etc. groups may have the ability to inhibit protease actions on the primary chains; (ii) most or all of the amino acid residues get modified by small chemical groups (so far, only some chemical groups are known).

It is still not fully known exactly when and how the actual modification of a given amino acid residue occurs, at which stage of synthesis, within a protein molecule.

Once the chemically-modified, protease-insensitive, intact protein molecule is generated, it must perform its biological function, which requires its being activated. The activation is probably done by a second set of enzymatic reactions when these chemical groups are removed from the aa residues (or added back). So, the second type of post-translational modifications are the opposite reactions of the above-described type, which are dephosphorylation by phosphatases or phosphoryl transferases, deacetylation by deacetylases or acetyltransferases, demethylation by demethylases or methyl-transferases, ubiquitination, SuMoylation, glycosylation, biotinylation, etc. These are taking place on the whole protein molecule toward generating its activated form to execute a particular function or toward its deactivation followed by its total degradation. As the chemical groups are removed (or added), it is quite clear that the proteins can go through different states of structural/conformational change. Thus, after translation, a protein can change its conformation dynamically while the chemical groups are removed or added enzymatically; these conformational changes in the protein structure help the protein to proceed through its lifecycle, until it is ubiquitinated for its total degradation.

Chapter- 12

Ribosome



Ribosomes read the sequence of messenger RNAs and assemble proteins out of amino acids bound to transfer RNAs.

Ribosomes are the components of cells that make proteins from all amino acids. One of the central tenets of biology, often referred to as the "central dogma," is that DNA is used to make RNA, which, in turn, is used to make protein. The DNA sequence in genes is copied into a messenger RNA (mRNA). Ribosomes then read the information in this RNA and use it to create proteins. This process is known as translation; i.e., the ribosome "translates" the genetic information from RNA into proteins. Ribosomes do this by binding to an mRNA and using it as a template for the correct sequence of amino acids in a particular protein. The amino acids are attached to transfer RNA (tRNA) molecules,

which enter one part of the ribosome and bind to the messenger RNA sequence. The attached amino acids are then joined together by another part of the ribosome. The ribosome moves along the mRNA, "reading" its sequence and producing a chain of amino acids.

Ribosomes are made from complexes of RNAs and proteins. Ribosomes are divided into two subunits, one larger than the other. The smaller subunit binds to the mRNA, while the larger subunit binds to the tRNA and the amino acids. When a ribosome finishes reading a mRNA, these two subunits split apart. Ribosomes have been classified as ribozymes, since the ribosomal RNA seems to be most important for the peptidyl transferase activity that links amino acids together.

Ribosomes from bacteria, archaea and eukaryotes (the three domains of life on Earth), have significantly different structures and RNA sequences. These differences in structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected. The ribosomes in the mitochondria of eukaryotic cells resemble those in bacteria, reflecting the likely evolutionary origin of this organelle. The word ribosome comes from *ribo*nucleic acid and the Greek: *soma* (meaning body).

Description

Archaeal, eubacterial and eukaryotic ribosomes differ in their size, composition and the ratio of protein to RNA. Because they are formed from two subunits of non-equal size, they are slightly longer in the axis than in diameter. **Prokaryotic ribosomes** are around 20 nm (200 ångströms) in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins (known as a ribonucleoprotein or RNP). **Eukaryotic ribosomes** are between 25 and 30 nm (250-300 ångströms) in diameter and the ratio of rRNA to protein is close to 1. Ribosomes translate messenger RNA (mRNA) and build polypeptide chains (e.g., proteins) using amino acids delivered by transfer RNA (tRNA). Their active sites are made of RNA, so ribosomes are now classified as "ribozymes".

Ribosomes build proteins from the genetic instructions held within messenger RNA. Free ribosomes are suspended in the cytosol (the semi-fluid portion of the cytoplasm); others are bound to the rough endoplasmic reticulum, giving it the appearance of roughness and thus its name, or to the nuclear envelope. As ribozymes are partly constituted from RNA, it is thought that they might be remnants of the RNA world. Although catalysis of the peptide bond involves the C2 hydroxyl of RNA's P-site adenosine in a protein shuttle mechanism, other steps in protein synthesis (such as translocation) are caused by changes in protein conformations.

Ribosomes are sometimes referred to as organelles, but the use of the term *organelle* is often restricted to describing sub-cellular components that include a phospholipid membrane, which ribosomes, being entirely particulate, do not. For this reason, ribosomes may sometimes be described as "non-membranous organelles".

Ribosomes were first observed in the mid-1950s by Romanian cell biologist George Palade using an electron microscope as dense particles or granules for which he would win the Nobel Prize. The term "ribosome" was proposed by scientist Richard B. Roberts in 1958:

During the course of the symposium a semantic difficulty became apparent. To some of the participants, "microsomes" mean the ribonucleoprotein particles of the microsome fraction contaminated by other protein and lipid material; to others, the microsomes consist of protein and lipid contaminated by particles. The phrase “microsomal particles” does not seem adequate, and “ribonucleoprotein particles of the microsome fraction” is much too awkward. During the meeting, the word "ribosome" was suggested, which has a very satisfactory name and a pleasant sound. The present confusion would be eliminated if “ribosome” were adopted to designate ribonucleoprotein particles in sizes ranging from 35 to 100S.

– Roberts, R. B., Microsomal Particles and Protein Synthesis

The structure and function of the ribosomes and associated molecules, known as the *translational apparatus*, has been of research interest since the mid-twentieth century and is a very active field of study today.

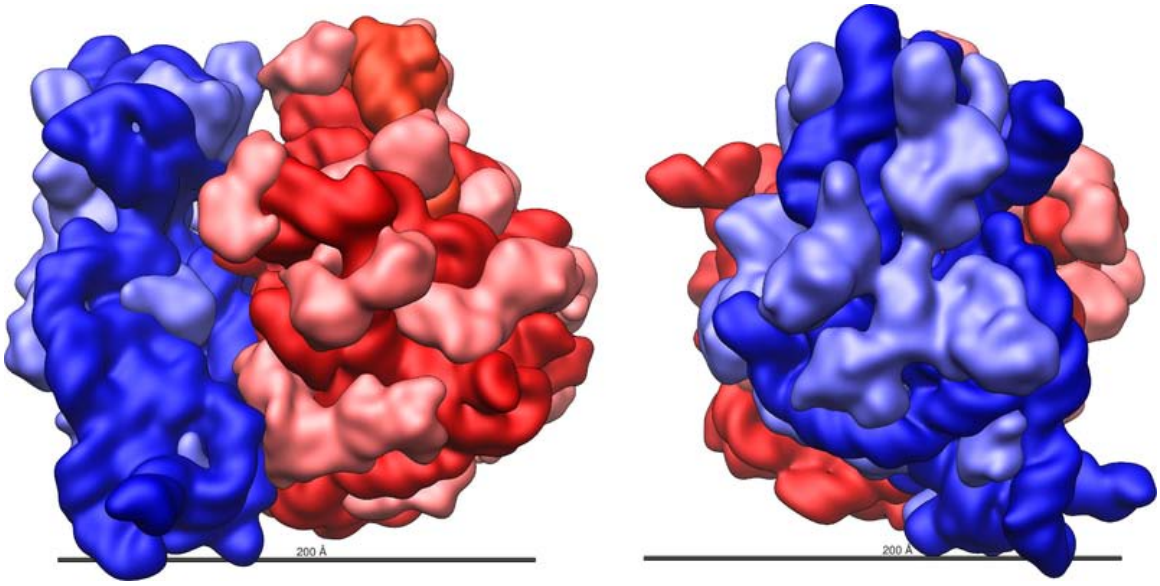


Figure 2 : Large (red) and small (blue) subunit fit together

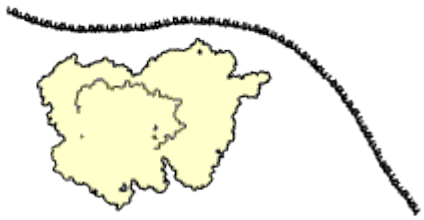
Ribosomes consist of two subunits (Figure 1) that fit together (Figure 2) and work as one to translate the mRNA into a polypeptide chain during protein synthesis (Figure 3). Bacterial subunits consist of one or two and eukaryotic of one or three very large RNA molecules (known as ribosomal RNA or rRNA) and multiple smaller protein molecules. Crystallographic work has shown that there are no ribosomal proteins close to the reaction site for polypeptide synthesis. This suggests that the protein components of

ribosomes act as a scaffold that may enhance the ability of rRNA to synthesize protein rather than directly participating in catalysis (See: Ribozyme).

Biogenesis

In bacterial cells, ribosomes are synthesized in the cytoplasm through the transcription of multiple ribosome gene operons. In eukaryotes, the process takes place both in the cell cytoplasm and in the nucleolus, which is a region within the cell nucleus. The assembly process involves the coordinated function of over 200 proteins in the synthesis and processing of the four rRNAs, as well as assembly of those rRNAs with the ribosomal proteins.

Ribosome locations



A ribosome translating a protein that is secreted into the endoplasmic reticulum.

Free and membrane-bound ribosomes differ only in their spatial distribution; they are identical in structure. Whether the ribosome exists in a free or membrane-bound state depends on the presence of an ER-targeting signal sequence on the protein being synthesized, so an individual ribosome might be membrane-bound when it is making one protein, but free in the cytosol when it makes another protein.

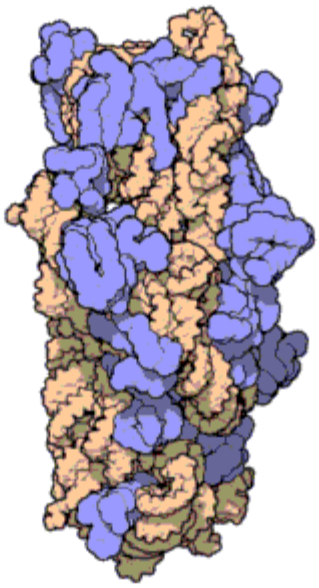
Free ribosomes

Free ribosomes can move about anywhere in the cytosol, but are excluded from the cell nucleus and other organelles. Proteins that are formed from free ribosomes are released into the cytosol and used within the cell. Since the cytosol contains high concentrations of glutathione and is, therefore, a reducing environment, proteins containing disulfide bonds, which are formed from oxidized cysteine residues, cannot be produced in this compartment.

Membrane-bound ribosomes

When a ribosome begins to synthesize proteins that are needed in some organelles, the ribosome making this protein can become "membrane-bound". In eukaryotic cells this happens in a region of the endoplasmic reticulum (ER) called the "rough ER". The newly produced polypeptide chains are inserted directly into the ER by the ribosome and are then transported to their destinations, through the secretory pathway. Bound ribosomes usually produce proteins that are used within the plasma membrane or are expelled from the cell via *exocytosis*.

Structure



Atomic structure of the 30S Subunit from *Thermus thermophilus*. Proteins are shown in blue and the single RNA strand in orange.

The ribosomal subunits of prokaryotes and eukaryotes are quite similar.

The unit of measurement is the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size and accounts for why fragment names do not add up (70S is made of 50S and 30S).

Prokaryotes have 70S ribosomes, each consisting of a small (30S) and a large (50S) subunit. Their large subunit is composed of a 5S RNA subunit (consisting of 120 nucleotides), a 23S RNA subunit (2900 nucleotides) and 34 proteins. The 30S subunit has a 1540 nucleotide RNA subunit (16S) bound to 21 proteins.

Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit. Their large subunit is composed of a 5S RNA (120 nucleotides), a 28S RNA (4700

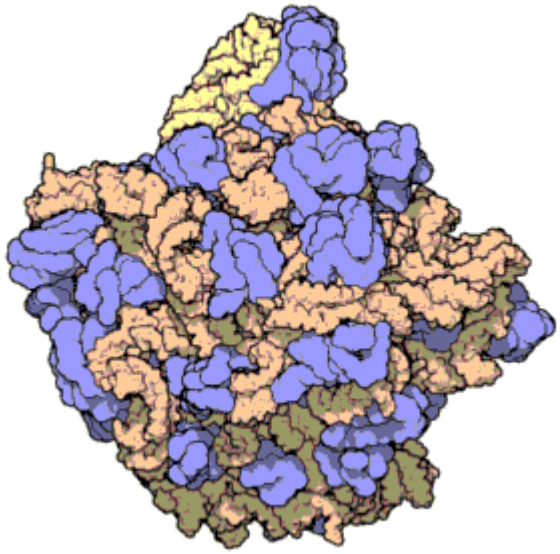
nucleotides), a 5.8S subunit (160 nucleotides) and ~49 proteins. The 40S subunit has a 1900 nucleotide (18S) RNA and ~33 proteins.

The ribosomes found in chloroplasts and mitochondria of eukaryotes also consist of large and small subunits bound together with proteins into one 70S particle. These organelles are believed to be descendants of bacteria and as such their ribosomes are similar to those of bacteria.

The various ribosomes share a core structure, which is quite similar despite the large differences in size. Much of the RNA is highly organized into various tertiary structural motifs, for example pseudoknots that exhibit coaxial stacking. The extra RNA in the larger ribosomes is in several long continuous insertions, such that they form loops out of the core structure without disrupting or changing it. All of the catalytic activity of the ribosome is carried out by the RNA; the proteins reside on the surface and seem to stabilize the structure.

The differences between the bacterial and eukaryotic ribosomes are exploited by pharmaceutical chemists to create antibiotics that can destroy a bacterial infection without harming the cells of the infected person. Due to the differences in their structures, the bacterial 70S ribosomes are vulnerable to these antibiotics while the eukaryotic 80S ribosomes are not. Even though mitochondria possess ribosomes similar to the bacterial ones, mitochondria are not affected by these antibiotics because they are surrounded by a double membrane that does not easily admit these antibiotics into the organelle.

High-resolution structure



Atomic structure of the 50S Subunit from *Haloarcula marismortui*. Proteins are shown in blue and the two RNA strands in orange and yellow. The small patch of green in the center of the subunit is the active site.

The general molecular structure of the ribosome has been known since the early 1970s. In the early 2000s the structure has been achieved at high resolutions, on the order of a few ångströms.

The first papers giving the structure of the ribosome at atomic resolution were published in rapid succession in late 2000. First, the 50S (large prokaryotic) subunit from the archaeon *Haloarcula marismortui* was published. Soon after, the structure of the 30S subunit from *Thermus thermophilus* was published. Shortly thereafter, a more detailed structure was published. These structural studies were awarded the Nobel Prize in Chemistry in 2009. Early the next year (May 2001) these coordinates were used to reconstruct the entire *T. thermophilus* 70S particle at 5.5 ångström resolution.

Two papers were published in November 2005 with structures of the *Escherichia coli* 70S ribosome. The structures of a vacant ribosome were determined at 3.5-ångström resolution using x-ray crystallography. Then, two weeks later, a structure based on cryo-electron microscopy was published, which depicts the ribosome at 11-15 ångström resolution in the act of passing a newly synthesized protein strand into the protein-conducting channel.

First atomic structures of the ribosome complexed with tRNA and mRNA molecules were solved by using X-ray crystallography by two groups independently, at 2.8 ångström and at 3.7 ångström. These structures allow one to see the details of interactions of the *Thermus thermophilus* ribosome with mRNA and with tRNAs bound at classical ribosomal sites. Interactions of the ribosome with long mRNAs containing Shine-Dalgarno sequences were visualized soon after that at 4.5- to 5.5-ångström resolution.

Function

Ribosomes are the workhorses of protein biosynthesis, the process of translating mRNA into protein. The mRNA comprises a series of codons that dictate to the ribosome the sequence of the amino acids needed to make the protein. Using the mRNA as a template, the ribosome traverses each codon (3 nucleotides) of the mRNA, pairing it with the appropriate amino acid provided by a tRNA. Molecules of transfer RNA (tRNA) contain a complementary anticodon on one end and the appropriate amino acid on the other. The small ribosomal subunit, typically bound to a tRNA containing the amino acid methionine, binds to an AUG codon on the mRNA and recruits the large ribosomal subunit. The ribosome then contains three RNA binding sites, designated A, P and E. The A site binds an aminoacyl-tRNA (a tRNA bound to an amino acid); the P site binds a peptidyl-tRNA (a tRNA bound to the peptide being synthesized); and the E site binds a free tRNA before it exits the ribosome. Protein synthesis begins at a start codon AUG near the 5' end of the mRNA. mRNA binds to the P site of the ribosome first. The ribosome is able to identify the start codon by use of the Shine-Dalgarno sequence of the mRNA in prokaryotes and Kozak box in eukaryotes.

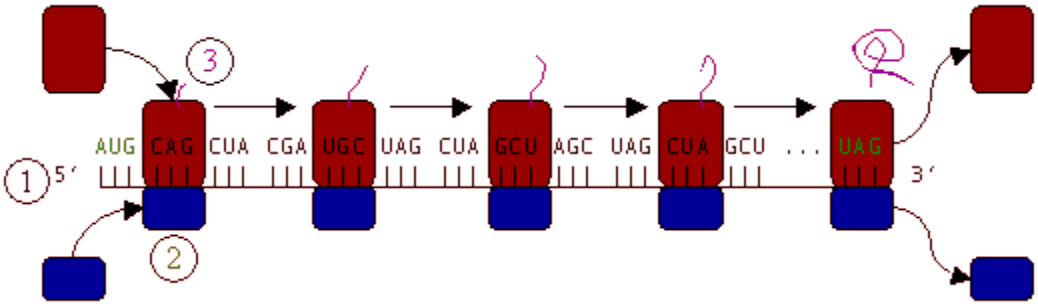


Figure 3 : Translation of mRNA (1) by a ribosome (2)(shown as small and large subunits) into a polypeptide chain (3). The ribosome begins at the start codon of mRNA (AUG) and ends at the stop codon (UAG).

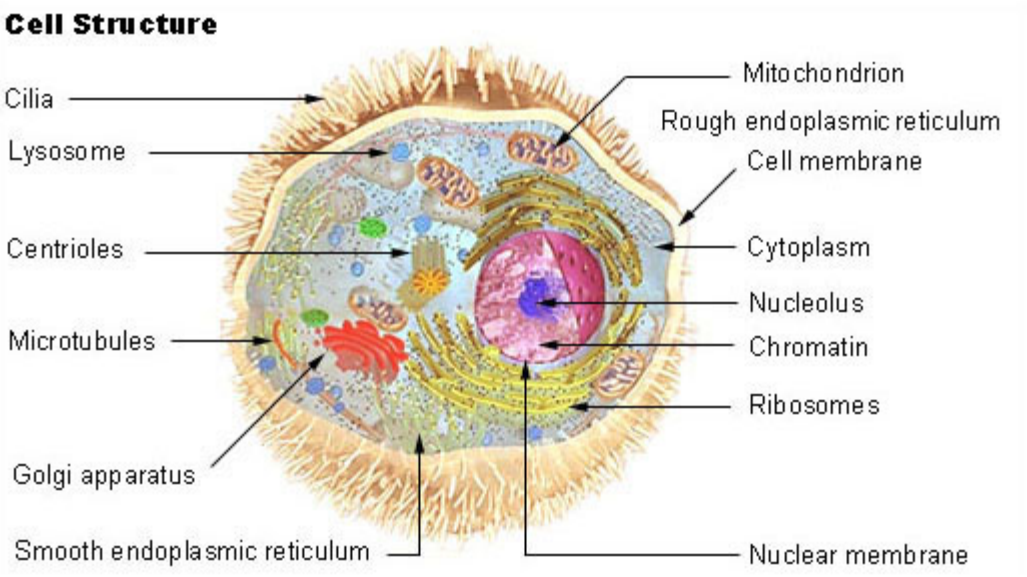
In Figure 3, both ribosomal subunits (small and large) assemble at the start codon (towards the 5' end of the mRNA). The ribosome uses tRNA that matches the current codon (triplet) on the mRNA to append an amino acid to the polypeptide chain. This is done for each triplet on the mRNA, while the ribosome moves towards the 3' end of the mRNA. Usually in bacterial cells, several ribosomes are working parallel on a single mRNA, forming what is called a *polyribosome* or *polysome*.

Nobel Prize

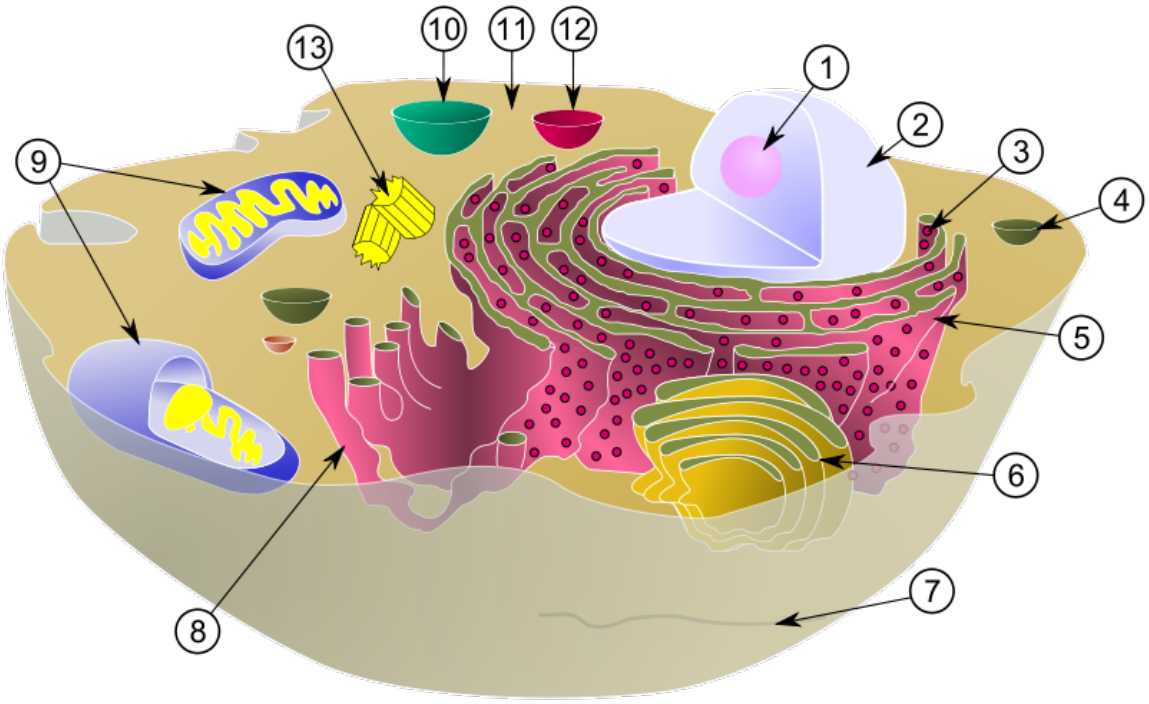
Together with Albert Claude and Christian de Duve, George Emil Palade was awarded the Nobel Prize in Physiology or Medicine, in 1974, for the discovery of the ribosomes. The Nobel Prize in Chemistry 2009 was awarded to Drs Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome"

Chapter- 13

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. 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.

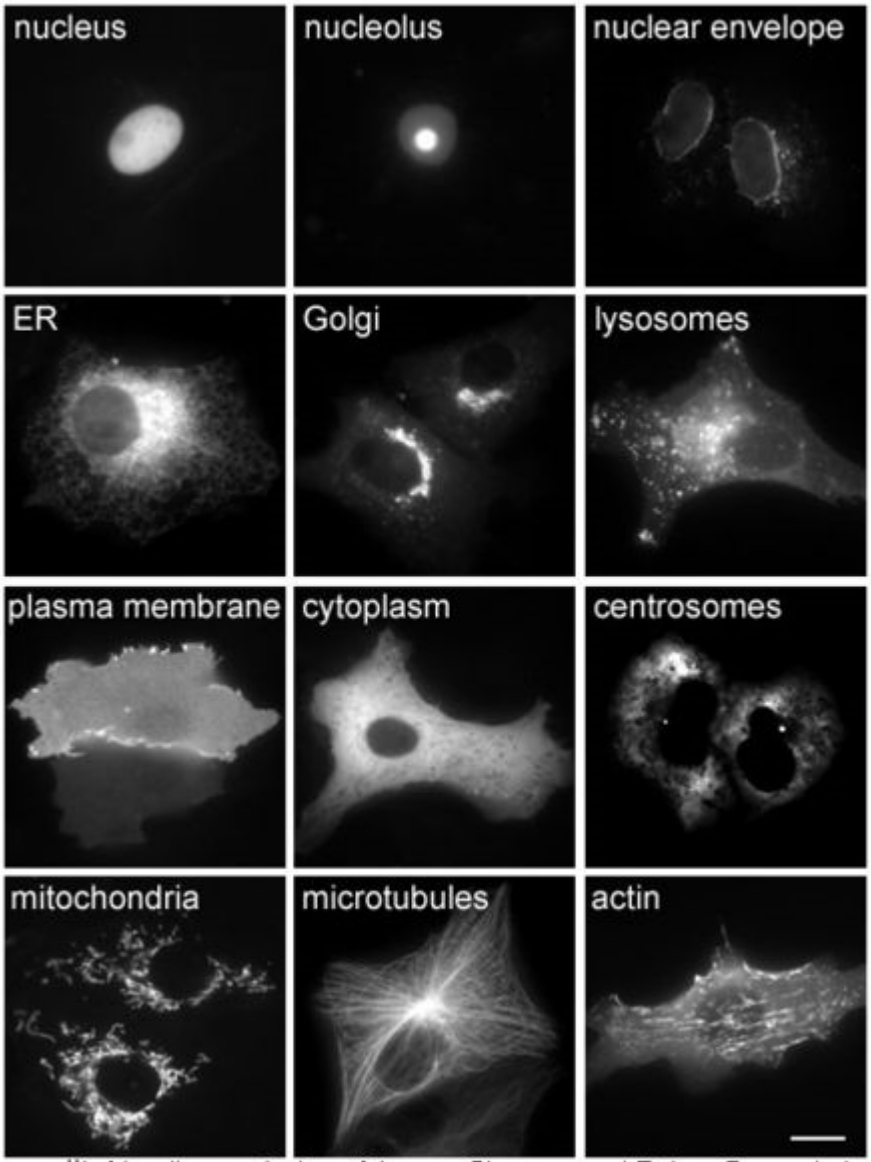
Other functions include digesting foreign bacteria (or other forms of waste) that invade a cell and helping repair damage to the plasma membrane by serving as a membrane patch, sealing the wound. In the past, lysosomes were thought to kill cells that are no longer wanted, such as those in the tails of 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.

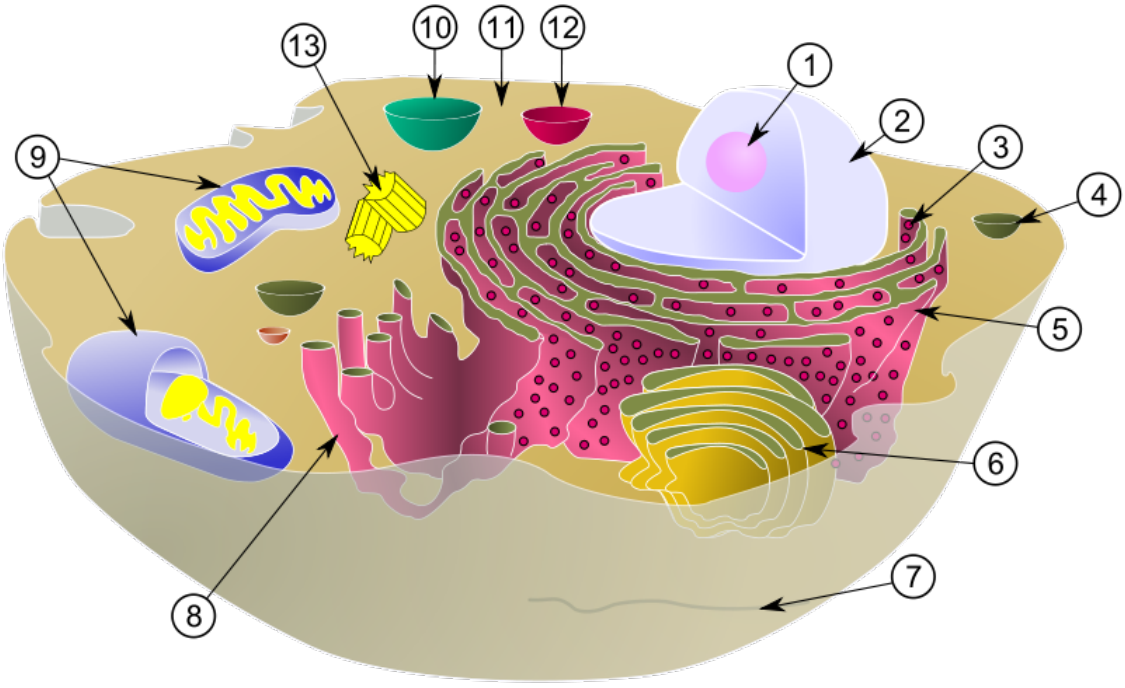


With friendly permission of Jeremy Simpson and Rainer Pepperkok

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

Chapter- 14

Centrosome



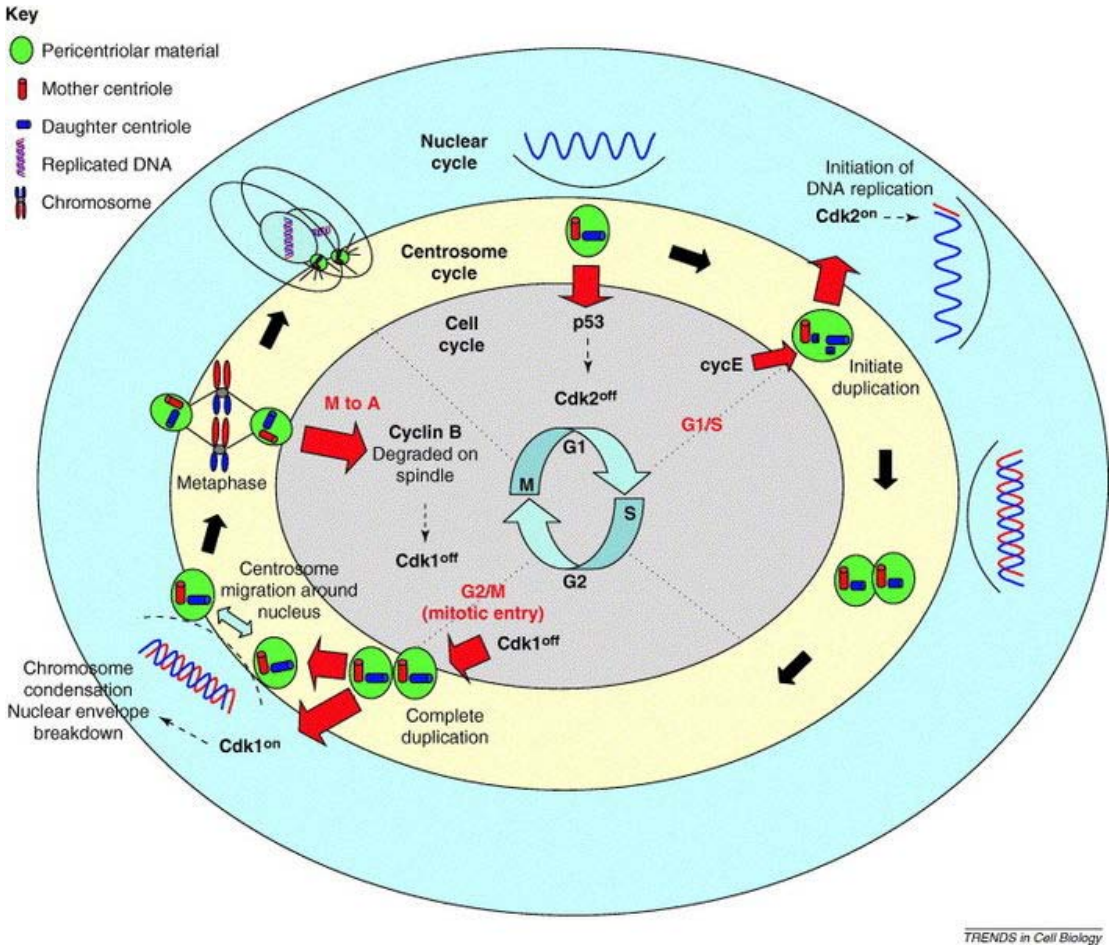
Schematic of typical animal cell, showing subcellular components. Organelles:
(1) Nucleolus
(2) Nucleus
(3) Ribosomes (little dots)
(4) Vesicle
(5) Rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(10) Vacuole
(11) Cytoplasm

(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 necessary.

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

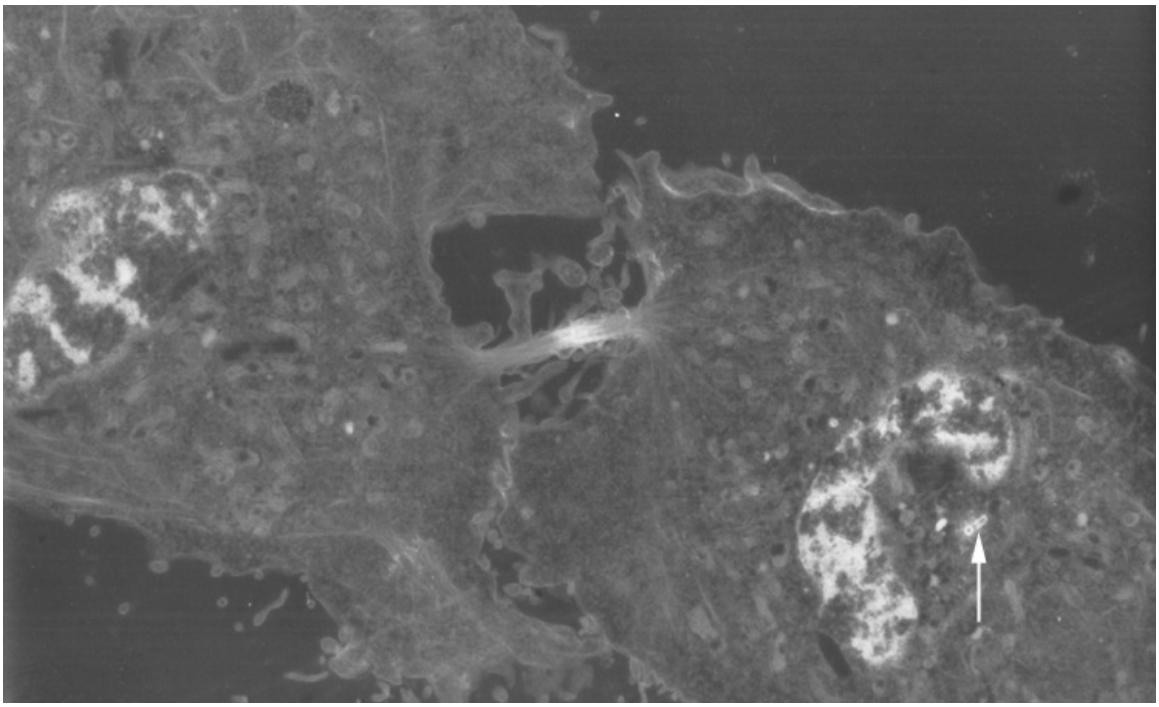


Role of the centrosome in cell cycle progression

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. In fact, these structures are not even present in the centrosomes of plant cells.

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.

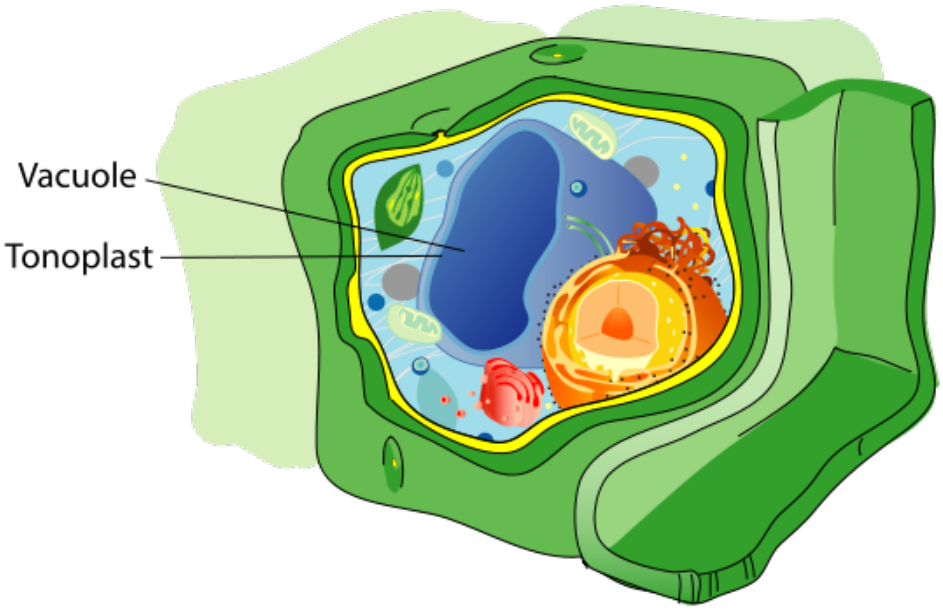
It is evident that some parts of the centrosome are highly diverged in the model species Drosophila melanogaster and Caenorhabditis elegans. For example, both species have lost one of the centrin subfamilies that are usually associated with centriole duplication. Drosophila melanogaster mutants that 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

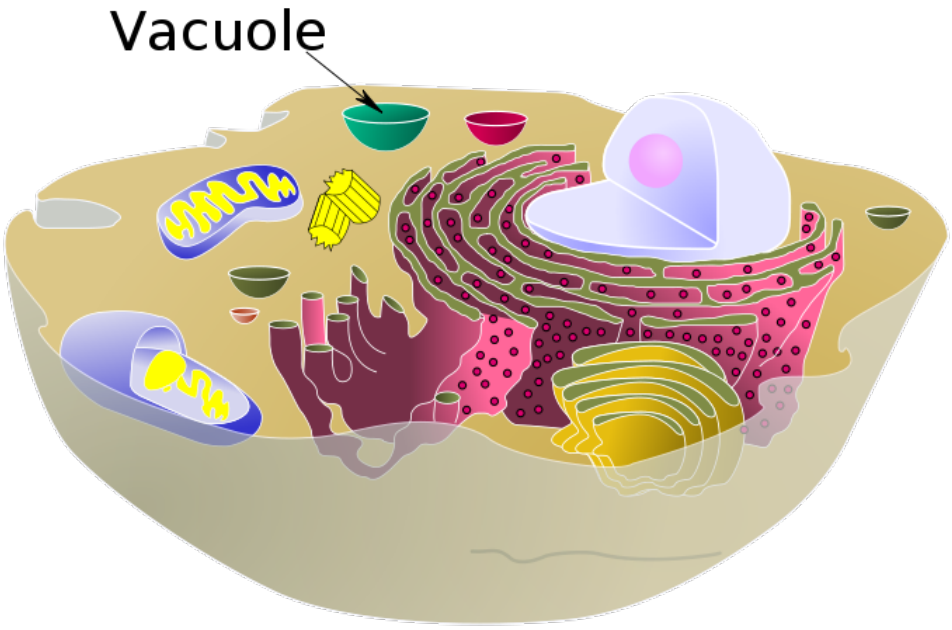
Research in 2006 indicated that centrosomes from Surf clam eggs contain RNA sequences. The sequences identified were found in "few to no" other places in the cell, and do not appear in existing genome databases. One identified RNA sequence contains a putative RNA polymerase, leading to the hypothesis 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- 15

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:

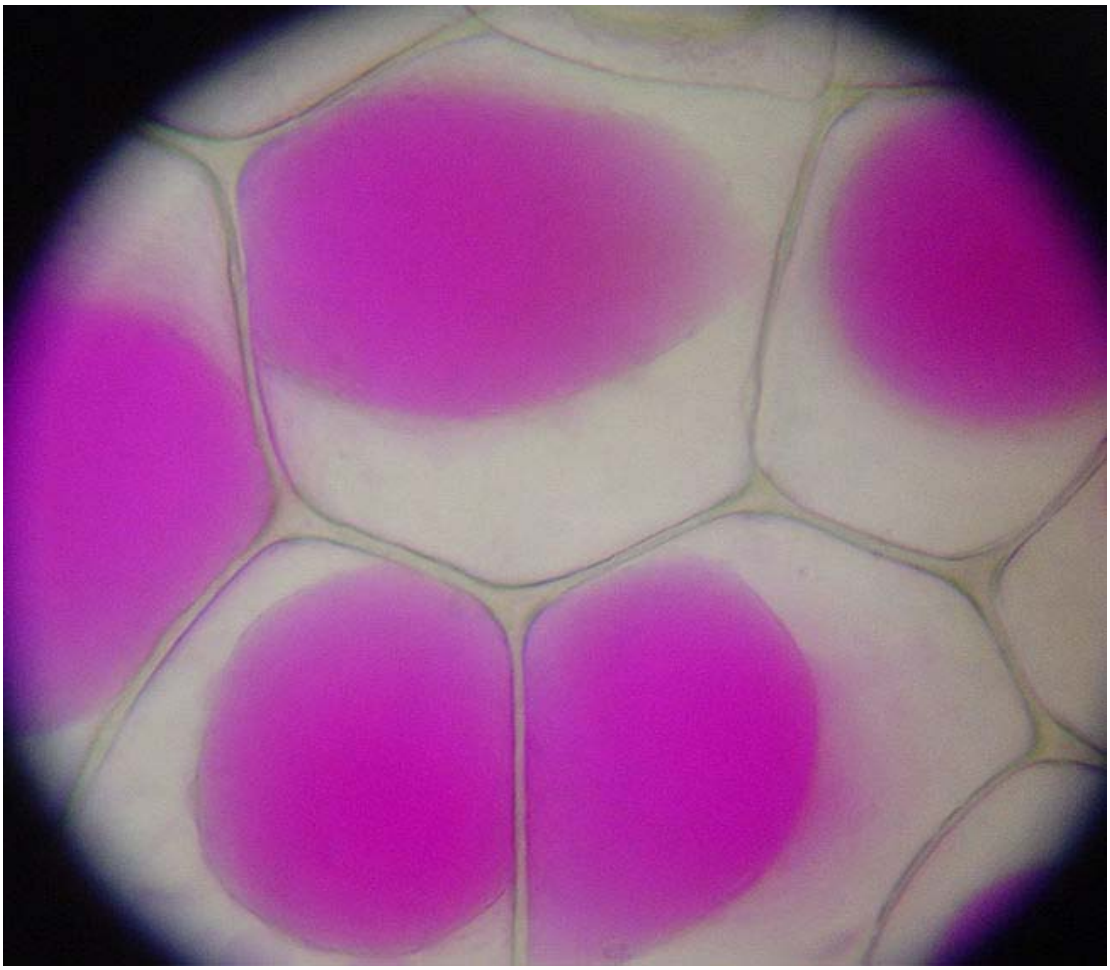
- Isolating materials that might be harmful or a threat to the cell
- Containing waste products
- Containing water in plant cells
- Maintaining internal hydrostatic pressure or turgor within the cell
- Maintaining an acidic internal pH
- Containing small molecules
- Exporting unwanted substances from 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 vacuoles contain high concentrations of nitrate ions and is therefore thought to be a storage organelle.

Plants



The vacuoles of spiderwort cells, stained in pink

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.

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.