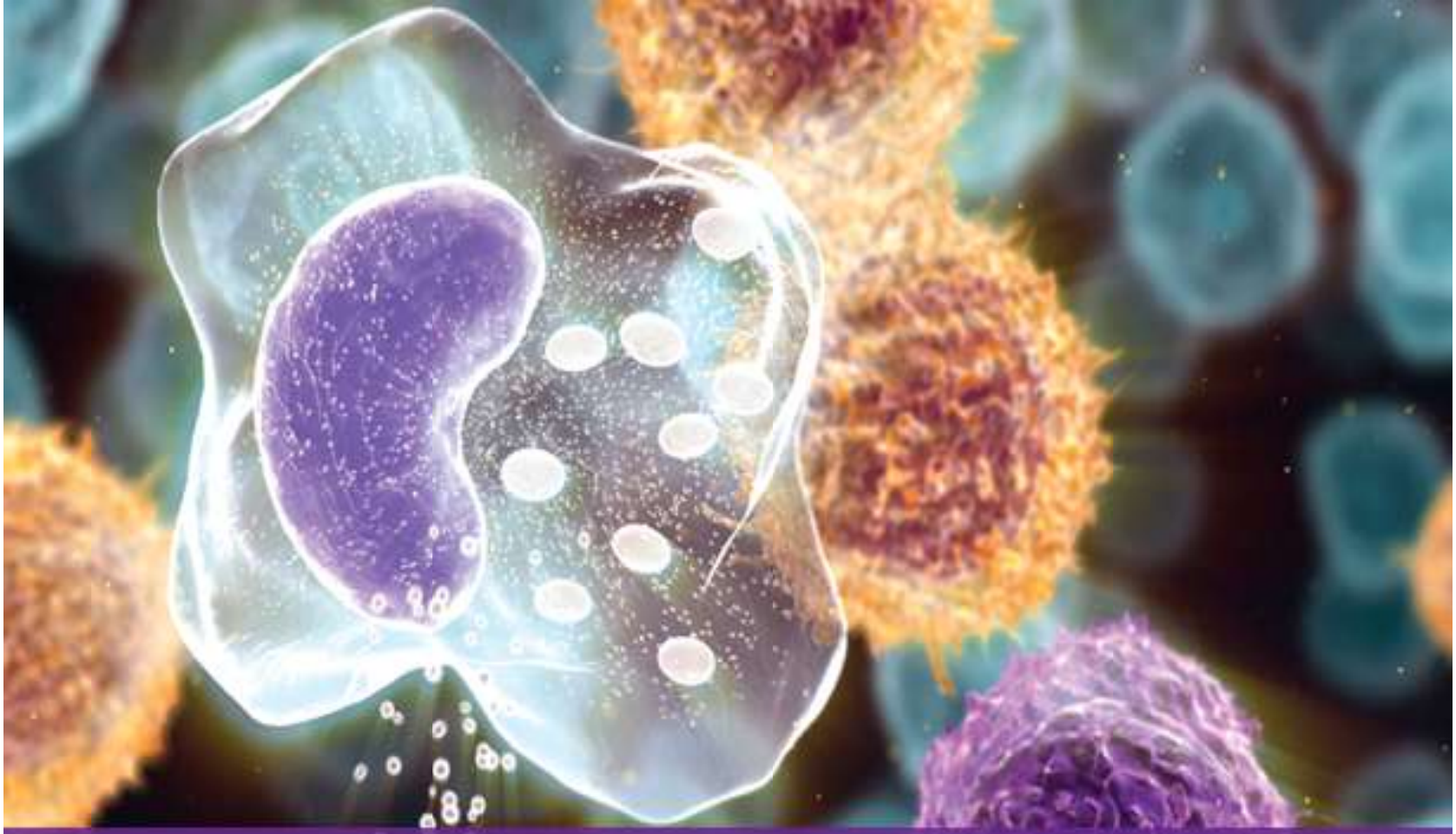

Cellular Anatomy & Reproduction

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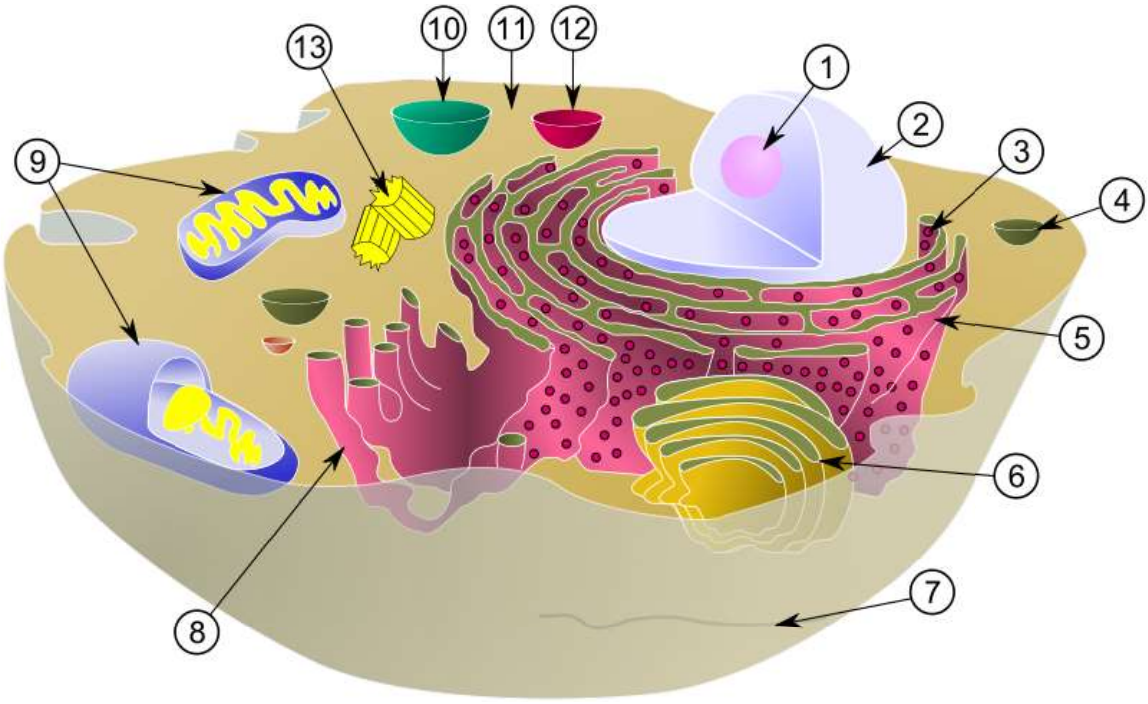
Organelle



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	single-membrane compartment	all eukaryotes	rough endoplasmic reticulum is covered with ribosomes, has

	endoplasmic reticulum), expression of lipids (smooth endoplasmic reticulum)			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

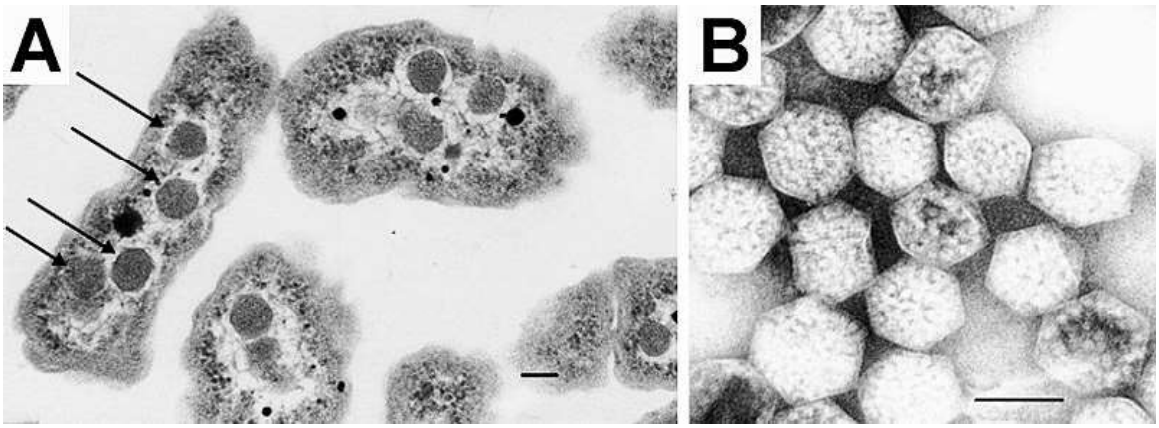
Organelle/Macromolecule	Main function	Structure	Organisms
acrosome	helps spermatoza fuse with ovum	single-membrane compartment	many animals
autophagosome	vesicle which sequesters cytoplasmic material and organelles for degradation	double-membrane compartment	all eukaryotic cells
centriole	anchor for 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-RNA	most eukaryotes
parenthesome	not characterized	not characterized	fungi
peroxisome	breakdown of metabolic hydrogen peroxide	single-membrane compartment	all eukaryotes
ribosome	translation of RNA into proteins	RNA-protein	eukaryotes, prokaryotes
vesicle	material transport	single-membrane compartment	all eukaryotes

Other related structures:

- cytosol
- endomembrane system
- nucleosome

- microtubule
- cell membrane



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

Prokaryotic organelles

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

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

Prokaryotic organelles and cell components

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

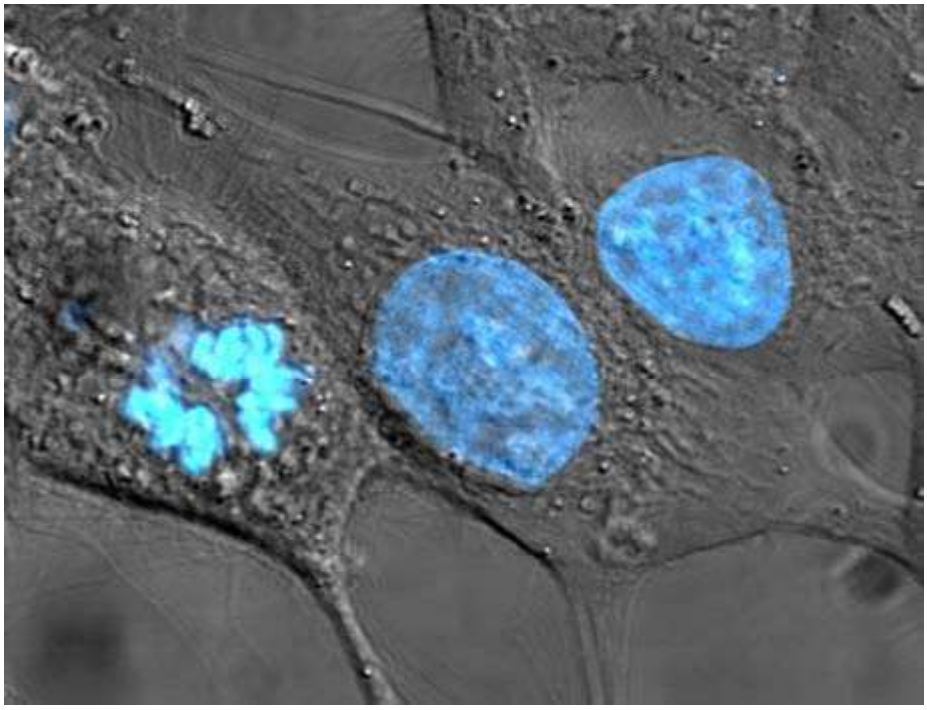
nucleoid	DNA maintenance, transcription to RNA	DNA-protein	prokaryotes
plasmid	DNA exchange	circular DNA	some bacteria
ribosome	translation of RNA into proteins	RNA-protein	eukaryotes, prokaryotes
thylakoid	photosynthesis	photosystem proteins and pigments	mostly cyanobacteria

Proteins and organelles

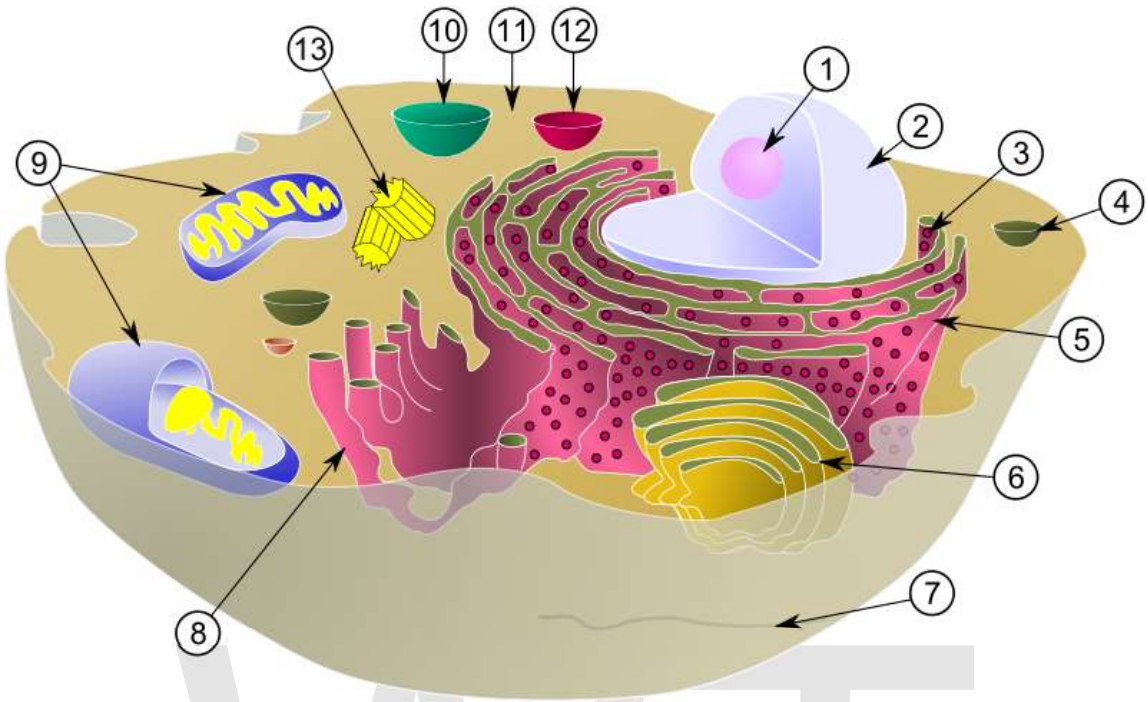
The function of a protein is closely correlated with the organelle in which it resides. Some methods were proposed for predicting the organelle in which an uncharacterized protein is located according to its amino acid composition and some methods were based on pseudo amino acid composition.

Chapter- 2

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.



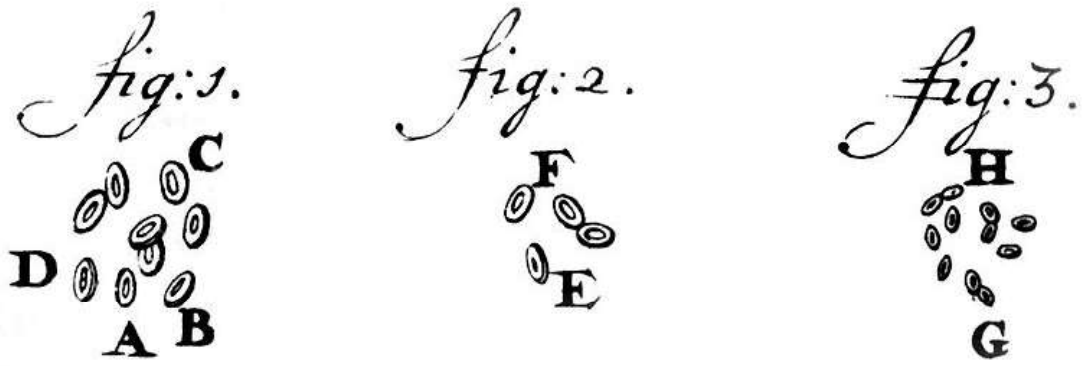
Schematic of typical animal cell, showing subcellular components. Organelles: (1) nucleolus (2) **nucleus** (3) ribosome (4) vesicle (5) rough endoplasmic reticulum (ER) (6) Golgi apparatus (7) Cytoskeleton (8) smooth ER (9) mitochondria (10) vacuole (11) cytoplasm (12) lysosome (13) centrioles

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.

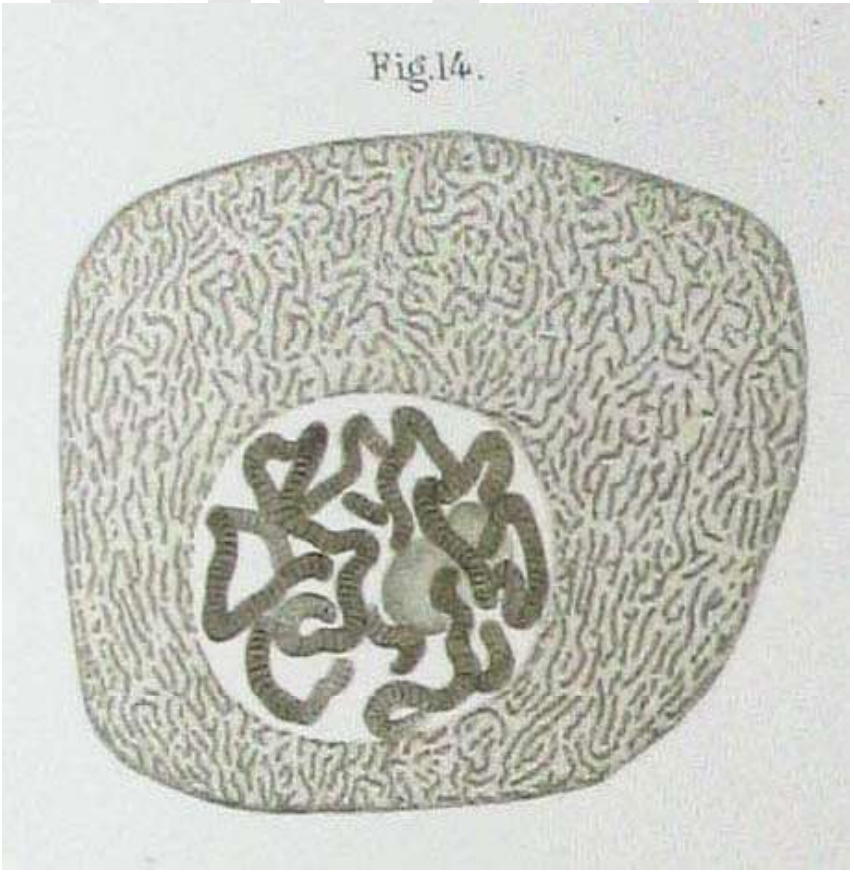
Although the interior of the nucleus does not contain any membrane-bound subcompartments, its contents are not uniform, and a number of *subnuclear bodies* exist, made up of unique proteins, RNA molecules, and particular parts of the chromosomes.

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.

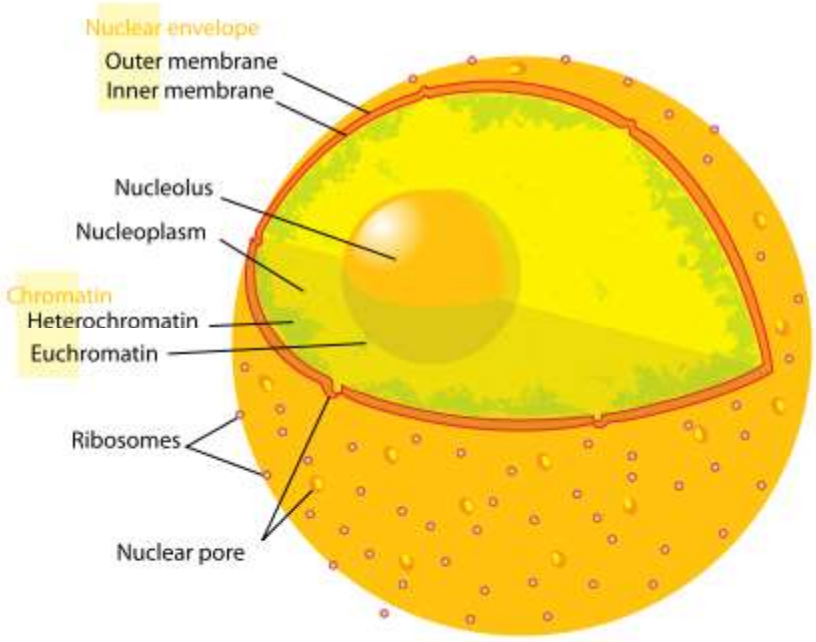
The nucleus was the first organelle to be discovered. The probably oldest preserved drawing dates back to the early microscopist Antonie van Leeuwenhoek (1632 – 1723). He observed a "Lumen", the nucleus, in the red blood cells of salmon. Unlike mammalian red blood cells, those of other vertebrates still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

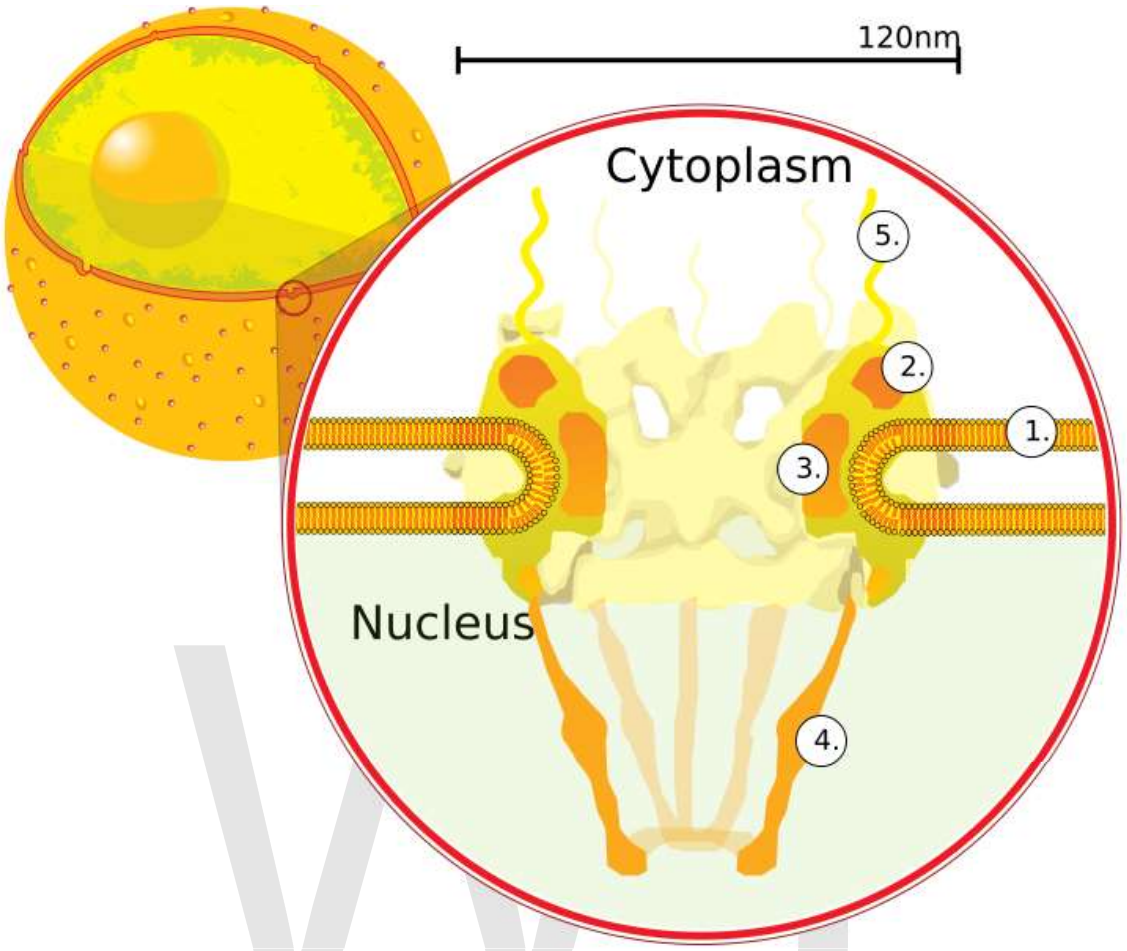
Structures

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.

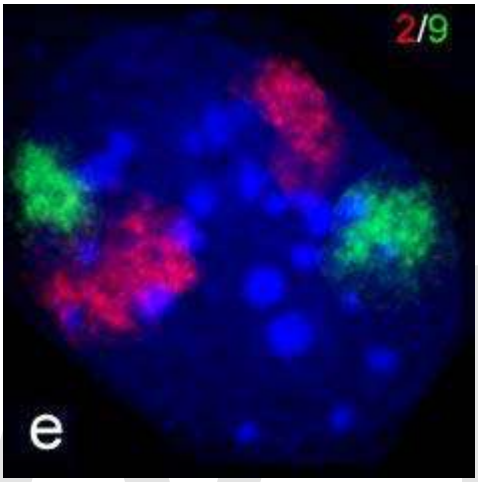
The nuclear lamina is mostly composed of lamin proteins. Like all proteins, lamins are synthesized in the cytoplasm and later transported into the nucleus interior, where they are assembled before being incorporated into the existing network of nuclear lamina. Lamins are also found inside the nucleoplasm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



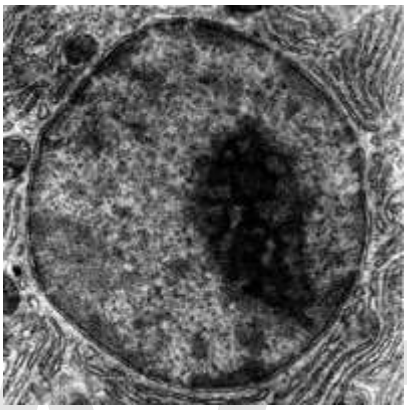
A mouse fibroblast nucleus in which DNA is stained blue. The distinct chromosome territories of chromosome 2 (red) and chromosome 9 (green) are visible stained with fluorescent in situ hybridization.

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.

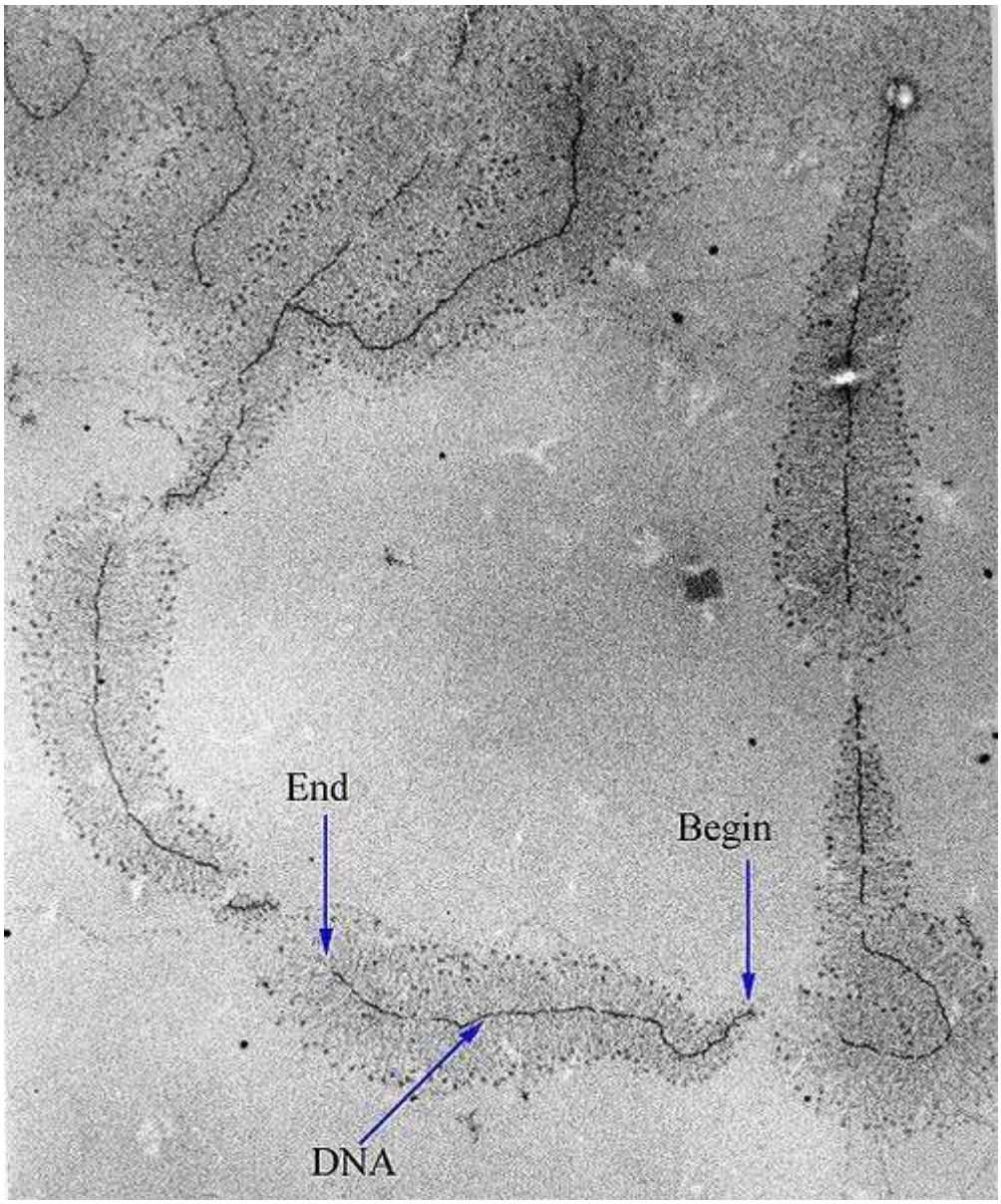
Cell compartmentalization

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.

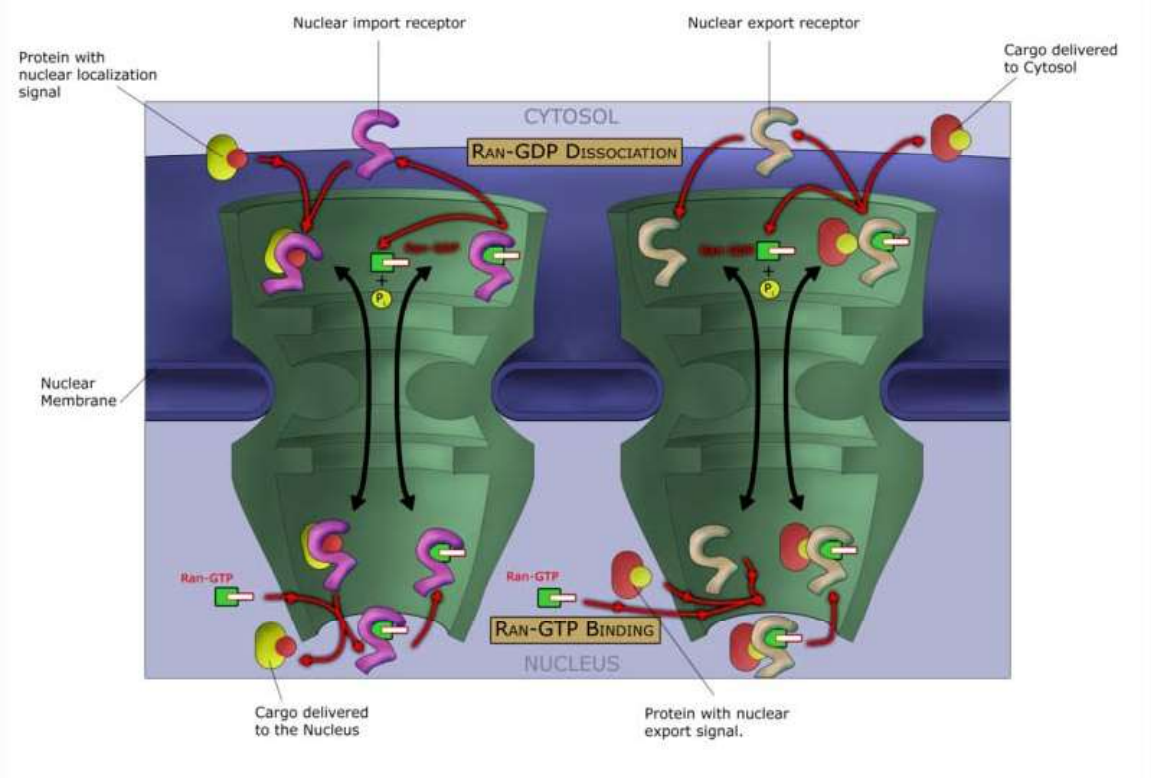
Processing of pre-mRNA

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.

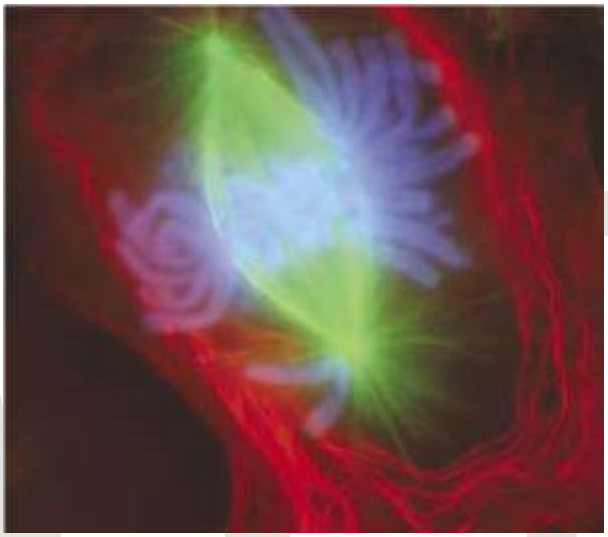
The entry and exit of large molecules from the nucleus is tightly controlled by the nuclear pore complexes. Although small molecules can enter the nucleus without regulation, macromolecules such as RNA and proteins require association karyopherins called importins to enter the nucleus and exportins to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

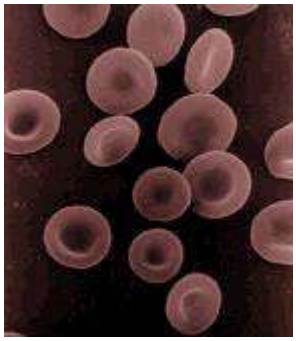
regulated by phosphorylation of the lamins. Towards the end of the cell cycle, the nuclear membrane is reformed, and around the same time, the nuclear lamina are reassembled by dephosphorylating the lamins.

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.

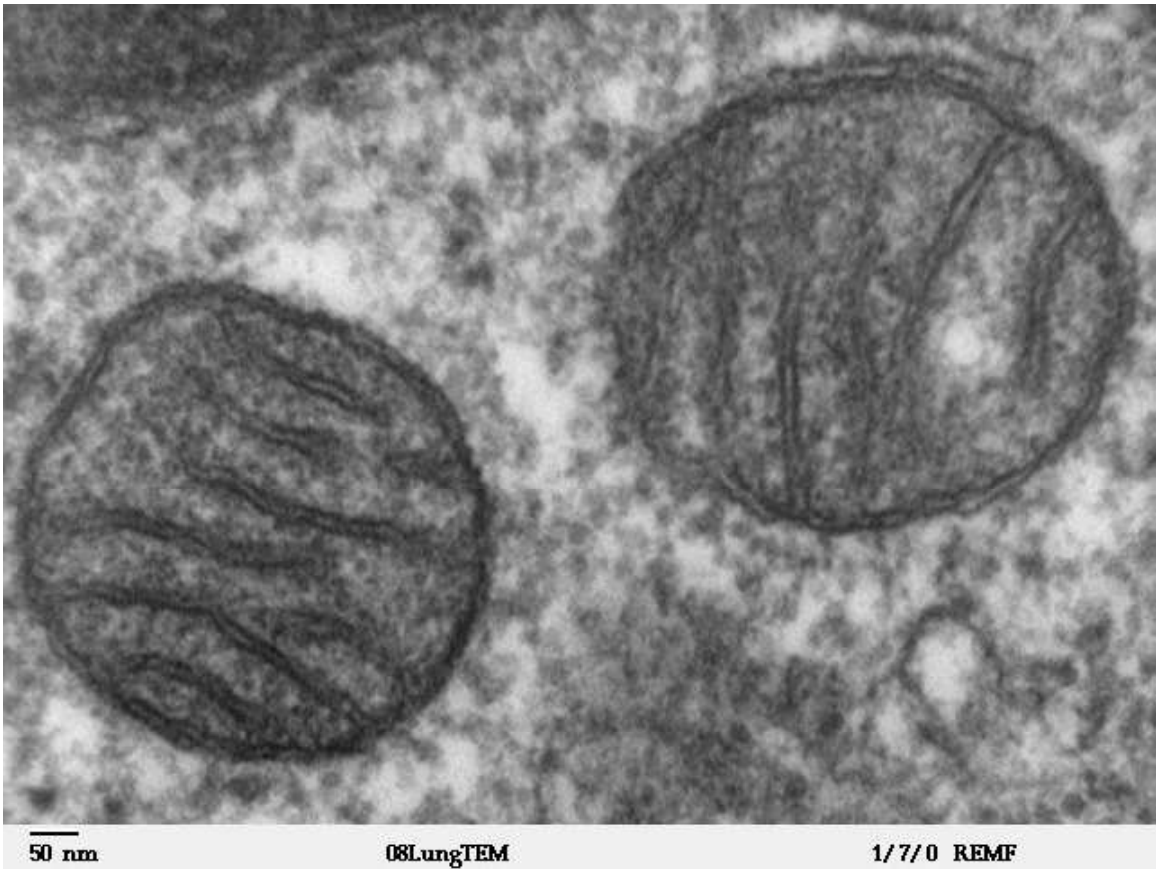
A second model proposes that proto-eukaryotic cells evolved from bacteria without an endosymbiotic stage. This model is based on the existence of modern planctomycetes bacteria that possess a nuclear structure with primitive pores and other compartmentalized membrane structures. A similar proposal states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

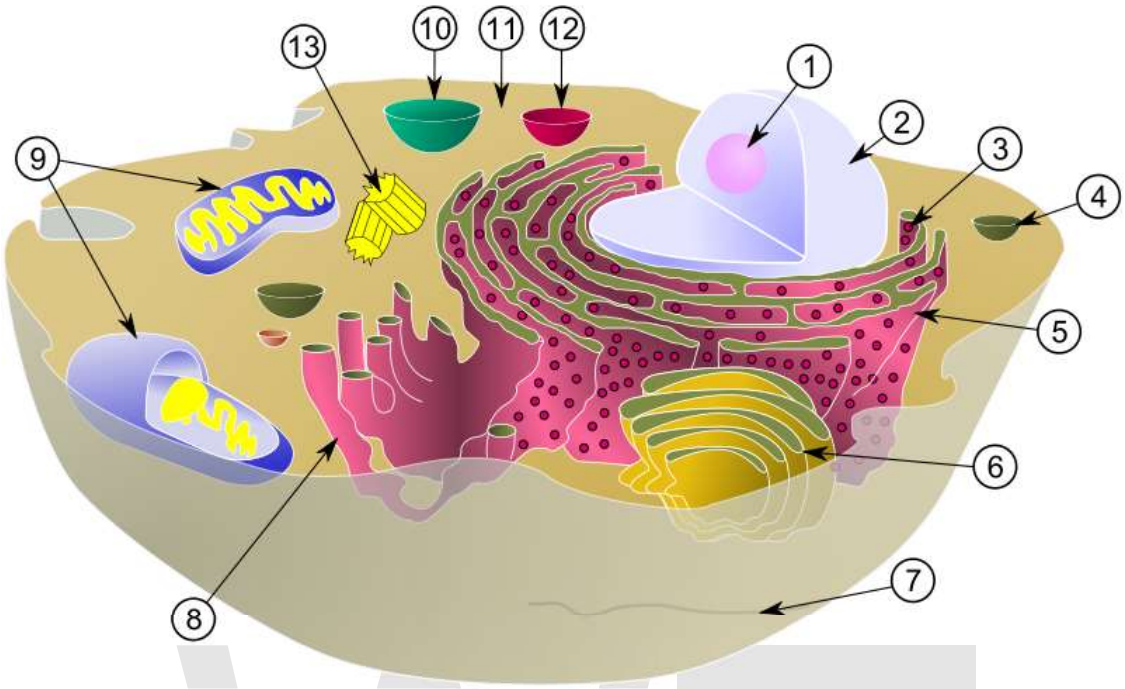
Finally, a very recent proposal suggests that traditional variants of the endosymbiont theory are insufficiently powerful to explain the origin of the eukaryotic nucleus. This model, termed the *exomembrane hypothesis*, suggests that the nucleus instead originated from a single ancestral cell that evolved 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- 3

Mitochondrion



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



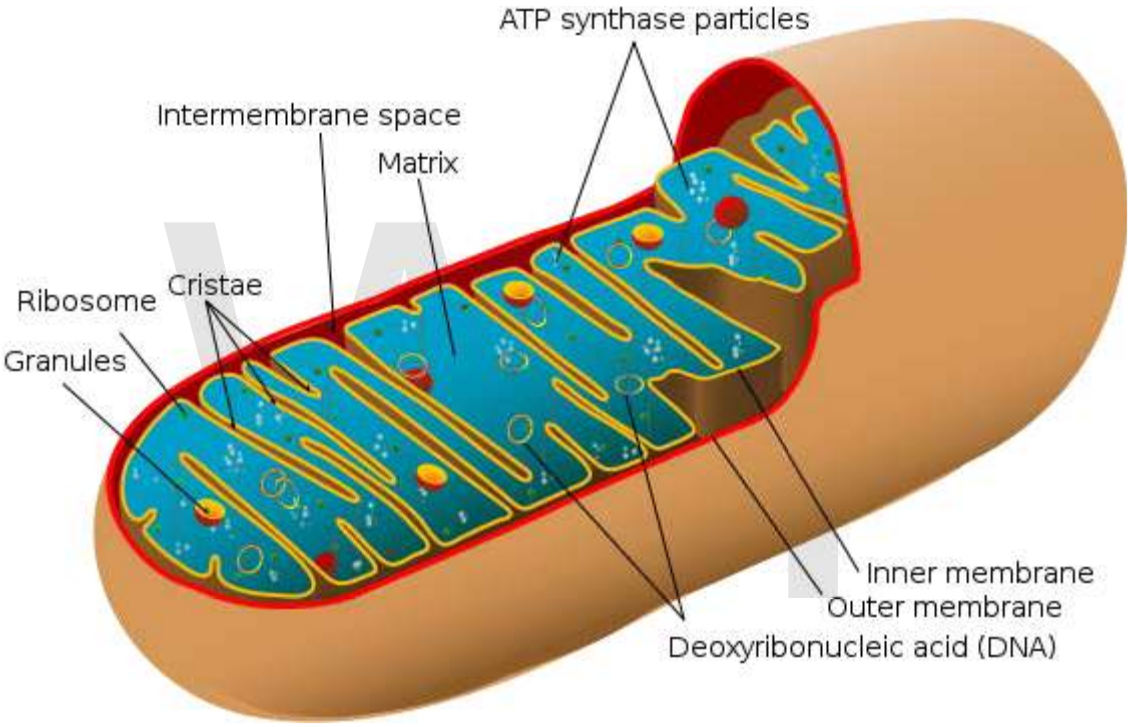
Schematic of typical animal cell, showing subcellular components. Organelles:
(1) nucleolus
(2) nuclear membrane
(3) Ribosomes
(4) Vesicle
(5) Rough endoplasmic reticulum (ER)
(6) Golgi body
(7) Cytoskeleton
(8) Smooth ER
(9) Mitochondria
(13) Centrioles within centrosome

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.

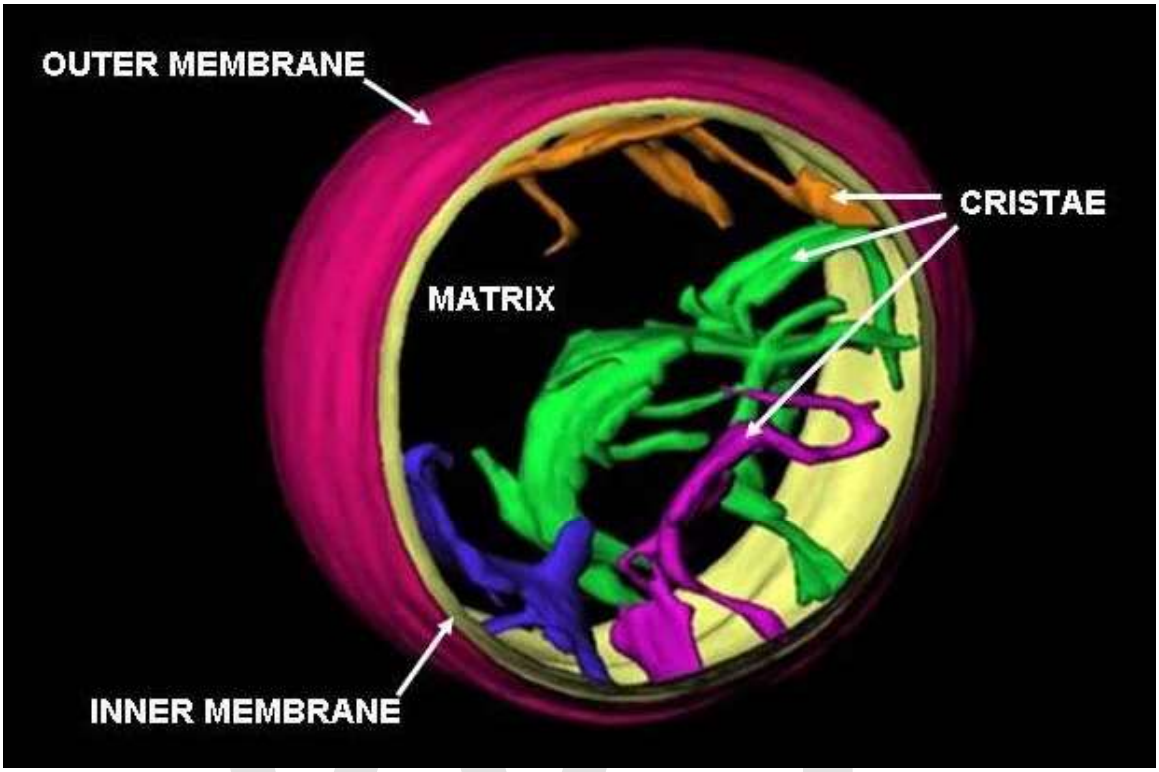
Inner membrane

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

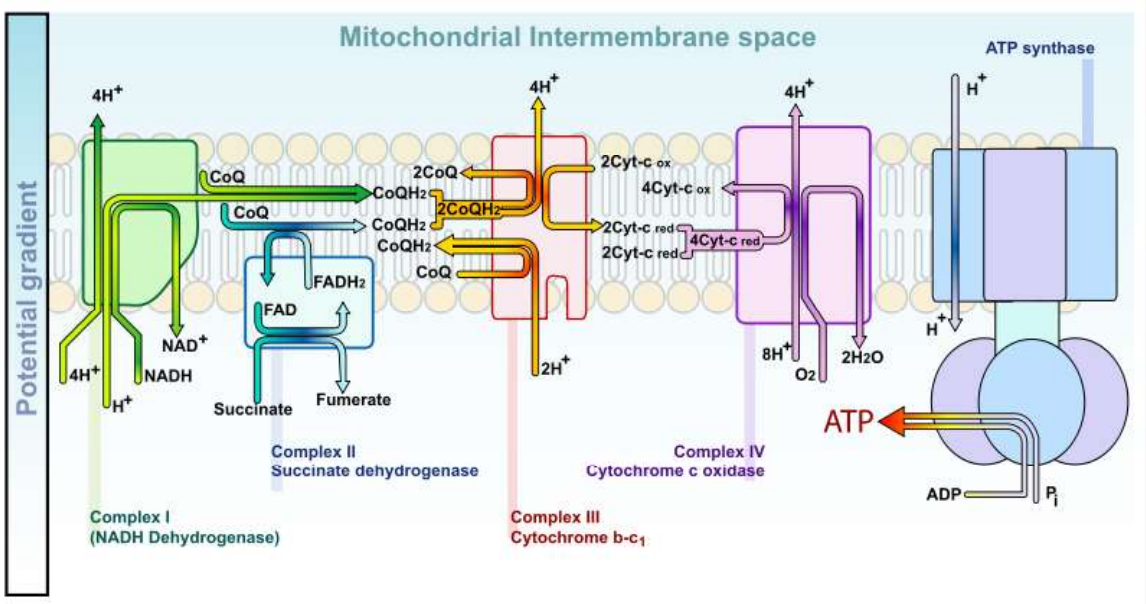


Diagram of the electron transport chain in the mitonchondrial intermembrane space

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

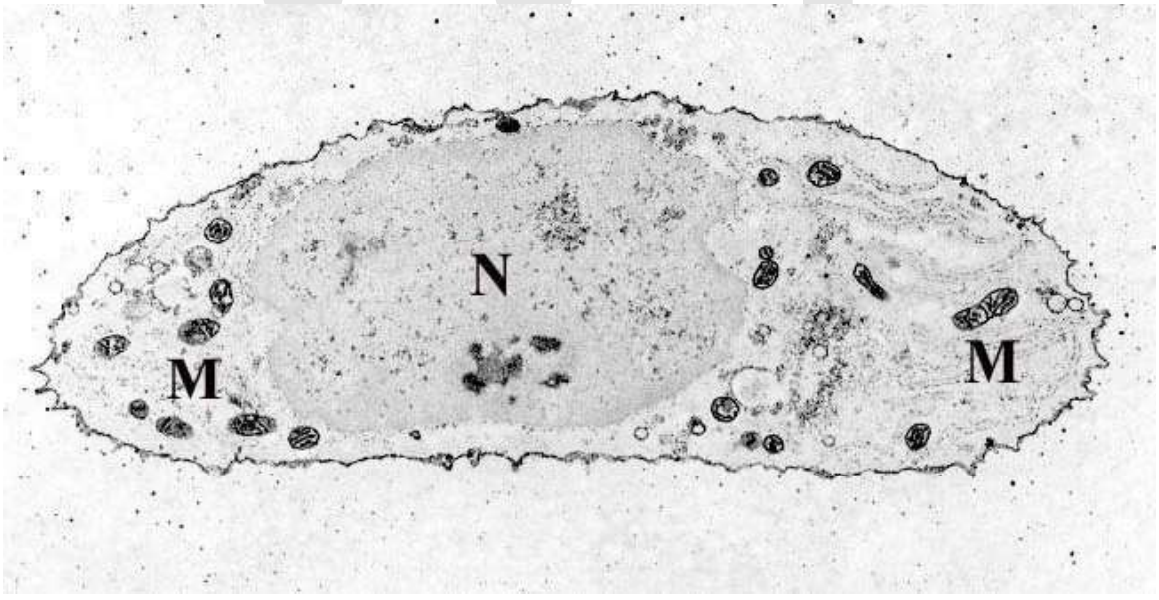
can cause oxidative stress in the mitochondria and may contribute to the decline in mitochondrial function associated with the aging process.

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.

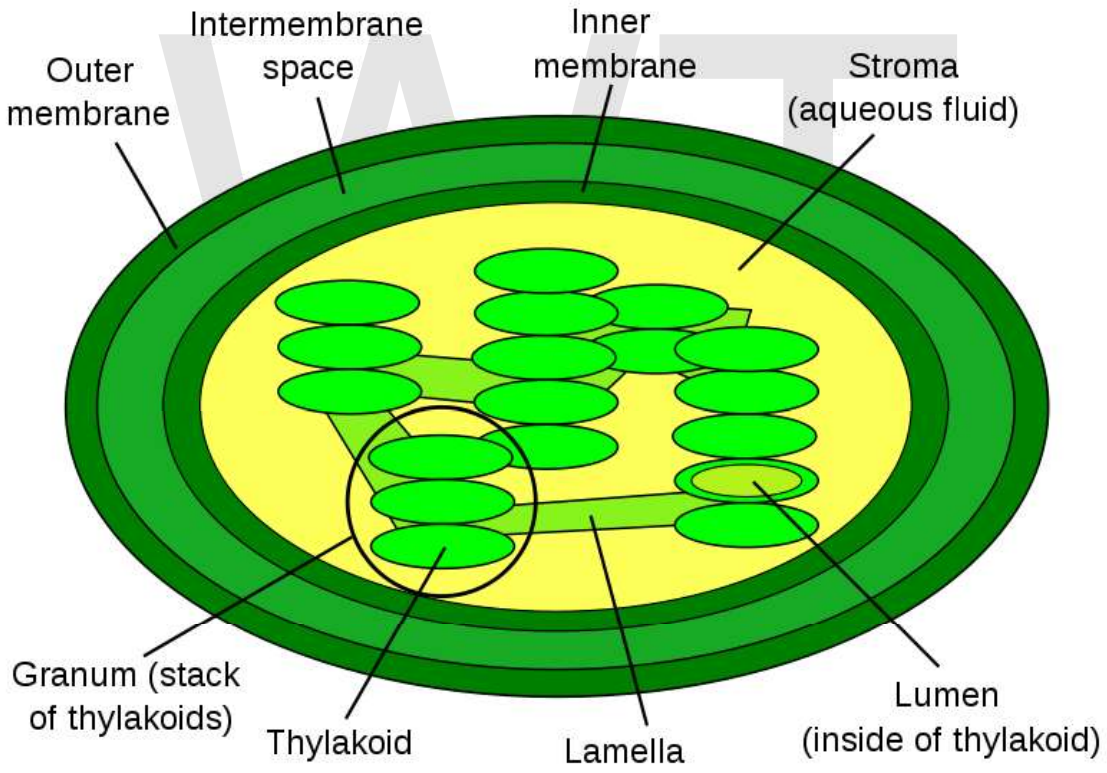
Possible relationships to aging

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- 4

Chloroplast and Lysosome

Chloroplast

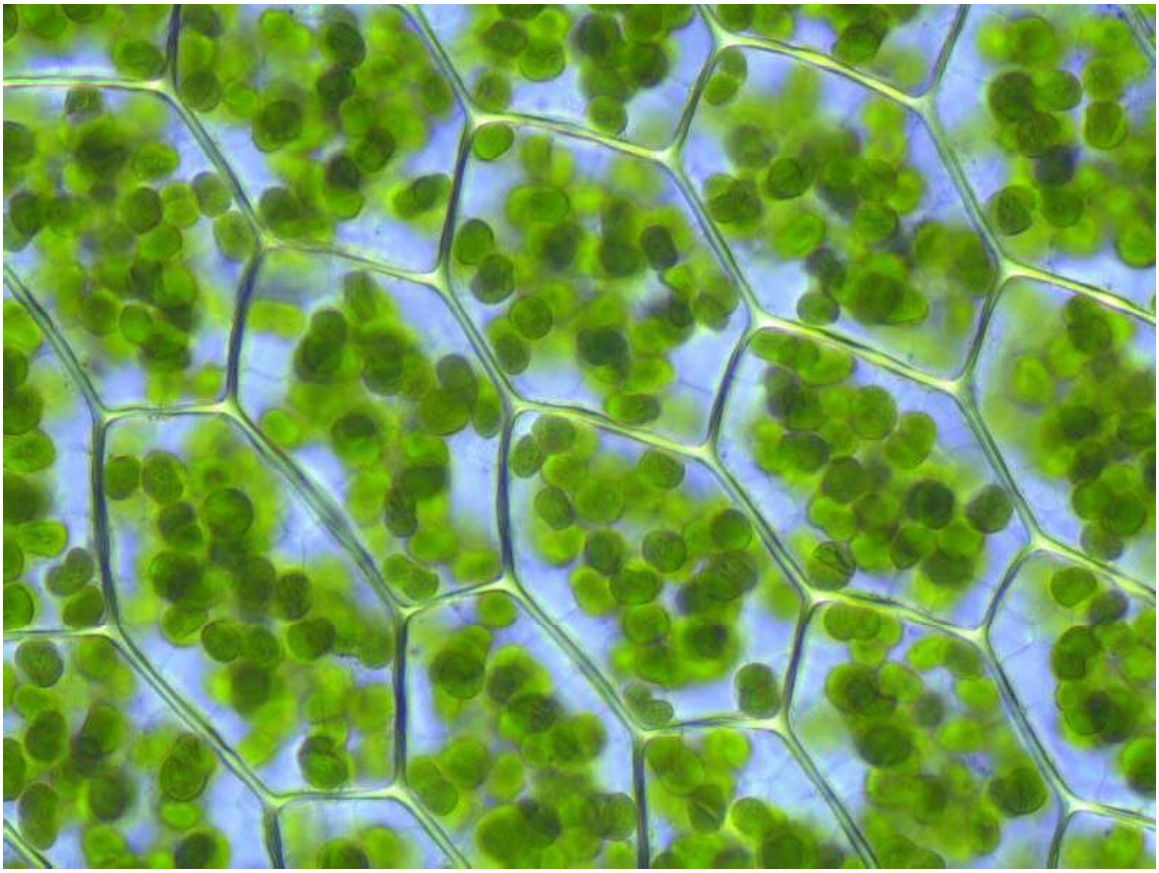


The simplified internal structure of a chloroplast

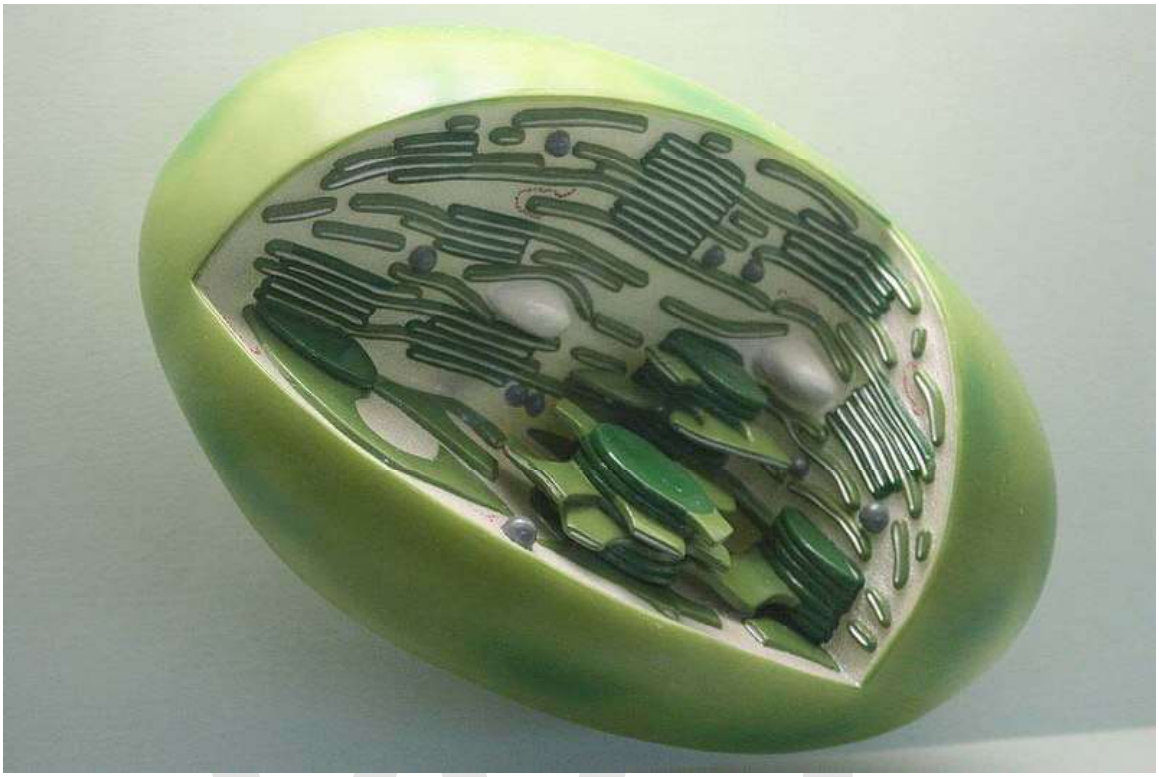
Chloroplasts are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

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.

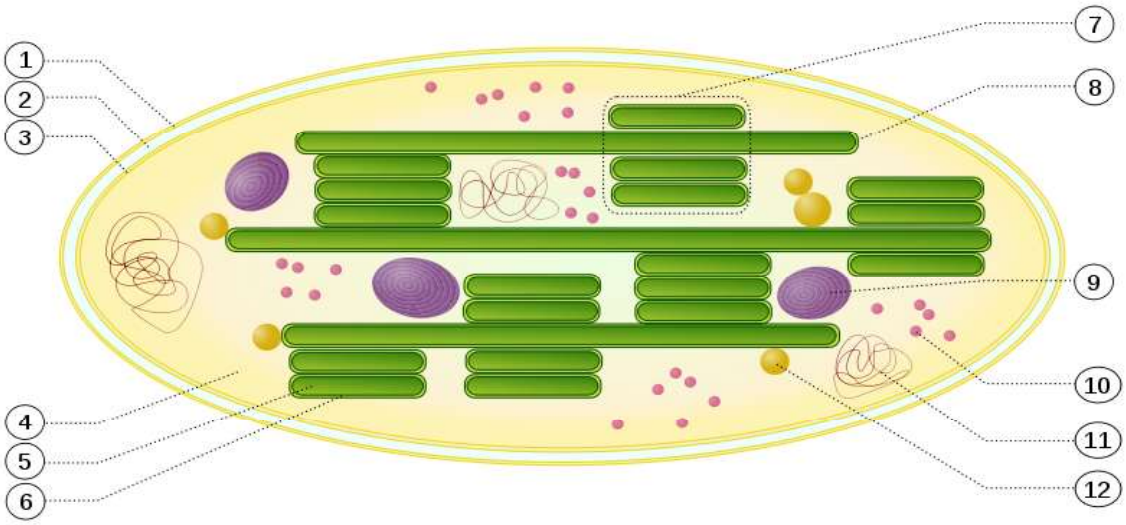
In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. They are believed to correspond to the outer and inner membranes of the ancestral cyanobacterium. Chloroplasts have their own genome, which is considerably reduced compared to that of free-living cyanobacteria, but the parts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

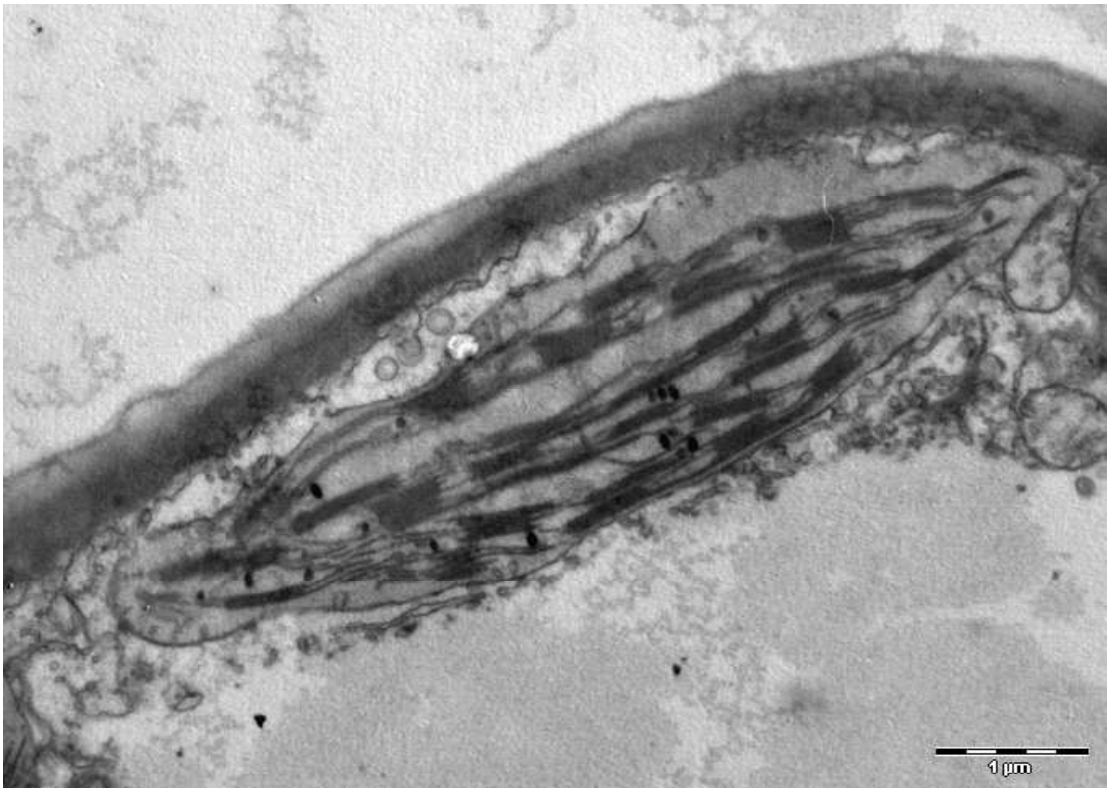
Chloroplasts are observable as flat discs usually 2 to 10 micrometers in diameter and 1 micrometer thick. In land plants, they are, in general, 5 μm in diameter and 2.3 μm thick. The chloroplast is contained by an envelope that consists of an inner and an outer phospholipid membrane. Between these two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

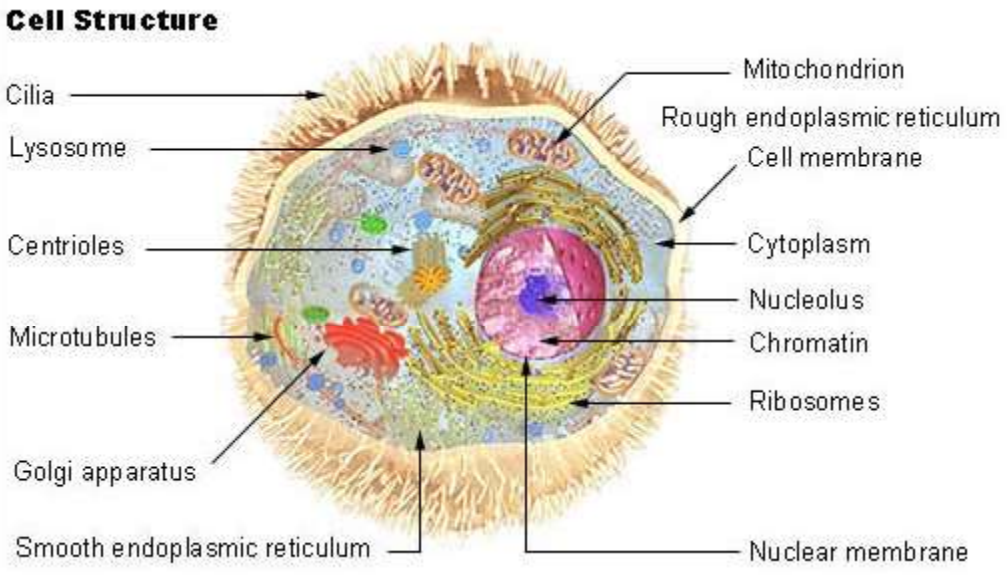
funneled to the reaction centre of this complex through resonance energy transfer. Two chlorophyll molecules are then ionised, producing an excited electron, which then passes onto the photochemical reaction centre.

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.

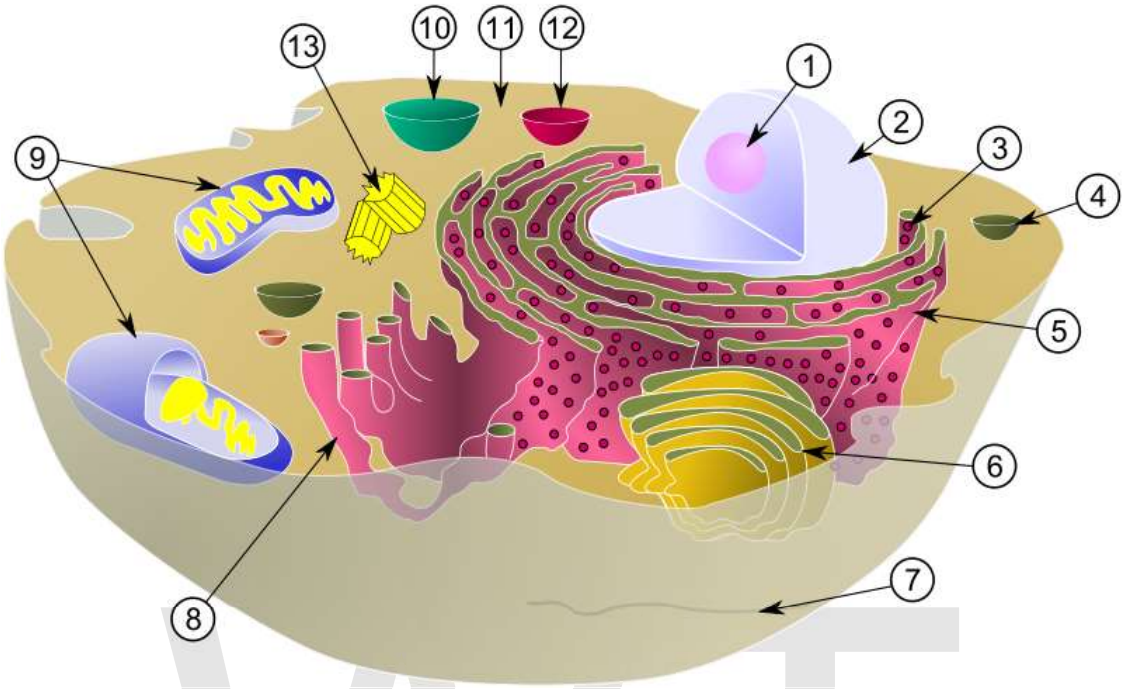
Transplastomic plants

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.

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

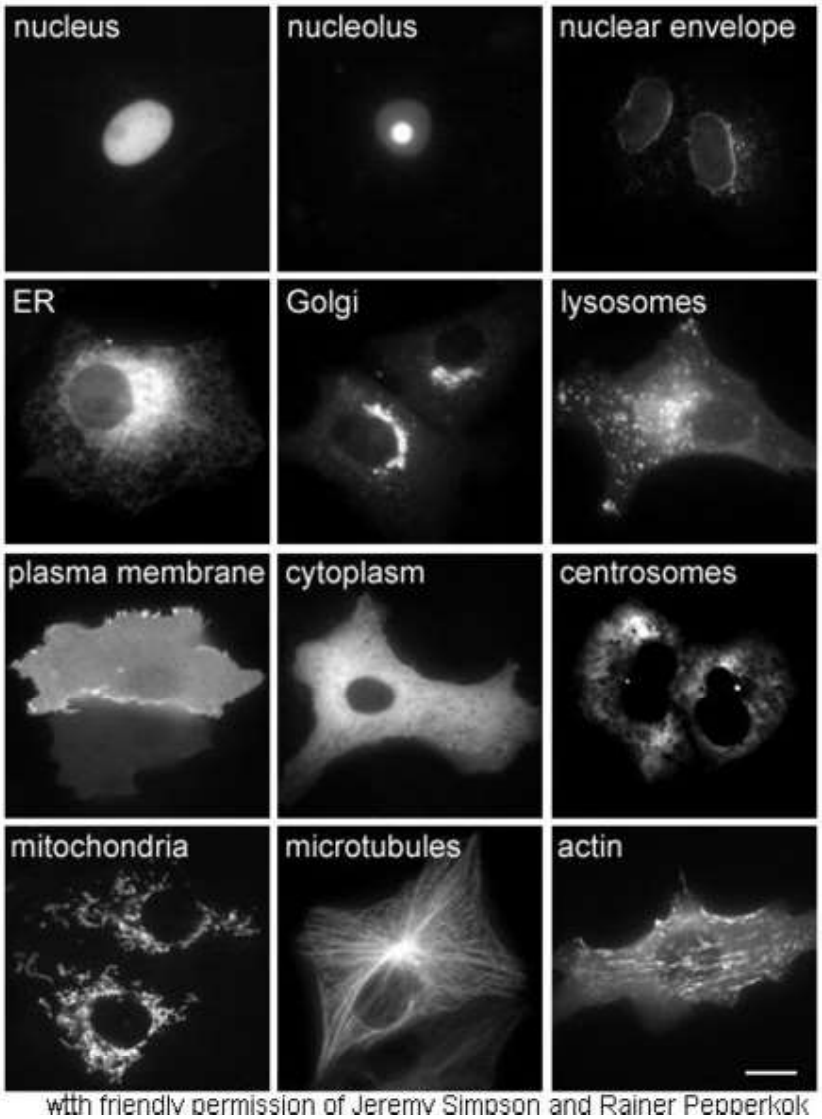
Lysosomes are the cell's waste disposal system and can break up anything. They digest almost everything. They are used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracellular material, like foreign invading microbes), endocytosis (where receptor proteins 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.

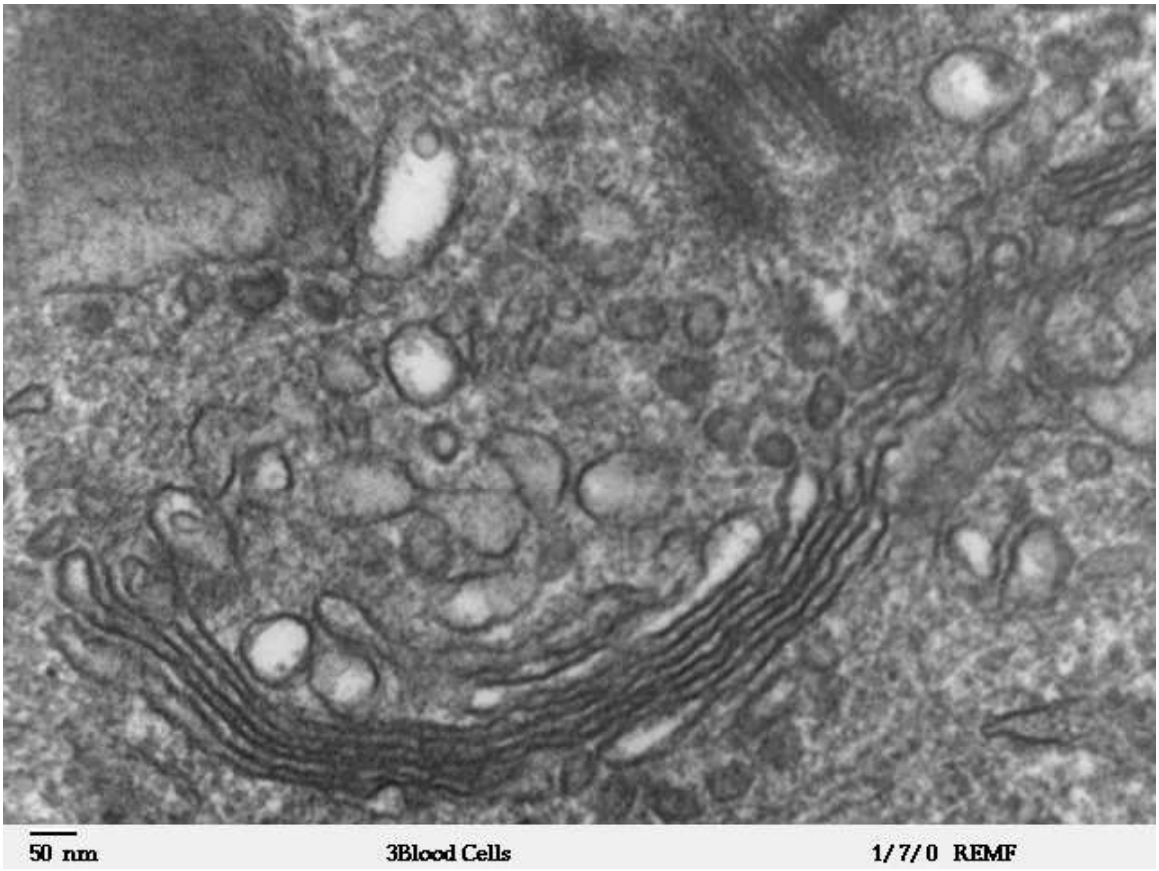


with friendly permission of Jeremy Simpson and Rainer Pepperkok

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

Chapter- 5

Golgi Apparatus



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

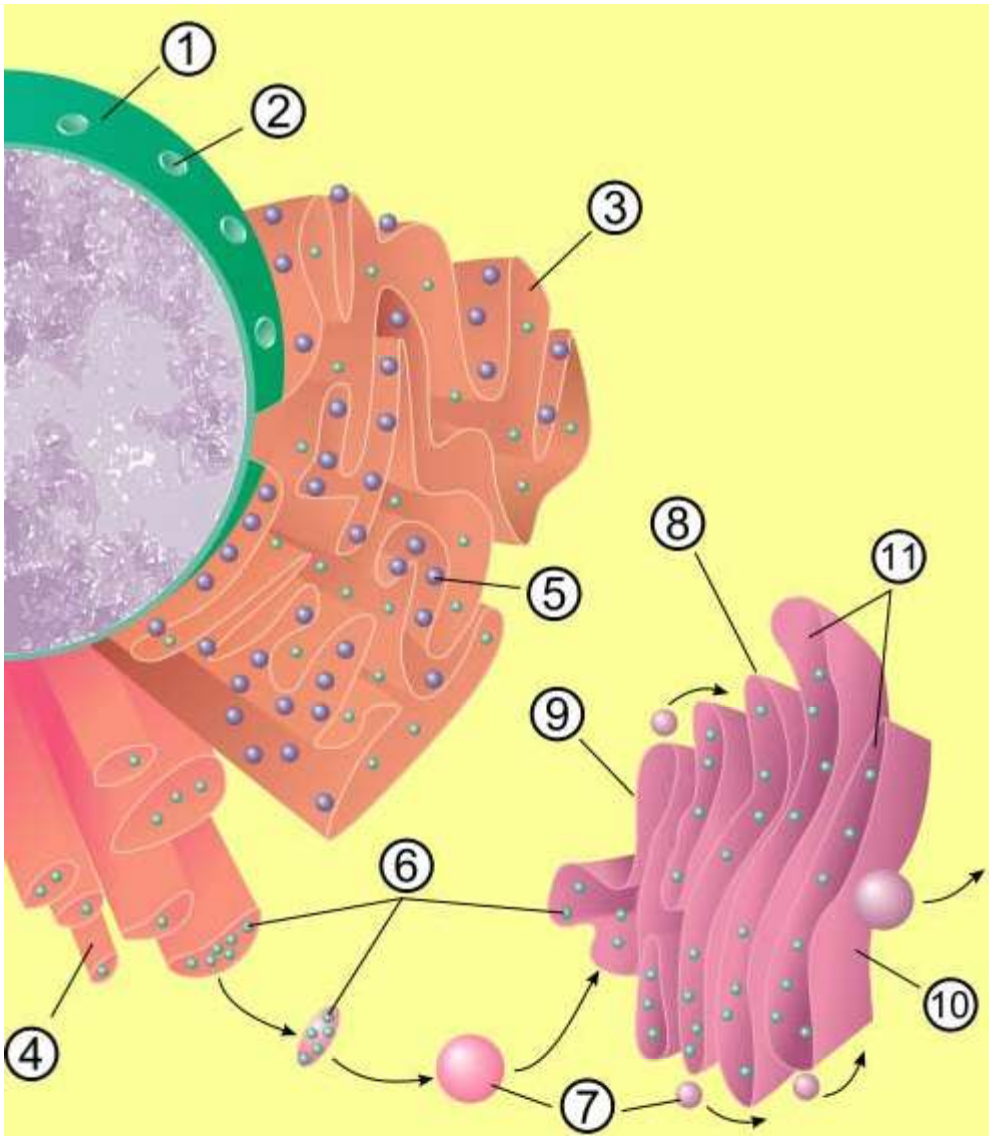


Diagram of secretory process from endoplasmic reticulum (orange) to Golgi apparatus (pink). 1. Nuclear membrane; 2. Nuclear pore; 3. Rough endoplasmic reticulum (RER); 4. Smooth endoplasmic reticulum (SER); 5. Ribosome attached to RER; 6. Macromolecules; 7. Transport vesicles; 8. Golgi apparatus; 9.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

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

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

Evolution

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

Discovery

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

Structure

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

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

Function

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

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

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

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

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

Vesicular transport

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

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

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

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

Transport mechanism

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

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

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

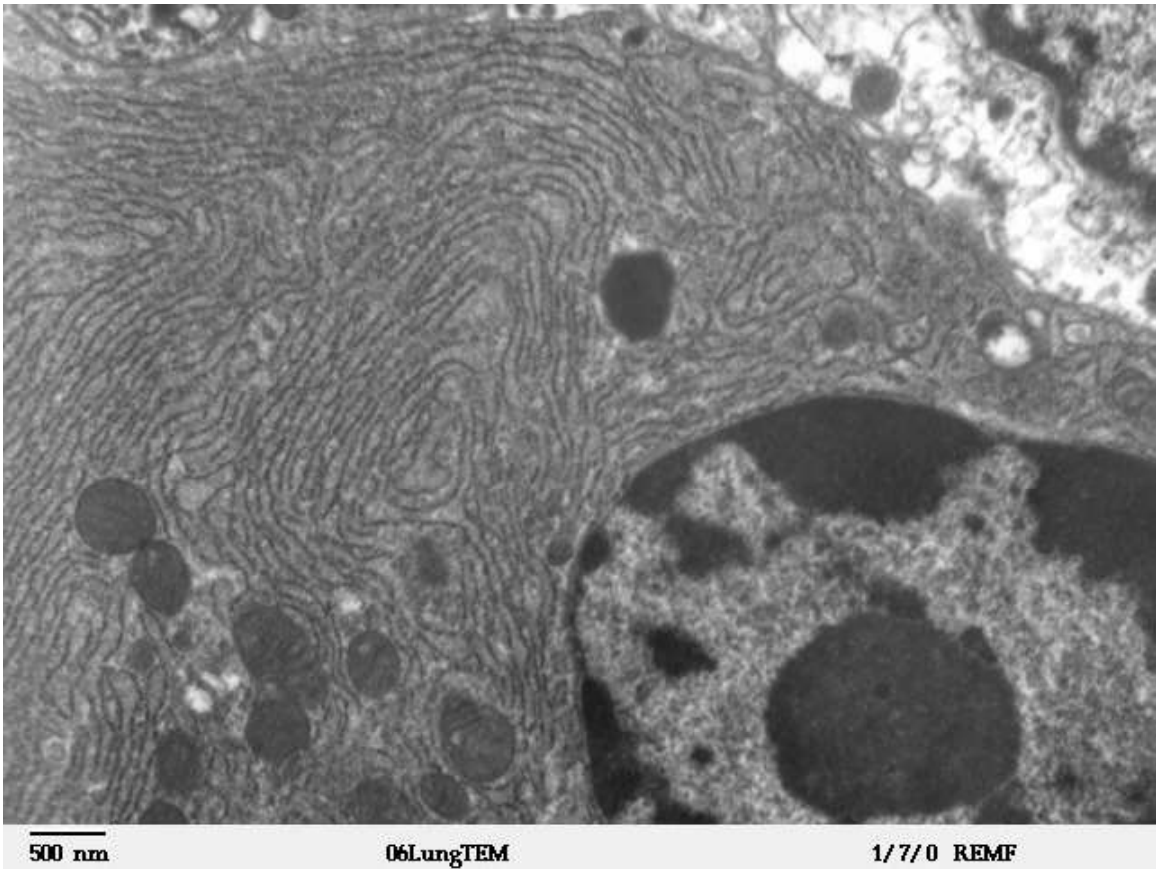
- ***Vesicular transport model***: Vesicular transport views the Golgi as a very stable organelle, divided into compartments in the cis to trans direction. Membrane bound carriers transport material between the ER and the different compartments of the Golgi. Experimental evidence includes the abundance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

Golgi apparatus during mitosis

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

Chapter- 6

Endoplasmic Reticulum

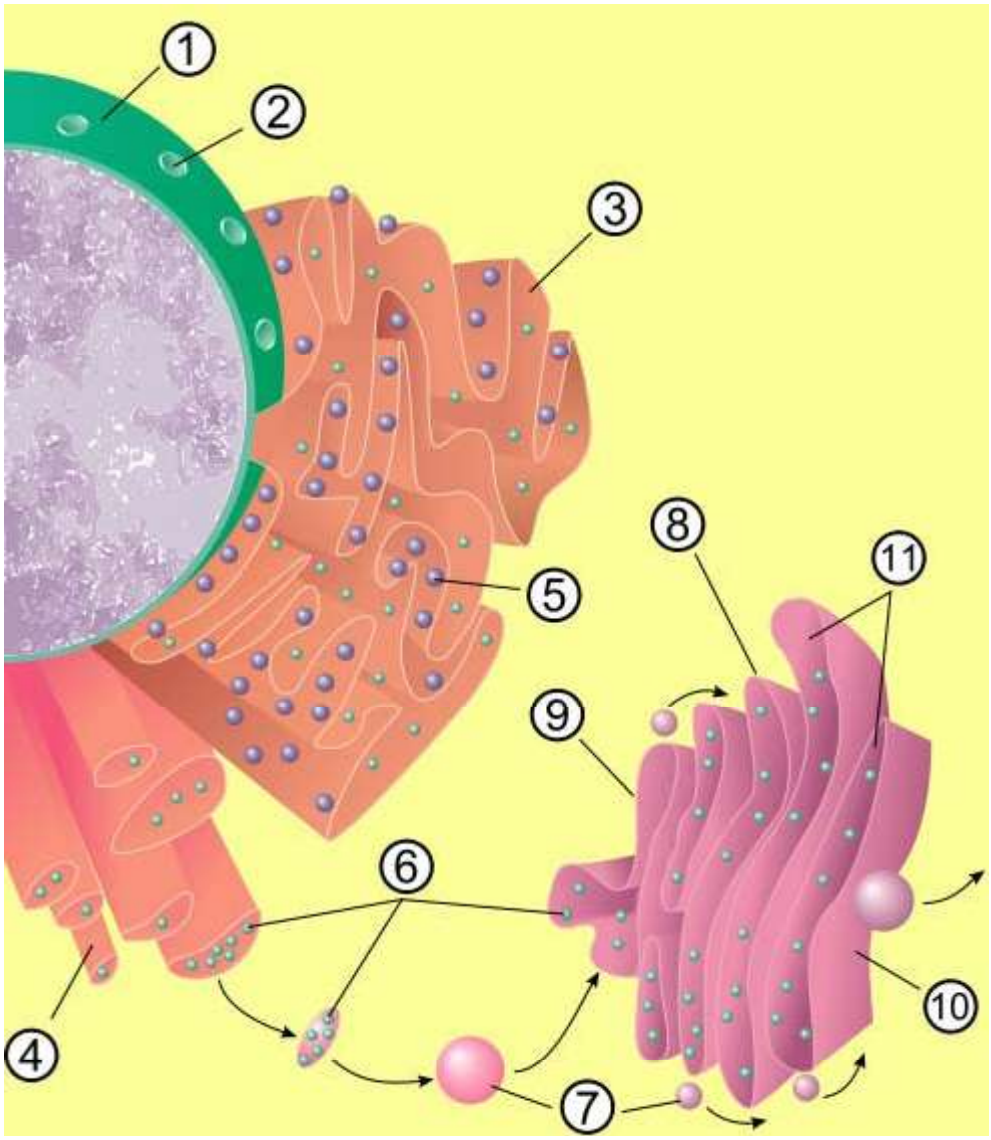


Micrograph of rough endoplasmic reticulum network around the nucleus (shown in lower right-hand side of the picture). Dark small circles in the network are mitochondria.

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.

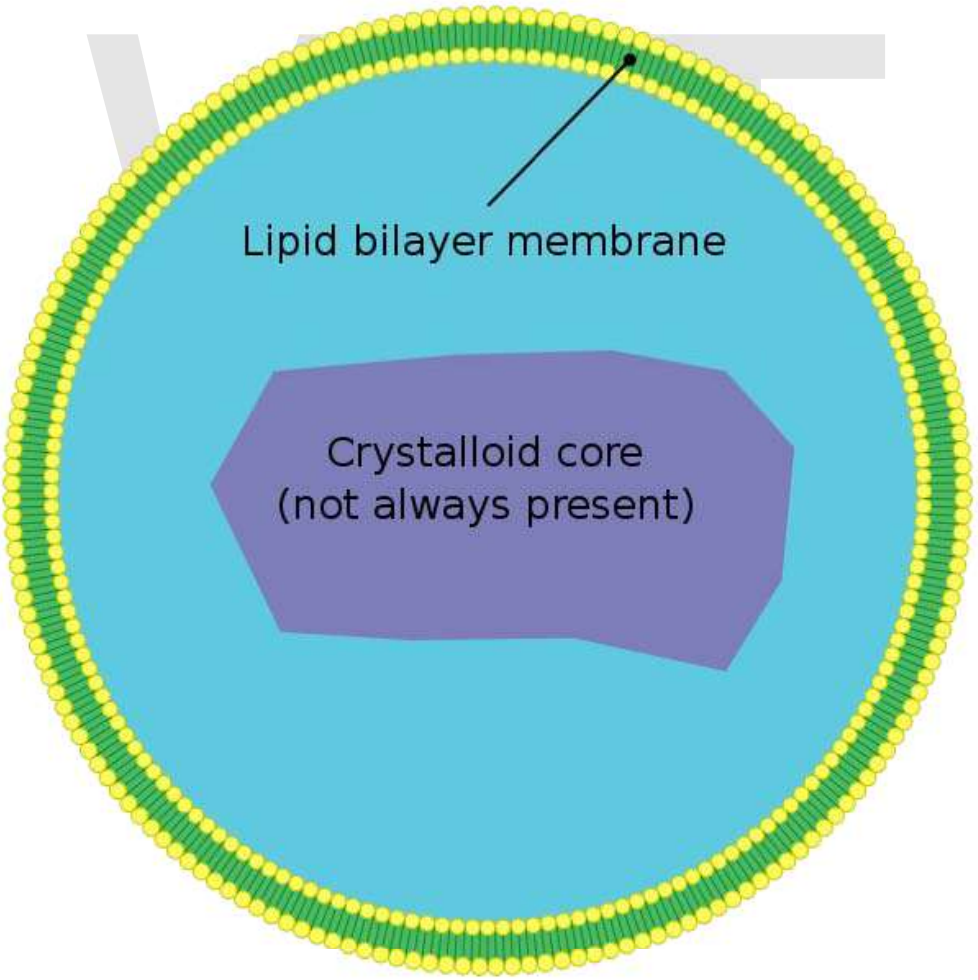
Other functions

- **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- 7

Peroxisome and Vacuole

Peroxisome



Basic structure of a peroxisome

Peroxisomes'also called microbodies' are organelles found in virtually all eukaryotic cells. They are involved in the catabolism of very long chain fatty acids, branched chain fatty acids, D-amino acids, polyamines, and biosynthesis of plasmalogens, etherphospholipids critical for the normal function of mammalian brains and lungs. They also contain approximately 10% of the total activity of two enzymes in the pentose phosphate pathway, which is important for energy metabolism. It is rigorously debated if peroxisomes are involved in isoprenoid and cholesterol synthesis in animals. Other known peroxisomal functions include the glyoxylate cycle in germinating seeds ("glyoxysomes"), photorespiration in leaves, glycolysis in trypanosomes ("glycosomes"), and methanol and/or amine oxidation and assimilation in some yeasts.

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

Metabolic functions

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

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

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

$$\mathrm{RH_2 + O_2 \rightarrow R + H_2O_2}$$

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

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

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

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

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

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

Peroxisome assembly

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

Associated medical conditions

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

Genes

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

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

Evolutionary origins

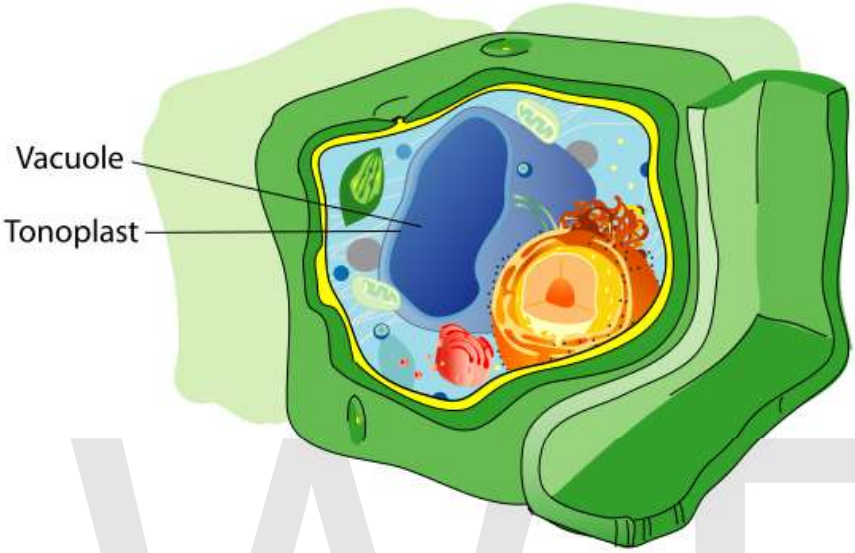
The protein content of peroxisomes varies across species, but the presence of proteins common to many species has been used to suggest an endosymbiotic origin; that is, peroxisomes evolved from bacteria that invaded larger cells as parasites, and very gradually evolved a symbiotic relationship. However,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

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

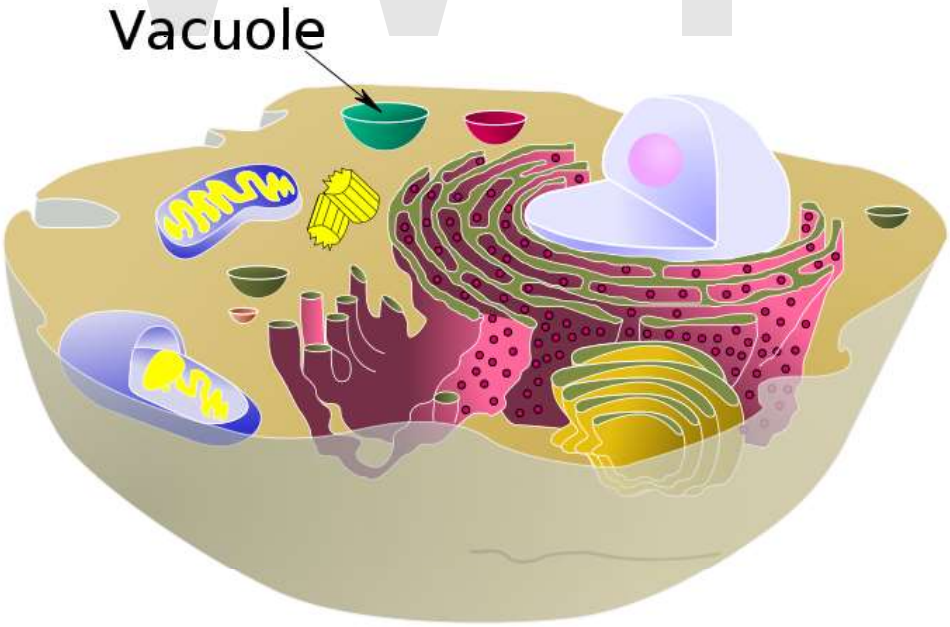
Other related organelles

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

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:

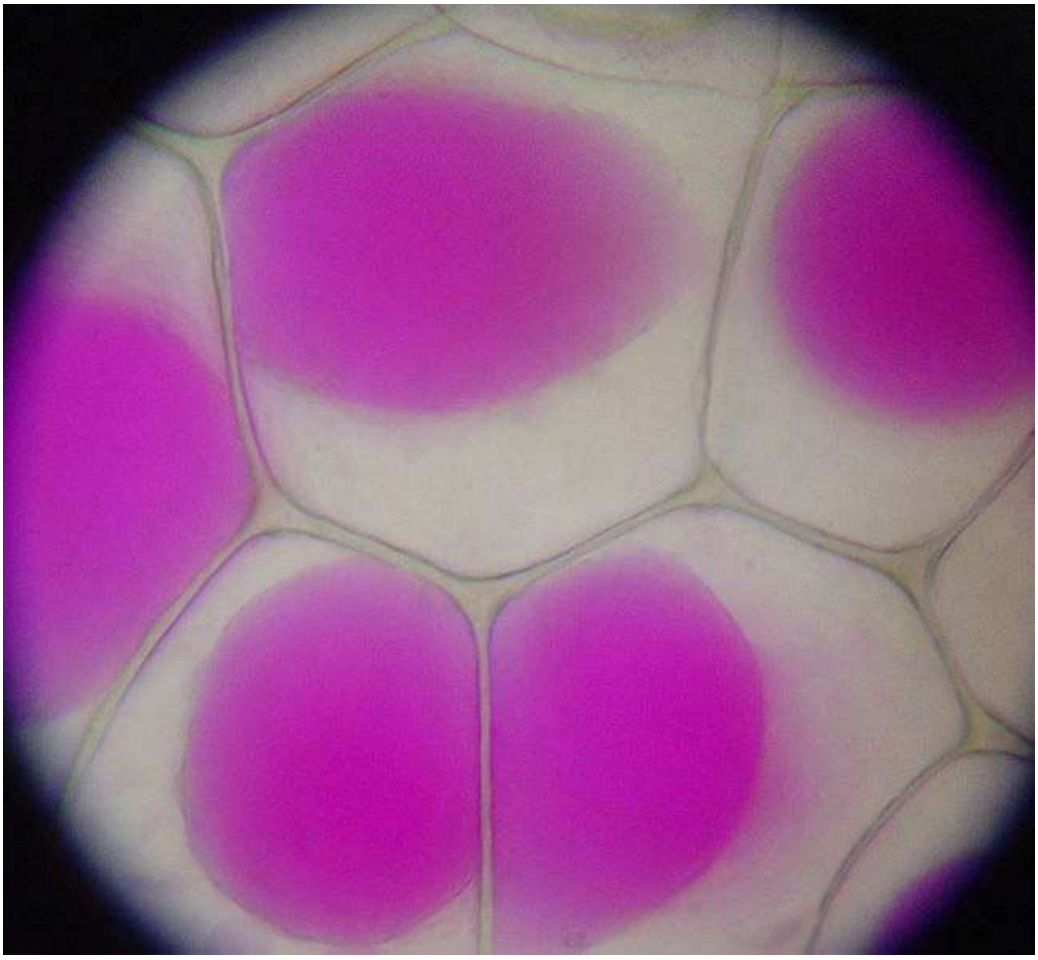
- Isolating materials that might be harmful or a threat to the cell
- Containing waste products
- Containing water in plant cells
- Maintaining internal hydrostatic pressure or turgor within the cell
- Maintaining an acidic internal pH
- Containing small molecules
- Exporting unwanted substances from 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.

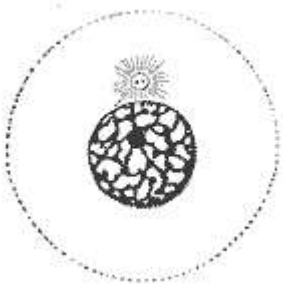
Exocytosis is the extrusion process of proteins and lipids from the cell. These materials are absorbed into secretory granules within the Golgi apparatus before being transported to the cell membrane and secreted into the extracellular environment. In this capacity, vacuoles are simply storage vesicles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

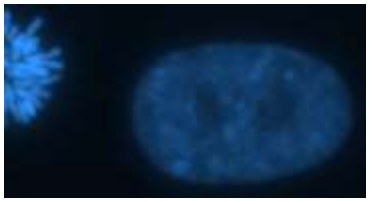
Chapter- 8

Interphase and Fission (Biology)

Interphase



An illustration of interphase. The chromatin has not yet condensed, and the cell is undergoing its normal functions.



An image of the nucleus of a cell (HT1080) currently in interphase (probably G1). Note: Cytoplasm of this cell or the neighboring cell is not visible (top-left), which is currently in the telophase of mitosis. Image taken using an optical microscope and DAPI staining of DNA.

Interphase is the phase of the cell cycle in which the cell spends the majority of its time and performs the majority of its purposes including preparation for cell division. In preparation for cell division, it increases its size and makes a copy of its DNA. Interphase is also considered to be the 'living' phase of the cell, in which the cell obtains nutrients, grows, reads its DNA, and conducts other "normal" cell functions. The majority of eukaryotic cells spend most of their time in interphase. Interphase does not describe a cell that is merely resting but is rather an active preparation for cell division. A common

misconception is that interphase is the first stage of mitosis. However, since mitosis is the division of the nucleus, prophase is actually the first stage.

In interphase, the cell gets itself ready for mitosis or meiosis. Somatic cells, or normal diploid cells of the body, go through mitosis in order to reproduce themselves through cell division, whereas diploid germ cells (i.e., primary spermatocytes and primary oocytes) go through meiosis in order to create haploid gametes (i.e., sperm and ova) for the purpose of sexual reproduction.

Identification

Under a microscope, prophase can be recognized because the nuclear membrane is still intact, the chromatin has not yet condensed, and chromosomes are not visible, though the nucleolus may be visible as an enlarged dark spot. The centrioles and spindle fibers are also not yet visible, though the centrosome, which contains and organizes them, may be visible near the nucleus.

Stages of interphase

There are three stages of interphase, with each phase ending when a cellular checkpoint checks the accuracy of the stage's completion before proceeding to the next. The stages of interphase are:

- G_1 (Gap 1), in which the cell grows and functions normally. During this time, much protein synthesis occurs and the cell grows (to about double its original size) - more organelles are produced, increasing the volume of the cytoplasm. If the cell is not to divide again, it will remain in this phase.

- Synthesis (S), in which the cell duplicates its DNA (via semiconservative replication). This is also known as the Swanson phase.

- G_2 (Gap 2), in which the cell resumes its growth in preparation for mitosis.

- In addition, some cells that do not divide often or ever, enter a stage called G_0 (Gap zero), which is either a stage separate from interphase or an extended G_1 phase, which follows the restriction point, a cell cycle checkpoint found at the end of G_1.

The duration of time spent in interphase and in each stage of interphase is variable and depends on both the type of cell and the species of organism it belongs to. Most cells of adult mammals spend about 20 hours in interphase, this accounts for about 90% of the total time involved in cell division.

Interphase within sequences of cellular processes

Interphase and the cell cycle

When G_2 is completed, the cell enters a relatively brief period of nuclear and cellular division, composed of mitosis and cytokinesis, respectively. After the successful completion of mitosis and cytokinesis, both resulting daughter cells re-enter G_1 of interphase.

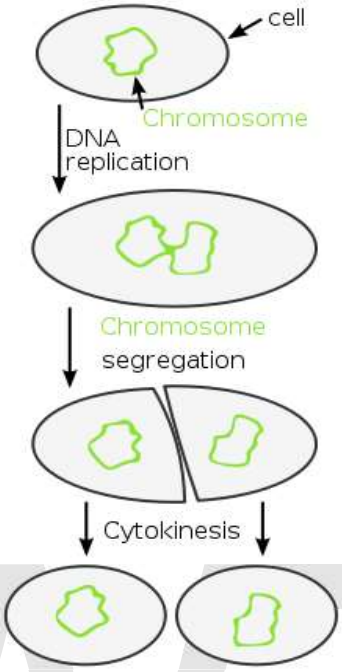
In the cell cycle, interphase is proceeded by telophase and cytokinesis of the M phase. In alternative fashion, interphase is sometimes interrupted by G_0 phase, which, in some circumstances, may then end and be followed by the remaining stages of interphase. After the successful completion of the G_2 checkpoint, the final checkpoint in interphase, the cell proceeds to prophase, or in plants to preprophase, which is the first stage of mitosis.

G_0 phase is viewed as either an extended G_1 phase where the cell is neither dividing nor preparing to divide and or as a distinct quiescent stage which occurs outside of the cell cycle.

Interphase and other cellular processes

In gamete production interphase is succeeded by meiosis. In programmed cell death, interphase is followed or preempted by apoptosis.

Fission (biology)

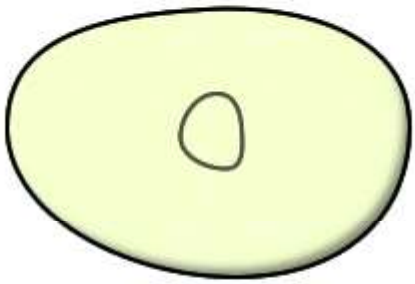


Binary fission

In biology, **fission** is the subdivision of a body, population, or species into parts and the regeneration of those parts into separate individuals. **Binary fission**, or **prokaryotic fission**, is a form of asexual reproduction and cell division used by all prokaryotes, some protozoa, and some organelles within eukaryotic organisms. This process results in the reproduction of a living prokaryotic cell by division into two parts which each have the potential to grow to the size of the original cell.

Mitosis and cytokinesis are not the same as binary fission; specifically, binary fission cannot be divided into prophase, metaphase, anaphase, and telophase because prokaryotes have no nucleus and no centromeres. The ability of some multicellular animals, such as echinoderms and flatworms, to regenerate two whole organisms after having been cut in half, is also not the same as binary fission. Neither is vegetative reproduction of plants.

Process



Complete process of binary fission.

Binary fission begins with DNA replication. DNA replication starts from an origin of replication, which opens up into a replication bubble (note: prokaryotic DNA replication usually has only 1 origin of replication, whereas eukaryotes have multiple origins of replication). The replication bubble separates the DNA double strand, each strand acts as template for synthesis of a daughter strand by semiconservative replication, until the entire prokaryotic DNA is duplicated.

Each circular DNA strand then attaches to the cell membrane. The cell elongates, causing the DNA to separate.

Cell division in bacteria is controlled by the FtsZ, a collection of about a dozen proteins that collect around the site of division. There, they direct assembly of the division septum. The cell wall and plasma membrane starts growing transversely from near the middle of the dividing cell. This separates the parent cell into two nearly equal daughter cells, each having a nuclear body.

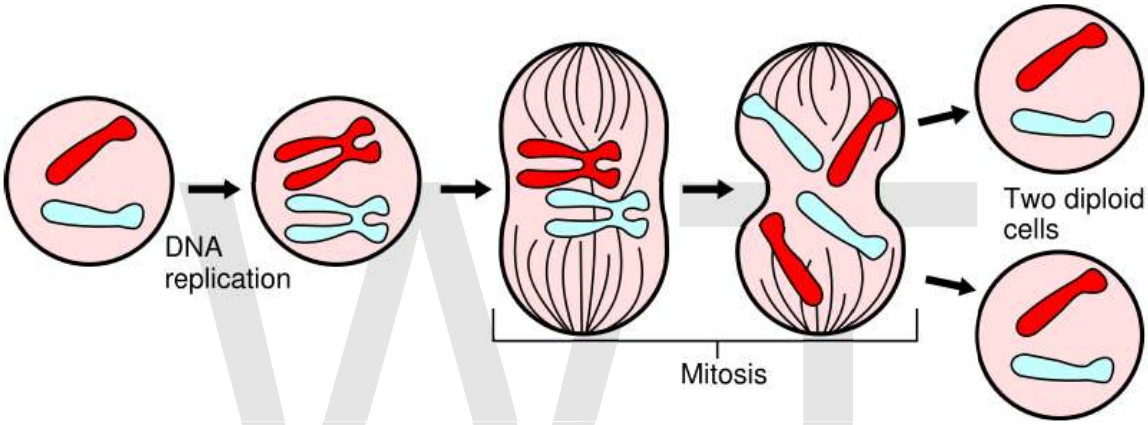
The cell membrane then invaginates (grows inwards) and splits the cell into two daughter cells, separated by a newly grown cell plate.

Use by eukaryotic organelles

Eukaryotic organelles such as mitochondria, chloroplasts, and peroxisomes also is not yet clear.

Chapter- 9

Cell Cycle

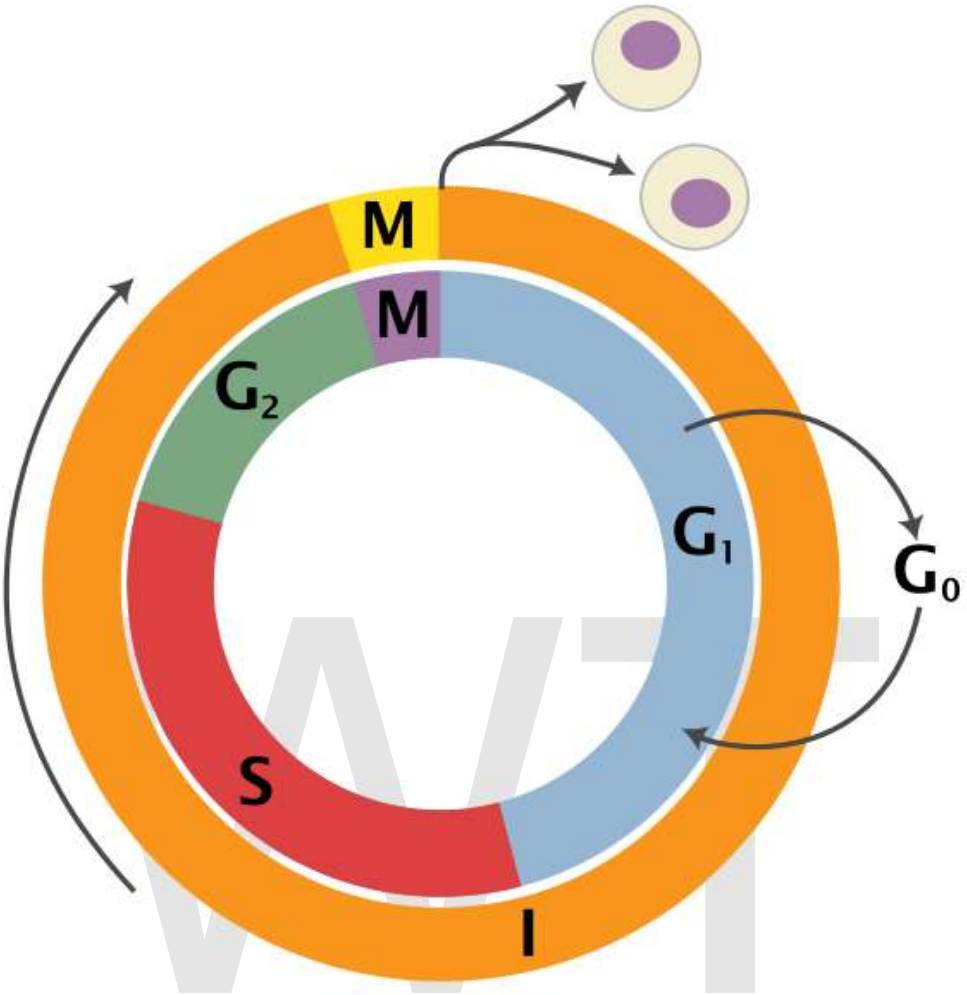


Each turn of the cell cycle divides the chromosomes in a cell nucleus.

The **cell cycle**, or **cell-division cycle**, is the series of events that takes place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two brief periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitosis (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells". The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed.

Phases

The cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase.



Schematic of the cell cycle. outer ring: I = Interphase, M = Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S = Synthesis; not in ring: G_0 = Gap 0/Resting.

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	**G_0**	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.

Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of cell division.

Resting (G_0 phase)

The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells in multicellular eukaryotes generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence is a state that occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

Before a cell can enter cell division, it needs to take in nutrients. All of the preparations are done during the interphase. Interphase proceeds in three stages, G1, S, and G2. Cell division operates in a cycle. Therefore, interphase is preceded by the previous cycle of mitosis and cytokinesis.

G_1 phase

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species.

S phase

The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled, though the ploidy of the cell remains the same. Rates of RNA transcription and protein synthesis are very low during this phase. An exception to this is histone production, most of which occurs during the S phase.

G_2 phase

The cell then enters the G_2 phase, which lasts until the cell enters mitosis. Again, significant biosynthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis. Inhibition of protein synthesis during G_2 phase prevents the cell from undergoing mitosis.

Mitosis (M Phase/Mitotic phase)

The relatively brief M phase consists of nuclear division (karyokinesis). The M phase has been broken down into several distinct phases, sequentially known as:

- prophase,
- metaphase,
- anaphase,
- telophase
- cytokinesis (strictly speaking, cytokinesis is not part of mitosis but is an event that directly follows mitosis in which cytoplasm is divided into two daughter cells)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle - the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

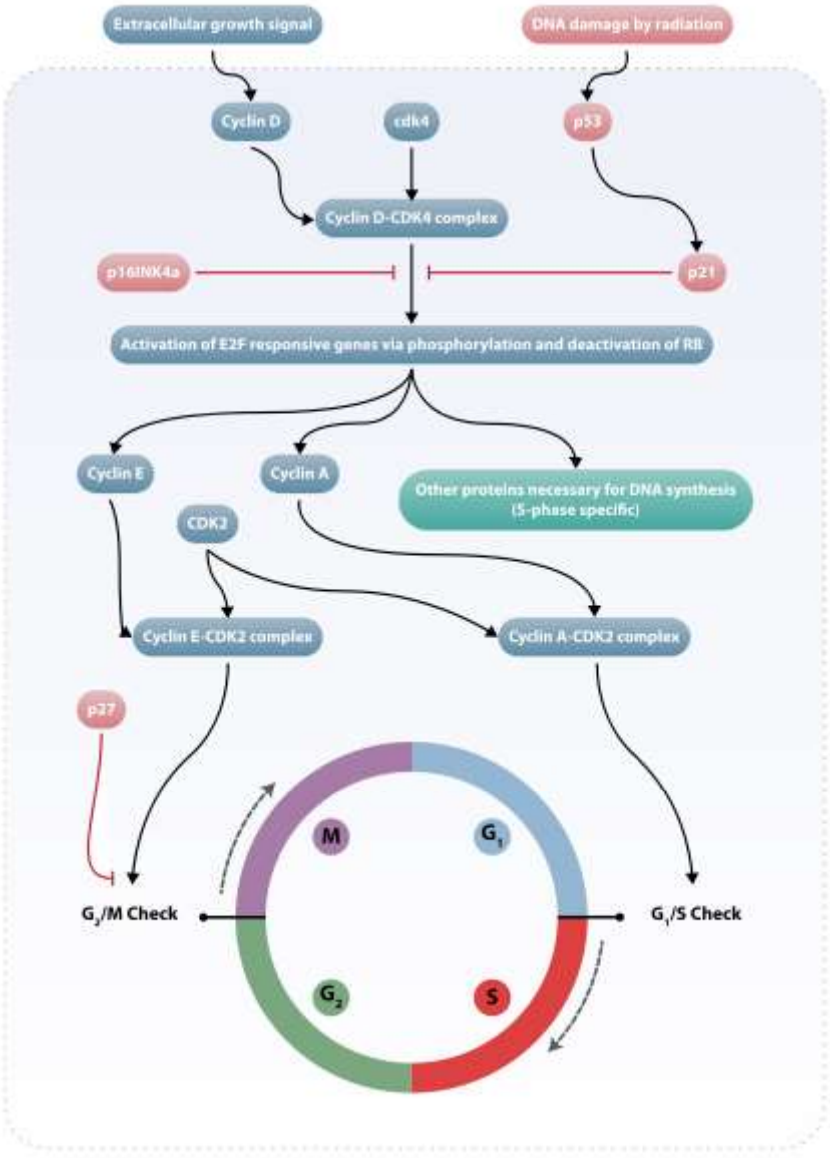
Mitosis occurs exclusively in eukaryotic cells, but occurs in different ways in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are prophase, prometaphase, metaphase, anaphase and telophase. During the process of mitosis the pairs of chromosomes condense and attach to fibers that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "M phase". However, there are many cells where mitosis and cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain

stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Regulation of eukaryotic cell cycle



Regulation of cell cycle: Schematic

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Role of cyclins and CDKs

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse won the 2001 Nobel Prize in Physiology or Medicine for their discovery of these central molecules. Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially *Saccharomyces cerevisiae*; genetic nomenclature in yeast dubs many these genes *cdc* (for "cell division cycle") followed by an identifying number, e.g., *cdc25* or *cdc20*.

Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.

General mechanism of cyclin-CDK interaction

Upon receiving a pro-mitotic extracellular signal, G_1 cyclin-CDK complexes become active to prepare the cell for S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and of enzymes required for DNA replication. The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S phase inhibitors by targeting them for ubiquitination. Once a protein has been ubiquitinated, it is targeted for proteolytic degradation by the proteasome.

Active S cyclin-CDK complexes phosphorylate proteins that make up the pre-replication complexes assembled during G_1 phase on DNA replication origins. The phosphorylation serves two purposes: to activate each already-assembled pre-replication complex, and to prevent new complexes from forming. This ensures that every portion of the cell's genome will be replicated once and only once. The reason for prevention of gaps in replication is fairly clear, because daughter cells that are missing all or part of crucial genes will die. However, for reasons related to gene copy number effects, possession of extra copies of certain genes is also deleterious to the daughter cells.

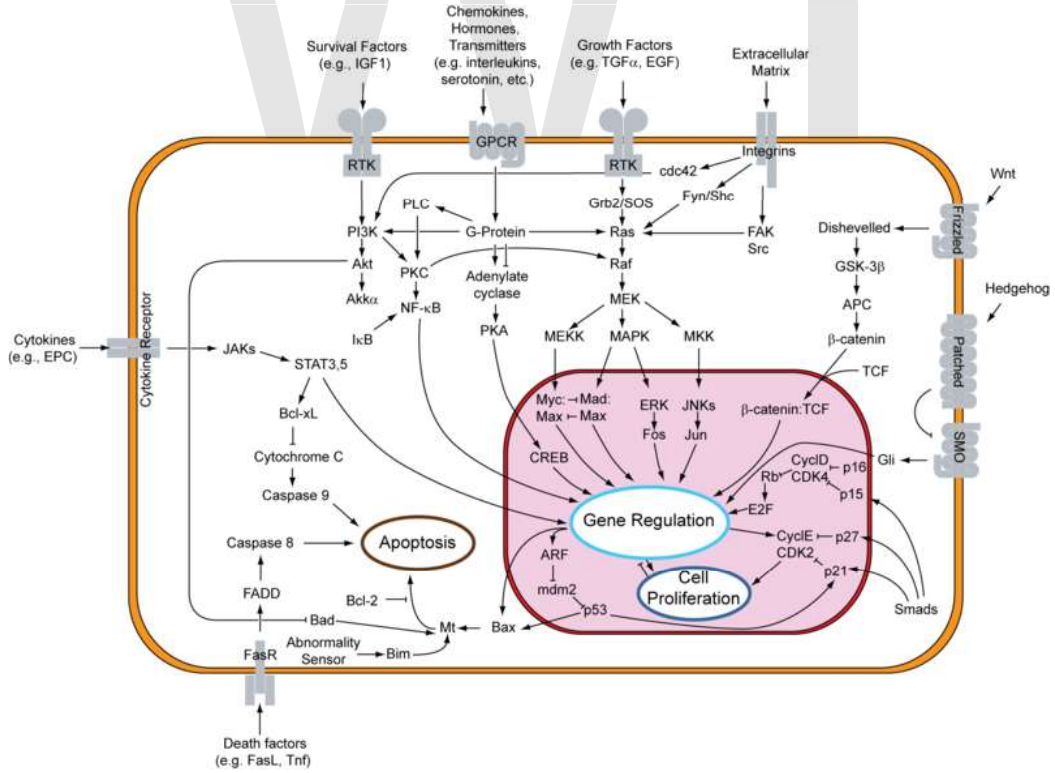
Mitotic cyclin-CDK complexes, which are synthesized but inactivated during S and G_2 phases, promote the initiation of mitosis by stimulating downstream proteins involved in chromosome condensation and mitotic spindle assembly. A critical complex activated during this process is a ubiquitin ligase known as the anaphase-promoting complex (APC), which promotes degradation of structural proteins associated with the chromosomal kinetochore. APC also targets the mitotic cyclins for degradation, ensuring that telophase and cytokinesis can proceed.

Interphase: Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G_0), Gap 1 (G_1), S (synthesis) phase, Gap 2 (G_2).

Specific action of cyclin-CDK complexes

Cyclin D is the first cyclin produced in the cell cycle, in response to extracellular signals (e.g. growth factors). Cyclin D binds to existing CDK4, forming the active cyclin D-CDK4 complex. Cyclin D-CDK4 complex in turn phosphorylates the retinoblastoma susceptibility protein (Rb). The hyperphosphorylated Rb dissociates from the E2F/DP1/Rb complex (which was bound to the E2F responsive genes, effectively "blocking" them from transcription), activating E2F. Activation of E2F results in transcription of various genes like cyclin E, cyclin A, DNA polymerase, thymidine kinase, etc. Cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex, which pushes the cell from G_1 to S phase (G_1/S transition). Cyclin B along with cdc2 (cdc2 - fission yeasts (CDK1 - mammalia)) forms the cyclin B-cdc2 complex, which initiates the G_2/M transition. Cyclin B-cdc2 complex activation causes breakdown of nuclear envelope and initiation of prophase, and subsequently, its deactivation causes the cell to exit mitosis.

Inhibitors



Overview of signal transduction pathways involved in apoptosis, also known as "programmed cell death".

Two families of genes, the *cip/kip* family and the INK4a/ARF (*In*hibitor of *K*inase 4/*A*lternative *R*eading *F*rame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The ***cip/kip* family** includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

The **INK4a/ARF family** includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p14arf which prevents p53 degradation.

Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.

Transcriptional Regulatory Network

Evidence suggests that a semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle. Several gene expression studies in *Saccharomyces cerevisiae* have identified approximately 800 to 1200 genes that change expression over the course of the cell cycle; they are transcribed at high levels at specific points in the cell cycle, and remain at lower levels throughout the rest of the cell cycle. While the set of identified genes differs between studies due to the computational methods and criterion used to identify them, each study indicates that a large portion of yeast genes are temporally regulated.

Many periodically expressed genes are driven by transcription factors that are also periodically expressed. One screen of single-gene knockouts identified 48 transcription factors (about 20% of all non-essential transcription factors) that show cell cycle progression defects. Genome-wide studies using high throughput technologies have identified the transcription factors that bind to the promoters of yeast genes, and correlating these findings with temporal expression patterns have allowed the identification of transcription factors that drive phase-specific gene expression. The expression profiles of these transcription factors are driven by the transcription factors that peak in the prior phase, and computational models have shown that a CDK-autonomous network of these transcription factors is sufficient to produce steady-state oscillations in gene expression).

Experimental evidence also suggests that gene expression can oscillate with the period seen in dividing wild-type cells independently of the CDK machinery. Orlando et. al. used microarrays to measure the expression of a set of 1,271 genes that they identified as periodic in both wild type cells and cells lacking all S-phase and mitotic cyclins (*clb1,2,3,4,5,6*). Of the 1,271 genes assayed, 882 continued to be expressed in the cyclin-deficient cells at the same time as in the wild type cells, despite the fact that the cyclin-deficient cells arrest at the border between G1 and S phase. However, 833 of the genes

assayed changed behavior between the wild type and mutant cells, indicating that these genes are likely directly or indirectly regulated by the CDK-cyclin machinery. Some genes that continued to be expressed on time in the mutant cells were also expressed at different levels in the mutant and wild type cells. These findings suggest that while the transcriptional network may oscillate independently of the CDK-cyclin oscillator, they are coupled in a manner that requires both to ensure the proper timing of cell cycle events. Other work indicates that phosphorylation, a post-translational modification, of cell cycle transcription factors by Cdk1 may alter the localization or activity of the transcription factors in order to tightly control timing of target genes (Ubersax et al 2003; Sidorova et al 1995; White et al 2009).

While oscillatory transcription plays a key role in the progression of the yeast cell cycle, the CDK-cyclin machinery operates independently in the early embryonic cell cycle. Before the midblastula transition, zygotic transcription does not occur and all needed proteins, such as the B-type cyclins, are translated from maternally loaded mRNA.

Checkpoints

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met.

Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G_1/S checkpoint and the G_2/M checkpoint. G_1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the postreplication checkpoint.

p53 plays an important role in triggering the control mechanisms at both G_1/S and G_2/M checkpoints.

Role in tumor formation

A disregulation of the cell cycle components may lead to tumor formation. As mentioned above, some genes like the cell cycle inhibitors, RB, p53 etc., when they mutate, may cause the cell to multiply uncontrollably, forming a tumor. Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G_0 phase) in tumors is much higher than that in normal tissue. Thus there is a net increase in cell number as the number of cells that die by apoptosis or senescence remains the same.

The cells which are actively undergoing cell cycle are targeted in cancer therapy as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. This fact is made use of in cancer treatment; by a process known as

debulking, a significant mass of the tumor is removed which pushes a significant number of the remaining tumor cells from G_0 to G_1 phase (due to increased availability of nutrients, oxygen, growth factors etc.). Radiation or chemotherapy following the debulking procedure kills these cells which have newly entered the cell cycle.

The fastest cycling mammalian cells in culture, crypt cells in the intestinal epithelium, have a cycle time as short as 9 to 10 hours. Stem cells in resting mouse skin may have a cycle time of more than 200 hours. Most of this difference is due to the varying length of G_1, the most variable phase of the cycle. M and S do not vary much.

In general, cells are most radiosensitive in late M and G_2 phases and most resistant in late S.

For cells with a longer cell cycle time and a significantly long G_1 phase, there is a second peak of resistance late in G_1

The pattern of resistance and sensitivity correlates with the level of sulfhydryl compounds in the cell. Sulfhydryls are natural radioprotectors and tend to be at their highest levels in S and at their lowest near mitosis.

Synchronization of cell cultures

Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G_1 phase, mitotic shake-off, treatment with colchicine and treatment with nocodazole halt the cell in M phase and treatment with 5-fluorodeoxyuridine halts the cell in S phase.

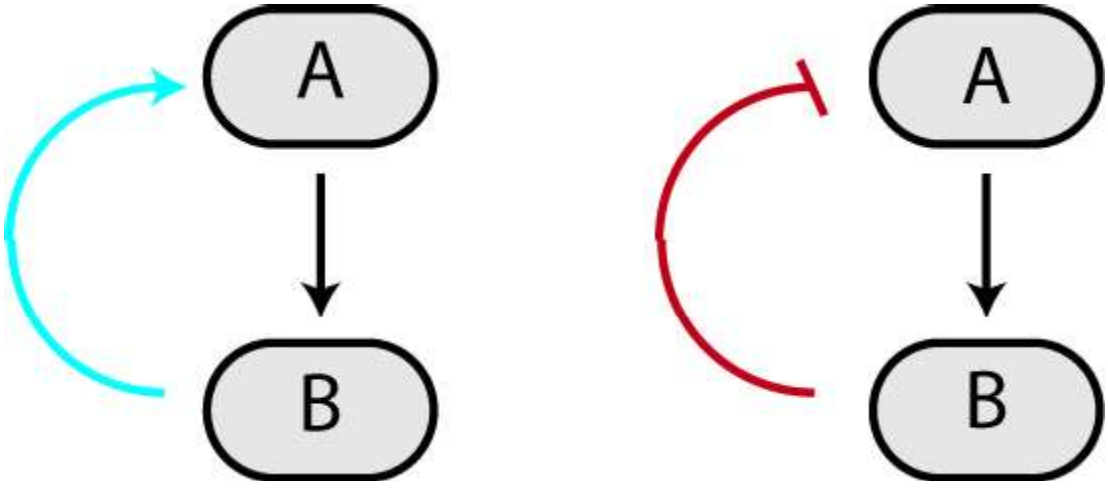
Chapter- 10

Biochemical Switches in the Cell Cycle

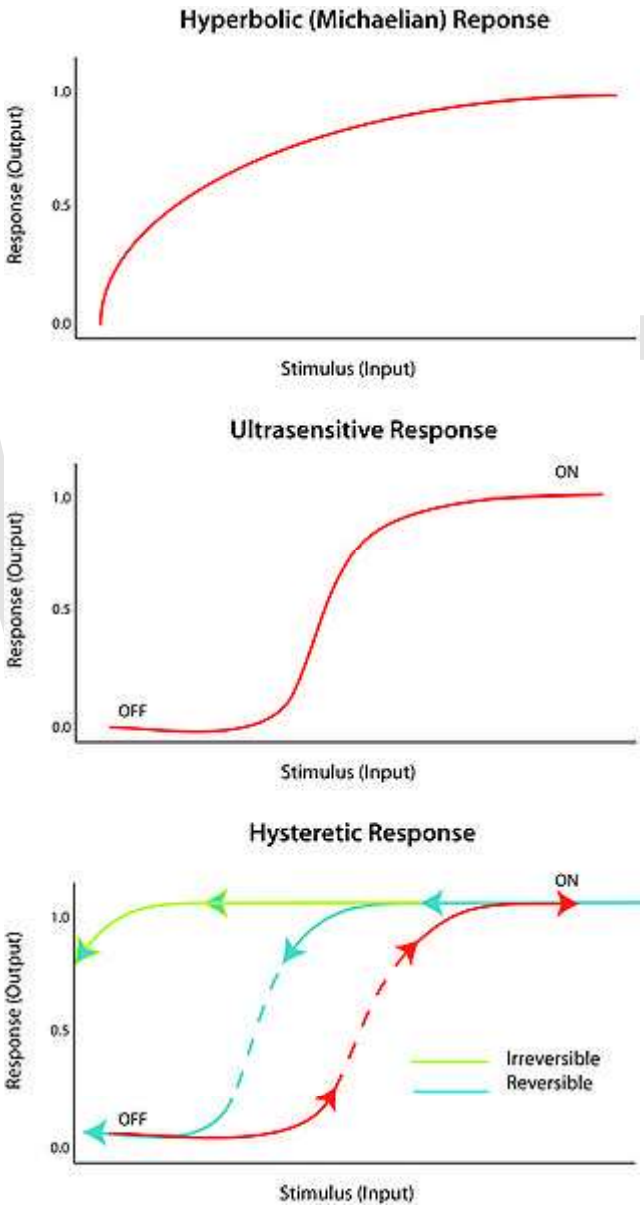
A series of **biochemical switches** control transitions between and within the various phases of the cell cycle. The cell cycle is a series of complex, ordered, sequential events that control how a single cell divides into two cells, and involves several different phases. The phases include the G1 and G2 phases, DNA replication or S phase, and the actual process of cell division, mitosis or M phase. During the M phase, the chromosomes separate and cytokinesis occurs.

The switches maintain the orderly progression of the cell cycle and act as checkpoints to ensure that each phase has been properly completed before progression to the next phase. For example, Cdk, or cyclin dependent kinase, is a major control switch for the cell cycle and it allows the cell to move from G1 to S or G2 to M by adding phosphate to protein substrates. Such multi-component (involving multiple inter-linked proteins) switches have been shown to generate decisive, robust (and potentially irreversible) transitions and trigger stable oscillations. As a result, they are a subject of active research that tries to understand how such complex properties are wired into biological control systems.

Feedback loops



Many biological circuits produce complex outputs by exploiting one or more feedback loops. In a sequence of biochemical events, feedback would refer to a downstream element in the sequence (B in the image on the left) affecting some upstream component (A in the image on the left) to affect its own production or activation (output) in the future. If this element acts to enhance its own output, then it engages in positive feedback (red arrow). A positive feedback loop is also known as a self-reinforcing loop, and it is possible that these loops can be part of a larger loop, as this is characteristic of regulatory circuits.



Conversely, if this element leads to its own inhibition through upstream elements, this is canonically negative feedback (blue arrow). A negative feedback loop is also known as a balancing loop, and it may be common to see oscillations in which a delayed negative feedback signal is used to maintain homeostatic balance in the system.

Feedback loops can be used for amplification (positive) or self-correction (negative). The right combination of positive and negative feedback loops can generate ultrasensitivity and bistability , which in turn can generate decisive transitions and oscillations.

Combination of Positive and Negative Feedback Loops

Positive and negative feedback loops do not always operate distinctly. In the mechanism of biochemical switches, they work together to create a flexible system. For example, according to Pfeuty et al., to overcome a drawback in biochemical systems, positive feedback regulation loops may interact with negative regulation loops to facilitate escape from stable states..The coexistence of two stable states is known as bistability, which is often the result of positive feedback regulations. An example that reveals the interaction of the multiple negative and positive feedback loops is the activation of cyclin-dependent protein kinases, or Cdks14. Positive feedback loops play a role by switching cells from low to high Cdk-activity. The interaction between the two types of loops is evident in mitosis. While positive feedback initiates mitosis, a negative feedback loop promotes the inactivation of the cyclin-dependent kinases by the anaphase-promoting complex. This example clearly shows the combined effects that positive and negative feedback loops have on cell-cycle regulation.

Ultrasensitivity

An "all-or-none" response to a stimulus is termed ultrasensitivity. In other words, a very small change in stimulus causes a very large change in response, producing a sigmoidal dose-response curve. An ultrasensitive response is described by the general equation V = S^n/S^n +Km, known as the Hill equation, when n, the Hill coefficient, is more than 1. The steepness of the sigmoidal curve depends on the value of n. A value of n = 1 produces a hyperbolic or Michaelian response. Ultrasensitivity is achieved in a variety of systems; a notable example is the cooperative binding of the enzyme Hemoglobin to its substrate. Since an ultrasensitive response is almost ‘digital’, it can be used to amplify a response to a stimulus or cause a decisive sharp transition (between ‘off’ and ‘on’ states).

Ultrasensitivity certainly plays a large role in cell-cycle regulation. For example, Cdk1 and Wee1 can mitotic regulators and they are able to inactivate each other through inhibitory phosphorylation. In essence, this represents a double negative feedback loop in which both regulators inactivate each other. According to Kim et al. (2007), there must be an ultra-sensitive element to generate a bistable response. It turns out that Wee1 has an ultrasensitive response to Cdk1, and this likely arises because of substrate competition among the various phosphorylation sites on Wee1.This example shows the role of ultrasensitivity in biochemical switches..

Bistability

Bistability implies hysteresis, and hysteresis implies multistability. Multistability indicates the presence of two or more stable states for a given input. Therefore, bistability

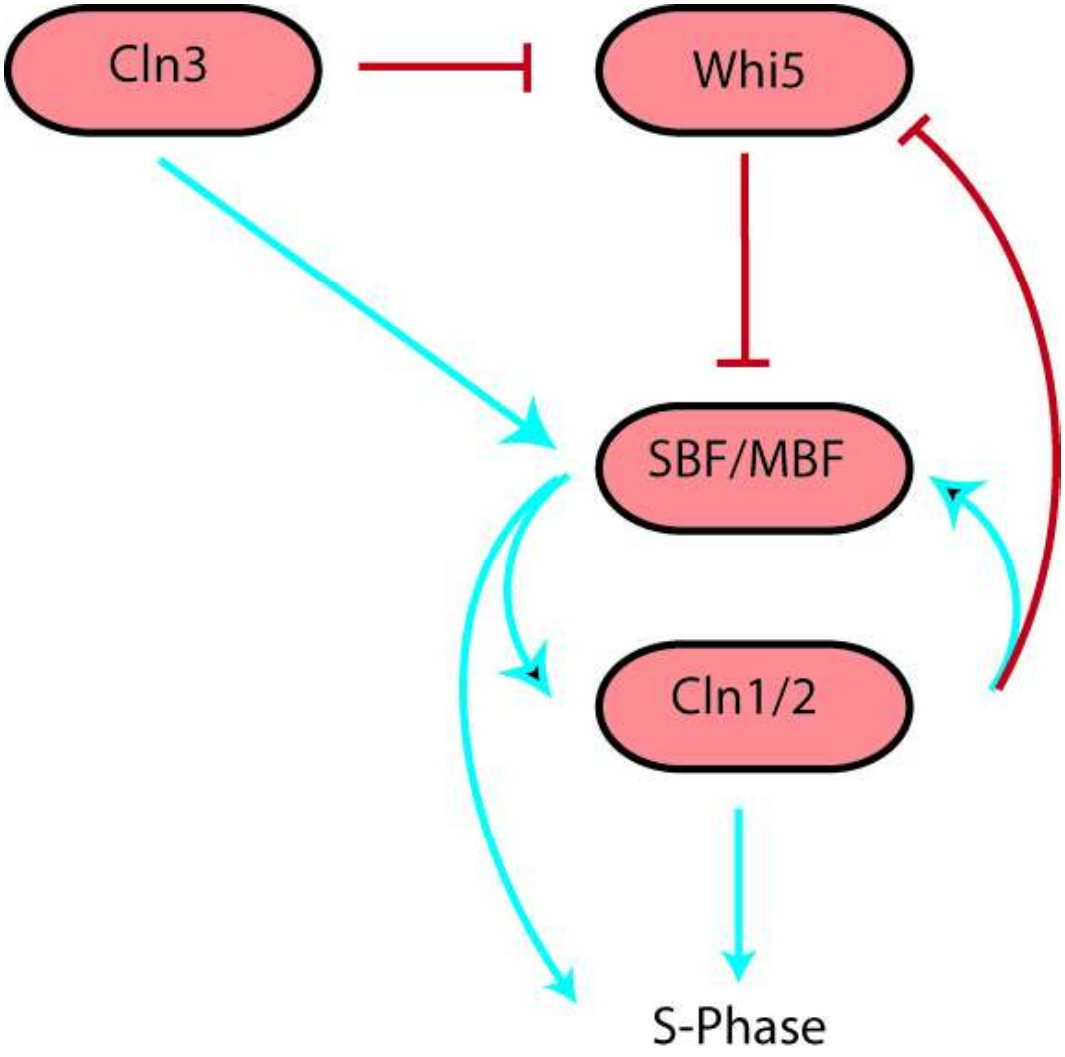
is the ability of a system to exist in two steady states. In other words, there is a range of stimulus values for which the response can have two steady-state values. Bistability is accompanied by hysteresis, which means that the system approaches one of the two steady states preferentially depending on its history. Bistability requires feedback as well as an ultrasensitive circuit element.

Under the proper circumstances, positive and negative feedback loops can provide the conditions for bistability; for example, by having positive feedback coupled to an ultrasensitive response element with the circuit. A hysteretic bistable system can act as a robust reversible switch because it is harder for the system to transition between 'on' and 'off' states (compared to the equivalent monostable ultransensitive response). The system could also be poised such that one of the transitions is physically unattainable; for example, no amount of reduction in the stimulus will return the system to the 'off'-state once it is already in the 'on' state. This would form a robust irreversible switch.

There is no one-to-one correspondence between network topology, since many networks have a similar input and output relationship. A network topology does not imply input or output, and similarly input or output does not imply network topology. It is for this reason that parameterization is very important for circuit function. If the dynamics of the input are comparable or faster than the response of the system, the response may appear hysteretic.

Three cell cycle switches are described below that achieve abrupt and/or irreversible transitions by exploiting some of the mechanisms described above.

The G1/S switch



The G1/S transition, more commonly known as the Start checkpoint in budding yeast (the restriction point in other organisms) regulates cell cycle commitment. At this checkpoint, cells either arrest before DNA replication (due to limiting nutrients or a pheromone signal), prolong G1 (size control), or begin replication and progress through the rest of the cell cycle. The G1/S regulatory network or regulon in budding yeast includes the G1 cyclins Cln1, Cln2 and Cln3, Cdc28 (Cdk1), the transcription factors SBF and MBF, and the transcriptional inhibitor Whi5. Cln3 interacts with Cdk1 to initiate the sequence of events by phosphorylating a large number of targets, including SBF, MBF and Whi5. Phosphorylation of Whi5 causes it to translocate out of the nucleus, preventing it from inhibiting SBF and MBF. Active SBF/MBF drive the G1/S transition by turning on the B-type cyclins and initiating DNA replication, bud formation and spindle body duplication. Moreover, SBF/MBF drives expression of Cln1 and Cln2, which can also interact with Cdk1 to promote phosphorylation of its targets.

This G1/S switch was initially thought to function as a linear sequence of events starting with Cln3 and ending in S phase. However, the observation that any one of the Clns was sufficient to activate the regulon indicated that Cln1 and Cln2 might be able to engage positive feedback to activate their own transcription. This would result in a continuously accelerating cycle that could act as an irreversible bistable trigger. Skotheim et al. used single-cell measurements in budding yeast to show that this positive feedback does indeed occur. A small amount of Cln3 induces Cln1/2 expression and then the feedback loop takes over, leading to rapid and abrupt exit of Whi5 from the nucleus and consequently coherent expression of G1/S regulon genes. In the absence of coherent gene expression, cells take longer to exit G1 and a significant fraction even arrest before S phase, highlighting the importance of positive feedback in sharpening the G1/S switch.

The G1/S cell cycle checkpoint controls the passage of eukaryotic cells from the first gap phase, G1, into the DNA synthesis phase, S. In this switch in mammalian cells, there are two cell cycle kinases that help to control the checkpoint: cell cycle kinases CDK4/6-cyclin D and CDK2-cyclin E. The transcription complex that includes Rb and E2F is important in controlling this checkpoint. In the first gap phase, the Rb-HDAC repressor complex binds to the E2F-DP1 transcription factors, therefore inhibiting the downstream transcription. The phosphorylation of Rb by CDK4/6 and CDK2 dissociates the Rb-repressor complex and serves as an on/off switch for the cell cycle. Once Rb is phosphorylated, the inhibition is released on the E2F transcriptional activity. This allows for the transcription of S phase genes encoding for proteins that amplify the G1 to S phase switch.

Many different stimuli apply checkpoint controls including TGFb, DNA damage, contact inhibition, replicative senescence, and growth factor withdrawal. The first four act by inducing members of the INK4 or Kip/Cip families of cell cycle kinase inhibitors. TGFb inhibits the transcription of Cdc25A, a phosphatase that activates the cell cycle kinases, and growth factor withdrawal activates GSK3b, which phosphorylates cyclin D. This leads to its rapid ubiquitination..

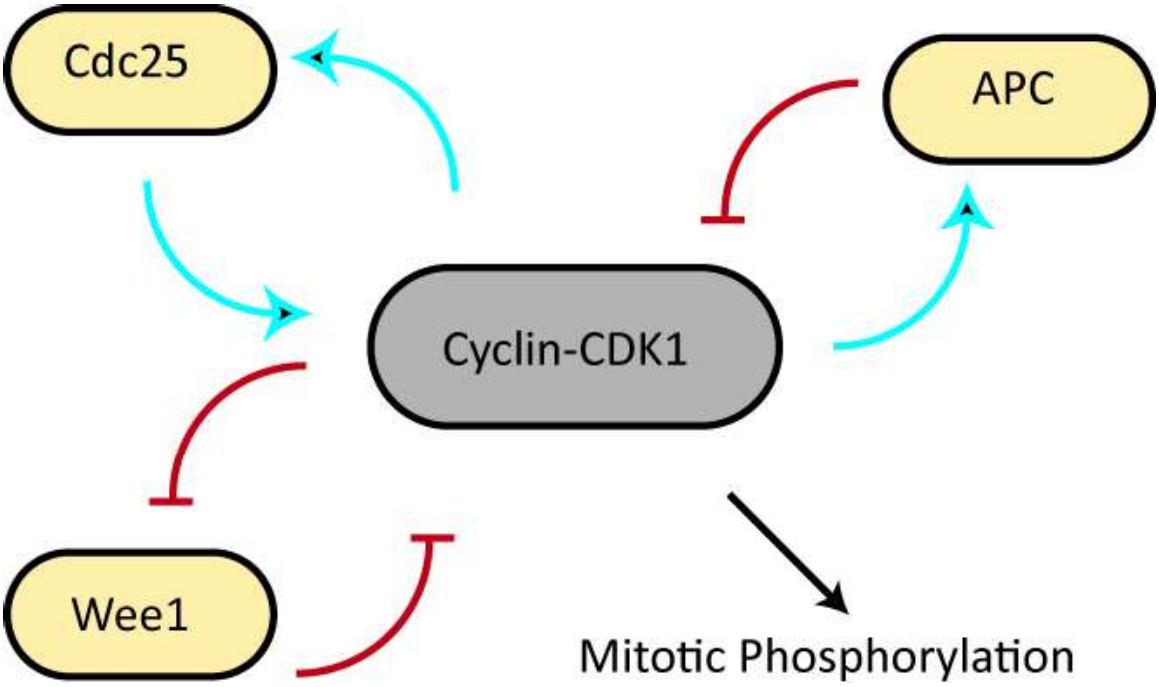
The G2/M switch

This transition is commenced by E2F-mediated transcription of cyclin A, forming the cyclin A-Cdk2 complex. This is useful in regulating events in prophase. In order to proceed past prophase, the cyclin B-Cdk1 complex (first discovered as MPF or M-phase promoting factor) is activated by Cdc 25, a protein phosphatase[1]. As mitosis starts, the nuclear envelope disintegrates, chromosomes condense and become visibile, and the cells prepares for division. The Cyclin B-Cdk1 activation results in nuclear envelope breakdown, which is a characteristic of the initiation of mitosis[1].It is evident that the cyclin A and B complexes with Cdks help regulate mitotic events at the G2/M transition.

As mentioned above, entry into mitosis is controlled by the Cyclin B-Cdk1 complex (first discovered as MPF or M-phase promoting factor; Cdk1 is also known as Cdc2 in fission yeast and Cdc28 in budding yeast). This complex forms an element of an interesting regulatory circuit in which Cdk1 can phosphorylate and activate its activator, the

phosphatase Cdc25 (positive feedback), and phosphorylate and inactivate its inactivator, the kinase Wee1 (double-negative feedback). It was suggested that this circuit could act as a bistable trigger with one stable steady state in G2 (Cdk and Cdc25 off, Wee1 on) and a second stable steady state in M phase (Cdk and Cdc25 active, Wee1 off). Once cells are in mitosis, Cyclin B-Cdk1 activates the Anaphase-promoting complex (APC), which in turn inactivates Cyclin B-Cdk1 by degrading Cyclin B, eventually leading to exit from mitosis. Coupling the bistable Cdk1 response function to the negative feedback from the APC could generate what is known as a relaxation oscillator, with sharp spikes of Cdk1 activity triggering robust mitotic cycles. However, in a relaxation oscillator, the control parameter moves slowly relative to the system's response dynamics which may be an accurate representation of mitotic entry, but not necessarily mitotic exit.

It is necessary to inactivate the cyclin B-Cdk1 complex in order to exit the mitotic stage of the cell cycle. The cells can then return to the first gap phase G1 and wait until the cycle proceeds yet again.



In 2003 Pomerening et al. provided strong evidence for this hypothesis by demonstrating hysteresis and bistability in the activation of Cdk1 in the cytoplasmic extracts of Xenopus oocytes. They first demonstrated a discontinuous sharp response of Cdk1 to changing concentrations of non-destructible Cyclin B (to decouple the Cdk1 response network from APC-mediated negative feedback). However, such a response would be consistent with both a monostable, ultransensitive transition and a bistable transition. To distinguish between these two possibilities, they measured the steady-state levels of active Cdk1 in response to changing cyclin levels, but in two separate experiments, one starting with an interphase extract and one starting with an extract already in mitosis. At intermediate concentrations of cyclin they found two steady-state concentrations of active Cdk1. Which of the two steady states was occupied depended on the history of the system,

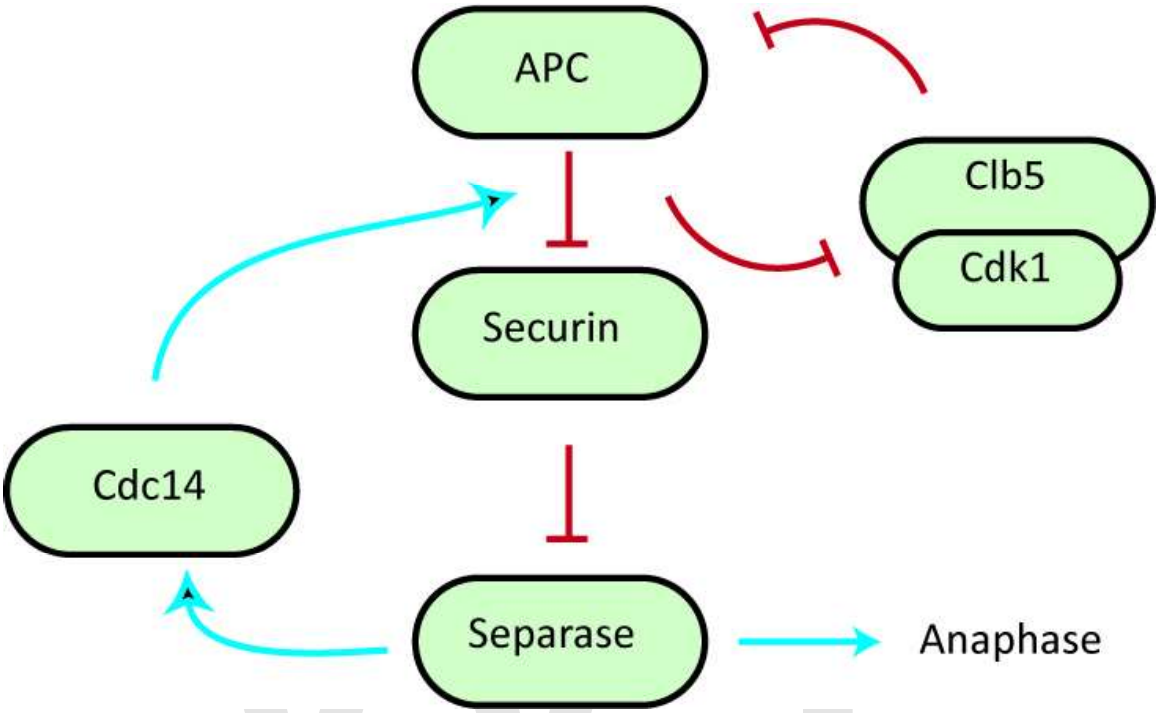
i.e.whether they started with interphase or mitotic extract, effectively demonstrating hysteresis and bistability.

In the same year, Sha et al. independently reached the same conclusion revealing the hysteretic loop also using Xenopus laevis egg extracts. Here, three predictions of the Novak-Tyson model were tested in an effort to conclude that hysteresis is the driving force for "cell-cycle transitions into and out of mitosis". The predictions of the Novak-Tyson model are generic to all saddle-node bifurcations. Saddle-node bifurcations are extremely useful bifurcations in an imperfect world because they help describe biological systems which are not perfect. The first prediction was that the threshold concentration of cyclin to enter mitosis is higher than the threshold concentration of cyclin to exit mitosis, and this was confirmed by supplementing cycling egg extracts with non-degradable cyclin B and measuring the activation and inactivation threshold after the addition of cycloheximide (CHX), which is a protein synthesis inhibitor.. Furthermore, the second prediction of the Novak-Tyson model was also validated: unreplicated deoxyribonucleic acid, or DNA, increases the threshold concentration of cyclin that is required to enter mitosis. In order to arrive at this conclusion, cytostatic factor released extracts were supplemented with CHX, APH (a DNA polymerase inhibitor), or both, and non-degradable cyclin B was added. The third and last prediction that was tested and proven true in this article was that the rate of Cdc2 activation slows down near the activation threshold concentration of cyclin. These predictions and experiments demonstrate the toggle-like switching behavior that can be described by hysteresis in a dynamical system..

Metaphase-anaphase switch

In the transition from metaphase to anaphase, it is crucial that sister chromatids are properly and simultaneously separated to opposite ends of the cell. Separation of sister-chromatids is initially strongly inhibited to prevent premature separation in late mitosis, but this inhibition is relieved through destruction of the inhibitory elements by the anaphase-promoting complex (APC) once sister-chromatid bi-orientation is achieved. One of these inhibitory elements is securin, which prevents the destruction of cohesin, the complex that holds the sister-chromatids together, by binding the protease separase which targets Scc1, a subunit of the cohesin complex, for destruction. In this system, the phosphatase Cdc14 can remove an inhibitory phosphate from securin, thereby facilitating the destruction of securin by the APC, releasing separase. As shown by Uhlmann et al., during the attachment of chromosomes to the mitotic spindle the chromatids remain paired because cohesion between the sisters prevents separation..SY.; Ferrell JE. (2007), "Substrate competition as a source of ultrasensitivity in the activation of Wee1," Cell 128(6): 1133-45 Cohesion is established during DNA replication and depends on cohesin, which is a multisubunit complex composed of Scc1, Scc3, Smc2, and Smc3. In yeast at the metaphase-to-anaphase transition, Scc1 dissociates from the chromosomes and the sister chromatids separate. This action is controlled by the Esp1 protein, which is tightly bound by the anaphase inhibitor Pds1 that is destroyed by the anaphase-promoting complex. In order to verify that Esp1 does play a role in regulating Scc1 chromosome association, cell strains were arrested in G1 with an alpha factor. These cells stayed in arrest during the development. Esp1-1 mutant cells were used and the experiment was

repeated, and Scc1 successfully bound to the chromosomes and remained associated even after the synthesis was terminated. This was crucial in showing that with Esp1, Scc1 is hindered in its ability to become stably associated with chromosomes during G1, and Esp1 can in fact directly remove Scc1 from chromosomes.

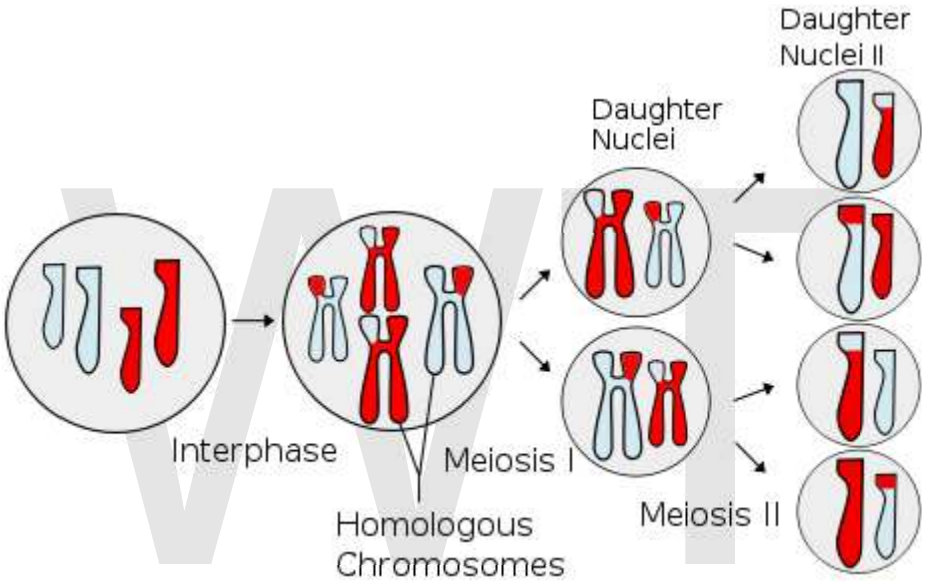


Esp1 and Scc1 diagram

It has been shown by Holt et al. that separase activates Cdc14, which in turn acts on securin, thus creating a positive feedback loop that increases the sharpness of the metaphase to anaphase transition and coordination of sister-chromatid separation. Holt et al. probed the basis for the effect of positive feedback in securin phosphophorlyation by using mutant 'securin' strains of yeast, and testing how changes in the phosphoregulation of securin affects the synchrony of sister chromatid separation. Their results indicate that interfering with this positive securin-separase-cdc14 loop decreases sister chromatid separation synchrony. This positive feedback can hypothetically generate bistability in the transition to anaphase, causing the cell to make the irreversible decision to separate sister-chromatids.

Chapter- 11

Meiosis



Events involving meiosis, showing chromosomal crossover

Meiosis is a special type of cell division necessary for sexual reproduction. In animals, meiosis produces gametes like sperm and egg cells, while in other organisms like fungi it generates spores. Meiosis begins with one diploid cell containing two copies of each chromosome—one from the organism's mother and one from its father—and produces four haploid cells containing one copy of each chromosome. Each of the resulting chromosomes in the gamete cells is a unique mixture of maternal and paternal DNA, ensuring that offspring are genetically distinct from either parent. This gives rise to genetic diversity in sexually reproducing populations, which enables them to adapt during the course of evolution.

Before meiosis, the cell's chromosomes are duplicated by a round of DNA replication. This leaves the maternal and paternal versions of each chromosome, called homologs, composed of two exact copies called sister chromatids and attached at the centromere region. In the beginning of meiosis, the maternal and paternal homologs pair to each other. Then they typically exchange parts by homologous recombination, leading to

crossovers of DNA from the maternal version of the chromosome to the paternal version and vice versa. Spindle fibers bind to the centromeres of each pair of homologs and arrange the pairs at the spindle equator. Then the fibers pull the recombined homologs to opposite poles of the cell. As the chromosomes move away from the center, the cell divides into two daughter cells, each containing a haploid number of chromosomes composed of two chromatids. After the recombined maternal and paternal homologs have separated into the two daughter cells, a second round of cell division occurs. There, meiosis ends as the two sister chromatids making up each homolog are separated and move into one of the four resulting gamete cells. Upon fertilization, for example when a sperm enters an egg cell, two gamete cells produced by meiosis fuse. The gamete from the mother and the gamete from the father each contribute half to the set of chromosomes that make up the new offsping's genome.

Meiosis uses many of the same mechanisms as mitosis, a type of cell division used by eukaryotes like plants and animals to split one cell into two identical daughter cells. In all plants, and in many protists, meiosis results in the formation of spores, haploid cells that can divide vegetatively without undergoing fertilization. Some eukaryotes, like Bdelloid rotifers, have lost the ability to carry out meiosis and have acquired the ability to reproduce by parthenogenesis. Meiosis does not occur in archaea or bacteria, which reproduce via asexual processes such as binary fission.

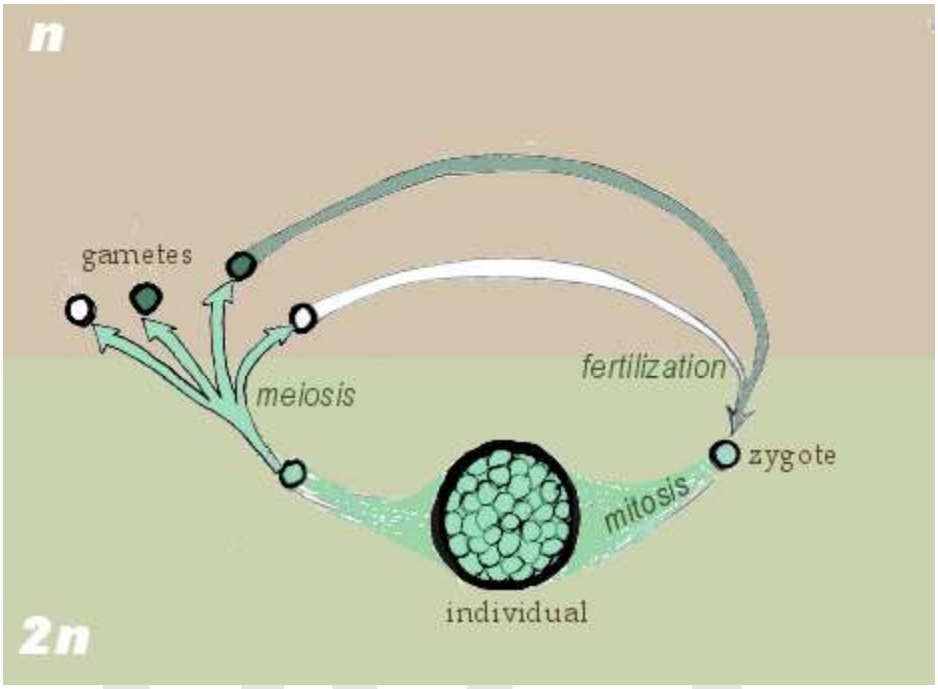
History

Meiosis was discovered and described for the first time in sea urchin eggs in 1876 by the German biologist Oscar Hertwig. It was described again in 1883, at the level of chromosomes, by the Belgian zoologist Edouard Van Beneden, in *Ascaris* worms' eggs. The significance of meiosis for reproduction and inheritance, however, was described only in 1890 by German biologist August Weismann, who noted that two cell divisions were necessary to transform one diploid cell into four haploid cells if the number of chromosomes had to be maintained. In 1911, the American geneticist Thomas Hunt Morgan observed crossover in *Drosophila melanogaster* meiosis and provided the first genetic evidence that genes are transmitted on chromosomes. The term meiosis was coined by J.B Farmer and J.B Moore in 1905.

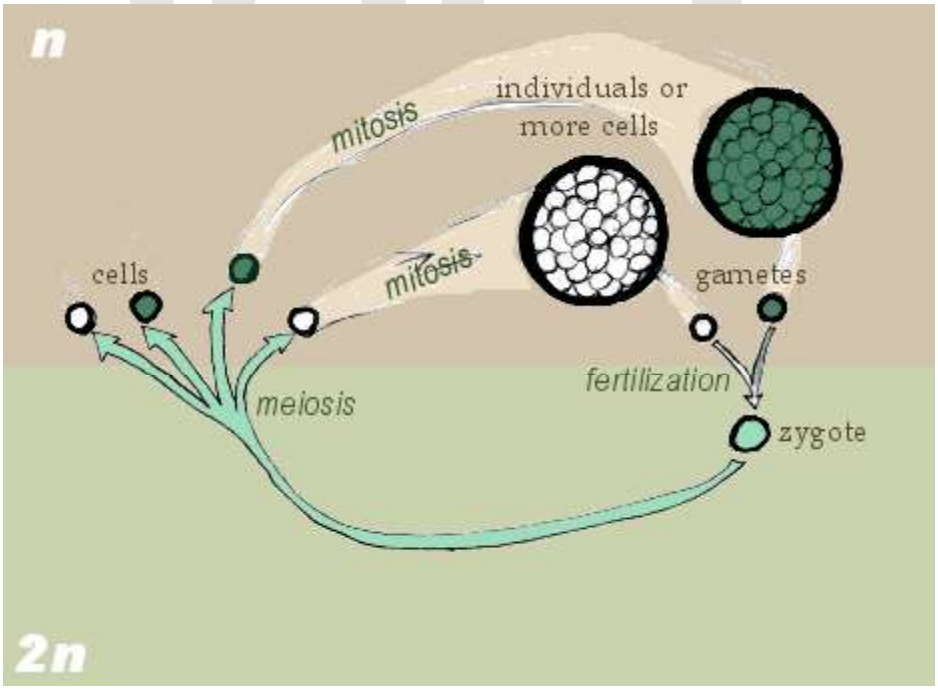
Evolution

Meiosis is thought to have appeared 1.4 billion years ago. The only supergroup of eukaryotes which does not have meiosis in all organisms is excavata. The other five major supergroups, opisthokonts, amoebozoa, rhizaria, archaeplastida and chromalveolates all seem to have genes for meiosis universally present, even if not always functional. Some excavata species do have meiosis which is consistent with the hypothesis that this group is an ancient, paraphyletic grade. An example of a eukaryotic organism in which meiosis does not exist is euglenoid.

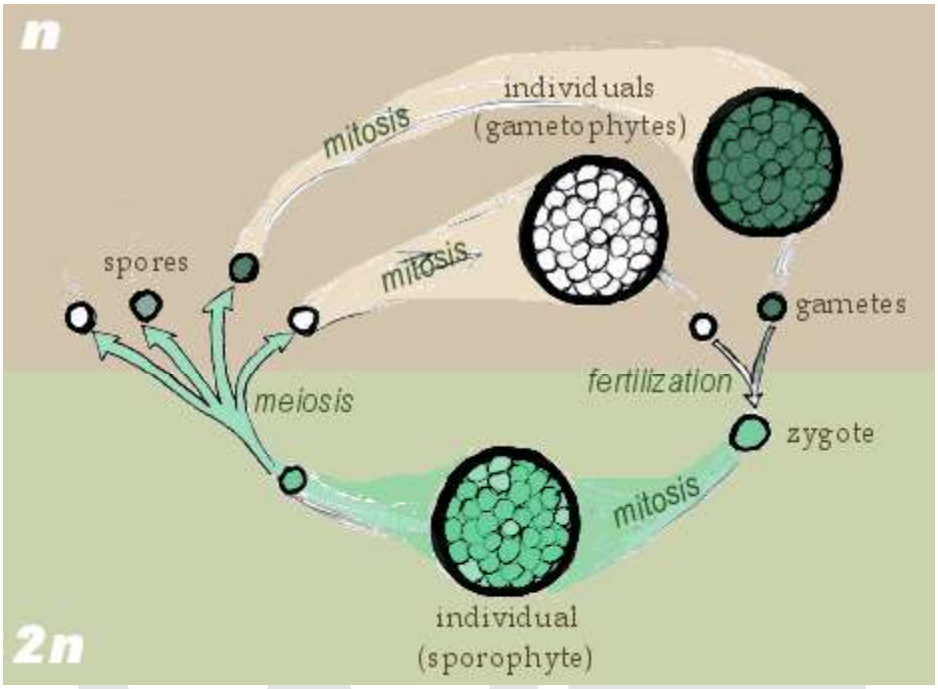
Occurrence of meiosis in eukaryotic life cycles



Gametic life cycle.



Zygotic life cycle.



Sporic life cycle.

Meiosis occurs in eukaryotic life cycles involving sexual reproduction, comprising of the constant cyclical process of meiosis and fertilization. This takes place alongside normal mitotic cell division. In multicellular organisms, there is an intermediary step between the diploid and haploid transition where the organism grows. The organism will then produce the germ cells that continue in the life cycle. The rest of the cells, called somatic cells, function within the organism and will die with it.

Cycling meiosis and fertilization events produces a series of transitions back and forth between alternating haploid and diploid states. The organism phase of the life cycle can occur either during the diploid state (*gametic* or *diploid* life cycle), during the haploid state (*zygotic* or *haploid* life cycle), or both (*sporic* or *haplodiploid* life cycle, in which there two distinct organism phases, one during the haploid state and the other during the diploid state). In this sense, there are three types of life cycles that utilize sexual reproduction, differentiated by the location of the organisms phase(s).

In the *gametic life cycle*, of which humans are a part, the species is diploid, grown from a diploid cell called the zygote. The organism's diploid germ-line stem cells undergo meiosis to create haploid gametes (the spermatozoa for males and ova for females), which fertilize to form the zygote. The diploid zygote undergoes repeated cellular division by mitosis to grow into the organism. Mitosis is a related process to meiosis that creates two cells that are genetically identical to the parent cell. The general principle is that mitosis creates somatic cells and meiosis creates germ cells.

In the *zygotic life cycle* the species is haploid instead, spawned by the proliferation and differentiation of a single haploid cell called the gamete. Two organisms of opposing

gender contribute their haploid germ cells to form a diploid zygote. The zygote undergoes meiosis immediately, creating four haploid cells. These cells undergo mitosis to create the organism. Many fungi and many protozoa are members of the zygotic life cycle.

Finally, in the *sporic life cycle*, the living organism alternates between haploid and diploid states. Consequently, this cycle is also known as the alternation of generations. The diploid organism's germ-line cells undergo meiosis to produce spores. The spores proliferate by mitosis, growing into a haploid organism. The haploid organism's germ cells then combine with another haploid organism's cells, creating the zygote. The zygote undergoes repeated mitosis and differentiation to become the diploid organism again. The sporic life cycle can be considered a fusion of the gametic and zygotic life cycles.

Process

Because meiosis is a "one-way" process, it cannot be said to engage in a cell cycle as mitosis does. However, the preparatory steps that lead up to meiosis are identical in pattern and name to the interphase of the mitotic cell cycle.

Interphase is divided into three phases:

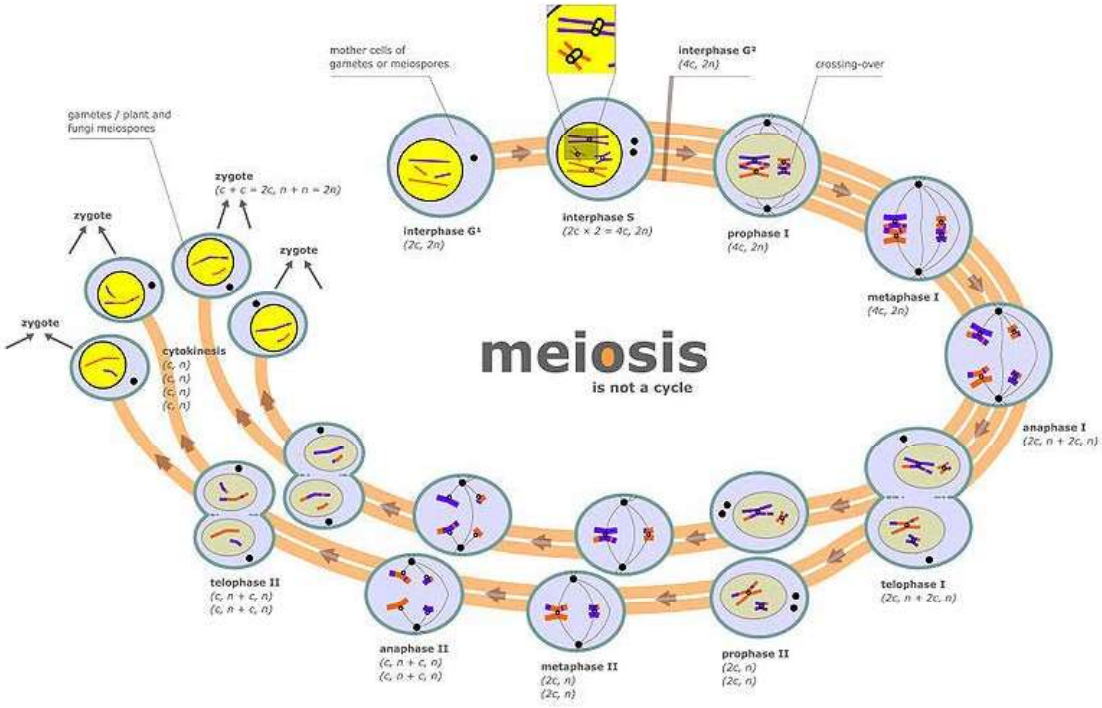
- **Growth 1 (G_1) phase**: This is a very active period, where the cell synthesizes its vast array of proteins, including the enzymes and structural proteins it will need for growth. In G_1 stage each of the chromosomes consists of a single (very long) molecule of DNA. In humans, at this point cells are **46 chromosomes, 2N**, identical to somatic cells.
- **Synthesis (S) phase**: The genetic material is replicated: each of its chromosomes duplicates, so that each of the 46 chromosomes becomes a complex of two identical sister chromatids. The cell is still considered diploid because it still contains the same number of centromeres. The identical sister chromatids have not yet condensed into the densely packaged chromosomes visible with the light microscope. This will take place during prophase I in meiosis.
- **Growth 2 (G_2) phase**: G_2 phase as seen before mitosis is not present in Meiosis. Actually, the first four stages of prophase I in many respects correspond to the G_2 phase of mitotic cell cycle.

Interphase is followed by meiosis I and then meiosis II. Meiosis I consists of separating the pairs of homologous chromosome, each made up of two sister chromatids, into two cells. One entire haploid content of chromosomes is contained in each of the resulting daughter cells; the first meiotic division therefore reduces the ploidy of the original cell by a factor of 2.

Meiosis II consists of decoupling each chromosome's sister strands (chromatids), and segregating the individual chromatids into haploid daughter cells. The two cells resulting from meiosis I divide during meiosis II, creating 4 haploid daughter cells. Meiosis I and II are each divided into prophase, metaphase, anaphase, and telophase stages, similar in purpose to their analogous subphases in the mitotic cell cycle. Therefore, meiosis

includes the stages of meiosis I (prophase I, metaphase I, anaphase I, telophase I), and meiosis II (prophase II, metaphase II, anaphase II, telophase II).

Meiosis generates genetic diversity in two ways: (1) independent alignment and subsequent separation of homologous chromosome pairs during the first meiotic division allows a random and independent selection of each chromosome segregates into each gamete; and (2) physical exchange of homologous chromosomal regions by homologous recombination during prophase I results in new combinations of DNA within chromosomes.



A diagram of the meiotic phases

Phases

Meiosis takes place in several stages.

Meiosis I

Meiosis I separates homologous chromosomes, producing two haploid cells (**N chromosomes, 23** in humans), so meiosis I is referred to as a **reductional division**. A regular diploid human cell contains 46 chromosomes and is considered 2N because it contains 23 pairs of homologous chromosomes. However, after meiosis I, although the cell contains 46 chromatids, it is only considered as being N, with 23 chromosomes. This is because later, in Anaphase I, the sister chromatids will remain together as the spindle fibres pulls the pair toward the pole of the new cell. In meiosis II, an **equational division**

similar to mitosis will occur whereby the sister chromatids are finally split, creating a total of 4 haploid cells (**23 chromosomes, N**) per daughter cell from the first division.

Prophase I

During prophase I, DNA is exchanged between homologous chromosomes in a process called homologous recombination. This often results in chromosomal crossover. The new combinations of DNA created during crossover are a significant source of genetic variation, and may result in beneficial new combinations of alleles. The paired and replicated chromosomes are called bivalents or tetrads, which have two chromosomes and four chromatids, with one chromosome coming from each parent. At this stage, non-sister chromatids may cross-over at points called chiasmata (plural; singular chiasma).

Leptotene

The first stage of prophase I is the *leptotene* stage, also known as *leptonema*, from Greek words meaning "thin threads".[:27]In this stage of prophase I, individual chromosomes—each consisting of two sister chromatids—change from the diffuse state they exist in during the cell's period of growth and gene expression, and condense into visible strands within the nucleus.[:27:353] However the two sister chromatids are still so tightly bound that they are indistinguishable from one another. During leptotene, lateral elements of the synaptonemal complex assemble.Leptotene is of very short duration and progressive condensation and coiling of chromosome fibers takes place. Chromosome assume a long thread like shape,they contract and become thick.At the beginning chromosomes are present in diploid number as in mitotic prophase.Each chromosome is made up of only one chromosome and half of the total chromosome are paternal and half maternal.For every paternal chromosome there is a corresponding maternal chromosome similar in size,shape and nature of inherited characters and are called homologous chromosome.

Zygotene

The *zygotene* stage, also known as *zygonema*, from Greek words meaning "paired threads",[:27] occurs as the chromosomes approximately line up with each other into homologous chromosome pairs. This is called the bouquet stage because of the way the telomeres cluster at one end of the nucleus. At this stage, the synapsis (pairing/coming together) of homologous chromosomes takes place, facilitated by assembly of central element of the synaptonemal complex.Pairing is brought about by a zipper like fashion and may start at the centromere(procentric),at the chromosome ends(proterminal),or at any other portion(intermediate).Individuals of a pair are equal in length and in position of centromere. Thus pairing is highly specific and exact.The paired chromosomes are called Bivalent chromosome.

Pachytene

The *pachytene* stage, also known as *pachynema*, from Greek words meaning "thick threads",[:27] is the stage when chromosomal crossover (crossing over) occurs. Nonsister

chromatids of homologous chromosomes randomly exchange segments over regions of homology. Sex chromosomes, however, are not wholly identical, and only exchange information over a small region of homology. At the sites where exchange happens, chiasmata form. The exchange of information between the non-sister chromatids results in a recombination of information; each chromosome has the complete set of information it had before, and there are no gaps formed as a result of the process. Because the chromosomes cannot be distinguished in the synaptonemal complex, the actual act of crossing over is not perceivable through the microscope, and chiasmata are not visible until the next stage.

Diplotene

During the *diplotene* stage, also known as *diplonema*, from Greek words meaning "two threads",[30] the synaptonemal complex degrades and homologous chromosomes separate from one another a little. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. However, the homologous chromosomes of each bivalent remain tightly bound at chiasmata, the regions where crossing-over occurred. The chiasmata remain on the chromosomes until they are severed in anaphase I.

In human fetal oogenesis all developing oocytes develop to this stage and stop before birth. This suspended state is referred to as the *dictyotene stage* and remains so until puberty. In males, only spermatogonia (spermatogenesis) exist until meiosis begins at puberty.

Diakinesis

Chromosomes condense further during the *diakinesis* stage, from Greek words meaning "moving through".[30] This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Other than this observation, the rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

Synchronous processes

During these stages, two centrosomes, containing a pair of centrioles in animal cells, migrate to the two poles of the cell. These centrosomes, which were duplicated during S-phase, function as microtubule organizing centers nucleating microtubules, which are essentially cellular ropes and poles. The microtubules invade the nuclear region after the nuclear envelope disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centriole, like a train on a track. There are four kinetochores on each tetrad, but the pair of kinetochores on each sister chromatid fuses and functions as a unit during meiosis I.

Microtubules that attach to the kinetochores are known as *kinetochore microtubules*. Other microtubules will interact with microtubules from the opposite centriole: these are called *nonkinetochore microtubules* or *polar microtubules*. A third type of microtubules, the aster microtubules, radiates from the centrosome into the cytoplasm or contacts components of the membrane skeleton.

Metaphase I

Homologous pairs move together along the metaphase plate: As **kinetochore microtubules** from both centrioles attach to their respective kinetochores, the homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line.

Anaphase I

Kinetochore (bipolar spindles) microtubules shorten, severing the recombination nodules and pulling homologous chromosomes apart. Since each chromosome has only one functional unit of a pair of kinetochores, whole chromosomes are pulled toward opposing poles, forming two haploid sets. Each chromosome still contains a pair of sister chromatids. Nonkinetochore microtubules lengthen, pushing the centrioles farther apart. The cell elongates in preparation for division down the center.

Telophase I

The last meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules that make up the spindle network disappear, and a new nuclear membrane surrounds each haploid set. The chromosomes uncoil back into chromatin. Cytokinesis, the pinching of the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells. Sister chromatids remain attached during telophase I.

Cells may enter a period of rest known as interkinesis or interphase II. No DNA replication occurs during this stage.

Meiosis II

Meiosis II is the second part of the meiotic process. Much of the process is similar to mitosis. The end result is production of four haploid cells (**23 chromosomes, N** in humans) from the two haploid cells (**23 chromosomes, N** * each of the chromosomes

consisting of two sister chromatids) produced in meiosis I. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II.

In **prophase II** we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrioles move to the polar regions and arrange spindle fibers for the second meiotic division.

In **metaphase II**, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate.

This is followed by **anaphase II**, where the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles.

The process ends with **telophase II**, which is similar to telophase I, and is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells.

Significance

Meiosis facilitates stable sexual reproduction. Without the halving of ploidy, or chromosome count, fertilization would result in zygotes that have twice the number of chromosomes as the zygotes from the previous generation. Successive generations would have an exponential increase in chromosome count. In organisms that are normally diploid, polyploidy, the state of having three or more sets of chromosomes, results in extreme developmental abnormalities or lethality. Polyploidy is poorly tolerated in most animal species. Plants, however, regularly produce fertile, viable polyploids. Polyploidy has been implicated as an important mechanism in plant speciation.

Most importantly, recombination and independent assortment of homologous chromosomes allow for a greater diversity of genotypes in the offspring. This produces genetic variation in gametes that promote genetic and phenotypic variation in a population of offspring. Therefore a gene for meiosis will be favoured by natural selection over an allele for mitotic reproduction, because any selection pressure which acts against any clone will act against all clones, whilst inevitably favoring some offspring which are the result of sexual reproduction.

Nondisjunction

The normal separation of chromosomes in meiosis I or sister chromatids in meiosis II is termed *disjunction*. When the separation is not normal, it is called **nondisjunction**. This

results in the production of gametes which have either too many or too few of a particular chromosome, and is a common mechanism for trisomy or monosomy. Nondisjunction can occur in the meiosis I or meiosis II, phases of cellular reproduction, or during mitosis.

This is a cause of several medical conditions in humans (such as):

- Down Syndrome - trisomy of chromosome 21
- Patau Syndrome - trisomy of chromosome 13
- Edward Syndrome - trisomy of chromosome 18
- Klinefelter Syndrome - extra X chromosomes in males - i.e. XXY, XXXY, XXXXY
- Turner Syndrome - lacking of one X chromosome in females - i.e. XO
- Triple X syndrome - an extra X chromosome in females
- XYY Syndrome - an extra Y chromosome in males

Meiosis in mammals

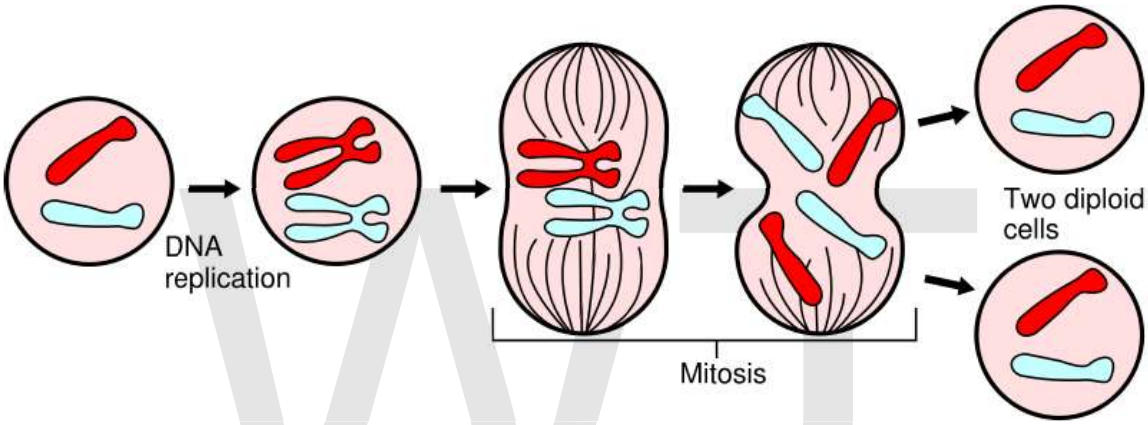
In females, meiosis occurs in cells known as oogonia (singular: oogonium). Each oogonium that initiates meiosis will divide twice to form a single oocyte and two polar bodies. However, before these divisions occur, these cells stop at the diplotene stage of meiosis I and lie dormant within a protective shell of somatic cells called the follicle. Follicles begin growth at a steady pace in a process known as folliculogenesis, and a small number enter the menstrual cycle. Menstruated oocytes continue meiosis I and arrest at meiosis II until fertilization. The process of meiosis in females occurs during oogenesis, and differs from the typical meiosis in that it features a long period of meiotic arrest known as the Dictyate stage and lacks the assistance of centrosomes.

In males, meiosis occurs during spermatogenesis in the seminiferous tubules of the testicles. Meiosis during spermatogenesis is specific to a type of cell called spermatocytes that will later mature to become spermatozoa.

In female mammals, meiosis begins immediately after primordial germ cells migrate to the ovary in the embryo, but in the males, meiosis begins years later at the time of puberty. It is retinoic acid, derived from the primitive kidney (mesonephros) that stimulates meiosis in ovarian oogonia. Tissues of the male testis suppress meiosis by degrading retinoic acid, a stimulator of meiosis. This is overcome at puberty when cells within seminiferous tubules called Sertoli cells start making their own retinoic acid. Sensitivity to retinoic acid is also adjusted by proteins called nanos and DAZL.

Chapter- 12

Mitosis



Mitosis divides the chromosomes in a cell nucleus.

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle—the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

Mitosis occurs exclusively in eukaryotic cells, but the process varies in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are interphase, prophase, prometaphase, metaphase, anaphase and telophase. During mitosis the pairs of chromosomes condense and attach to fibers

that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "mitotic phase". However, there are many cells where mitosis and cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Overview

The primary result of mitosis is the transferring of the parent cell's genome into two daughter cells. The genome is composed of a number of chromosomes—complexes of tightly-coiled DNA that contain genetic information vital for proper cell function. Because each resultant daughter cell should be genetically identical to the parent cell, the parent cell must make a copy of each chromosome before mitosis. This occurs during the S phase of interphase, the period that precedes the mitotic phase in the cell cycle where preparation for mitosis occurs.

Each new chromosome now contains two identical copies of itself, called *sister chromatids*, attached together in a specialized region of the chromosome known as the *centromere*. Each sister chromatid is not considered a chromosome in itself, and a chromosome always contains two sister chromatids.

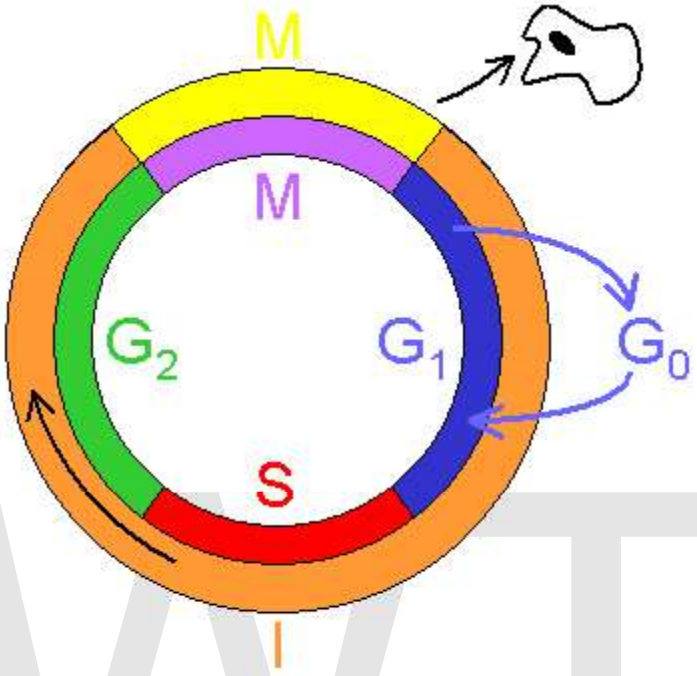
In most eukaryotes, the nuclear envelope that combines the DNA from the cytoplasm disassembles. The chromosomes align themselves in a line spanning the cell. Microtubules, essentially miniature strings, splay out from opposite ends of the cell and shorten, pulling apart the sister chromatids of each chromosome. As a matter of convention, each sister chromatid is now considered a chromosome, so they are renamed to *sister chromosomes*. As the cell elongates, corresponding sister chromosomes are pulled toward opposite ends. A new nuclear envelope forms around the separated sister chromosomes.

As mitosis completes cytokinesis is well underway. In animal cells, the cell pinches inward where the imaginary line used to be (the area of the cell membrane that pinches to form the two daughter cells is called the cleavage furrow), separating the two developing nuclei. In plant cells, the daughter cells will construct a new dividing cell wall between each other. Eventually, the mother cell will be split in half, giving rise to two daughter cells, each with an equivalent and complete copy of the original genome.

Prokaryotic cells undergo a process similar to mitosis called binary fission. However, prokaryotes cannot be properly said to undergo cytokinesis because they lack a nucleus and only have a single chromosome with no mitochondria.

Phases of cell cycle and mitosis

Interphase



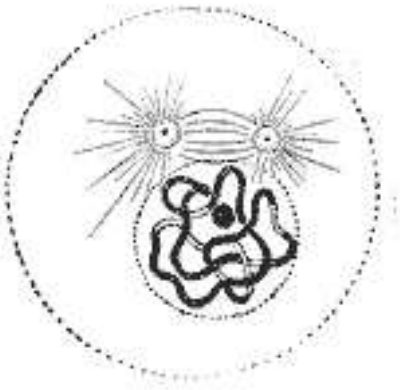
The cell cycle

The mitotic phase is a relatively short period of the cell cycle. It alternates with the much longer *interphase*, where the cell prepares itself for cell division. Interphase is therefore not part of mitosis. Interphase is divided into three phases, G_1 (first gap), S (synthesis), and G_2 (second gap). During all three phases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are replicated only during the S phase. Thus, a cell grows (G_1), continues to grow as it duplicates its chromosomes (S), grows more and prepares for mitosis (G_2), and finally it divides (M) before restarting the cycle.

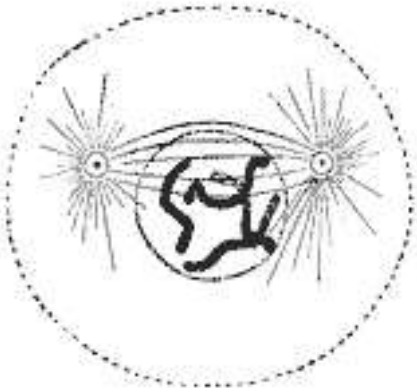
Preprophase

In plant cells only, prophase is preceded by a pre-prophase stage. In highly vacuolated plant cells, the nucleus has to migrate into the center of the cell before mitosis can begin. This is achieved through the formation of a phragmosome, a transverse sheet of cytoplasm that bisects the cell along the future plane of cell division. In addition to phragmosome formation, preprophase is characterized by the formation of a ring of microtubules and actin filaments (called preprophase band) underneath the plasma membrane around the equatorial plane of the future mitotic spindle. This band marks the position where the cell will eventually divide. The cells of higher plants (such as the flowering plants) lack centrioles; instead, microtubules form a spindle on the surface of the nucleus and are then being organized into a spindle by the chromosomes themselves,

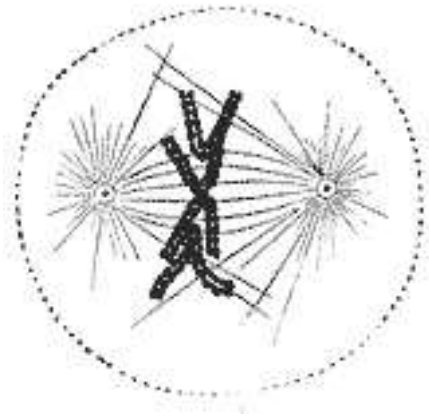
after the nuclear membrane breaks down. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase.



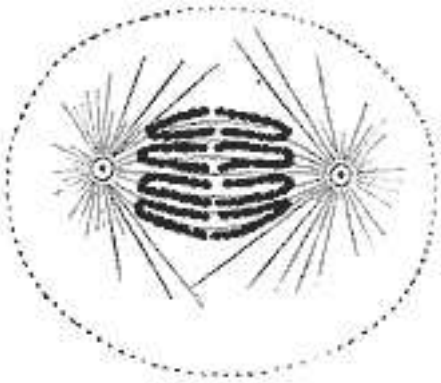
Prophase: The two round objects above the nucleus are the centrosomes. The chromatin has condensed.



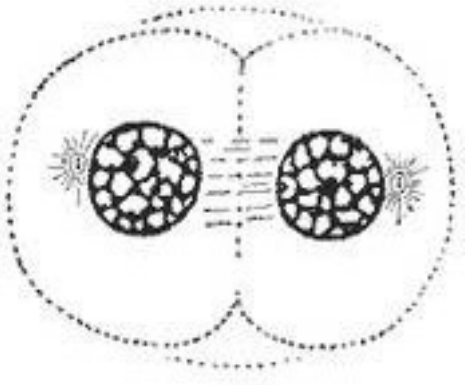
Prometaphase: The nuclear membrane has degraded, and microtubules have invaded the nuclear space. These microtubules can attach to kinetochores or they can interact with opposing microtubules.



Metaphase: The chromosomes have aligned at the metaphase plate.

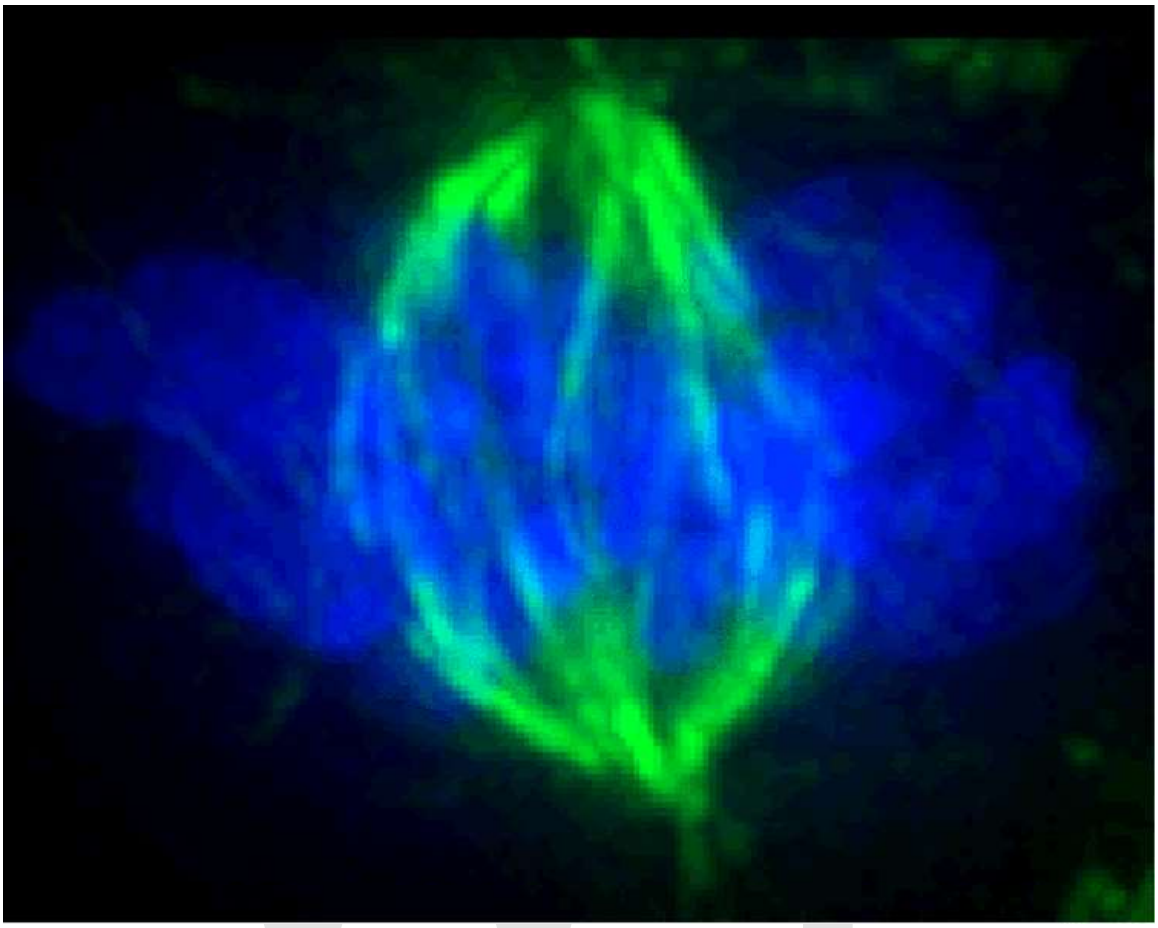


Early anaphase: The kinetochore microtubules shorten.



Telophase: The decondensing chromosomes are surrounded by nuclear membranes. Cytokinesis has already begun; the pinched area is known as the *cleavage furrow*.

Prophase



Micrograph showing condensed chromosomes in blue and the mitotic spindle in green during prometaphase of mitosis

Normally, the genetic material in the nucleus is in a loosely bundled coil called chromatin. At the onset of prophase, chromatin condenses together into a highly ordered structure called a chromosome. Since the genetic material has already been duplicated earlier in S phase, the replicated chromosomes have two sister chromatids, bound together at the centromere by the cohesion complex. Chromosomes are typically visible at high magnification through a light microscope.

Close to the nucleus are structures called centrosomes, which are made of a pair of centrioles. The centrosome is the coordinating center for the cell's microtubules. A cell inherits a single centrosome at cell division, which replicates before a new mitosis begins, giving a pair of centrosomes. The two centrosomes nucleate microtubules (which may be thought of as cellular ropes or poles) to form the spindle by polymerizing soluble tubulin. Molecular motor proteins then push the centrosomes along these microtubules to opposite sides of the cell. Although centrioles help organize microtubule assembly, they are not essential for the formation of the spindle, since they are absent from plants, and centrosomes are not always used in meiosis.

Prometaphase

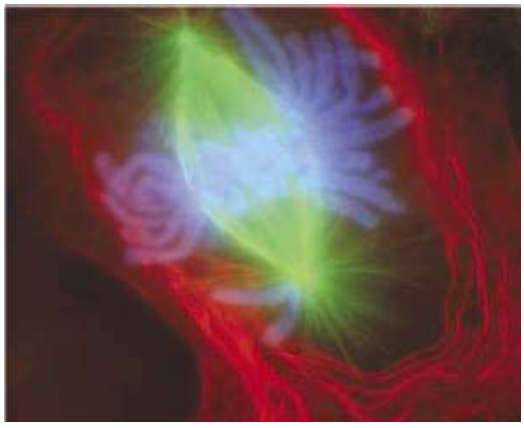
The nuclear envelope disassembles and microtubules invade the nuclear space. This is called *open mitosis*, and it occurs in most multicellular organisms. Fungi and some protists, such as algae or trichomonads, undergo a variation called *closed mitosis* where the spindle forms inside the nucleus, or its microtubules are able to penetrate an intact nuclear envelope.

Each chromosome forms two kinetochores at the centromere, one attached at each chromatid. A kinetochore is a complex protein structure that is analogous to a ring for the microtubule hook; it is the point where microtubules attach themselves to the chromosome. Although the kinetochore structure and function are not fully understood, it is known that it contains some form of molecular motor. When a microtubule connects with the kinetochore, the motor activates, using energy from ATP to "crawl" up the tube toward the originating centrosome. This motor activity, coupled with polymerisation and depolymerisation of microtubules, provides the pulling force necessary to later separate the chromosome's two chromatids.

When the spindle grows to sufficient length, *kinetochore microtubules* begin searching for kinetochores to attach to. A number of *nonkinetochore microtubules* find and interact with corresponding nonkinetochore microtubules from the opposite centrosome to form the mitotic spindle. Prometaphase is sometimes considered part of prophase.

In the fishing pole analogy, the kinetochore would be the "hook" that catches a sister chromatid or "fish". The centrosome acts as the "reel" that draws in the spindle fibers or "fishing line".

Metaphase



A cell in late metaphase. All chromosomes (blue) but one have arrived at the metaphase plate.

As microtubules find and attach to kinetochores in prometaphase, the centromeres of the chromosomes convene along the *metaphase plate* or *equatorial plane*, an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the

counterbalance of the pulling powers generated by the opposing kinetochores, analogous to a tug-of-war between people of equal strength. In certain types of cells, chromosomes do not line up at the metaphase plate and instead move back and forth between the poles randomly, only roughly lining up along the midline. Metaphase comes from the Greek *μετα* meaning "after."

Because proper chromosome separation requires that every kinetochore be attached to a bundle of microtubules (spindle fibres), it is thought that unattached kinetochores generate a signal to prevent premature progression to anaphase without all chromosomes being aligned. The signal creates the *mitotic spindle checkpoint*.

Anaphase

When every kinetochore is attached to a cluster of microtubules and the chromosomes have lined up along the metaphase plate, the cell proceeds to anaphase (from the Greek *ανα* meaning “up,” “against,” “back,” or “re-”).

Two events then occur: first, the proteins that bind sister chromatids together are cleaved, allowing them to separate. These sister chromatids, which have now become distinct sister chromosomes, are pulled apart by shortening kinetochore microtubules and move toward the respective centrosomes to which they are attached. Next, the nonkinetochore microtubules elongate, pulling the centrosomes (and the set of chromosomes to which they are attached) apart to opposite ends of the cell. The force that causes the centrosomes to move towards the ends of the cell is still unknown, although there is a theory that suggests that the rapid assembly and breakdown of microtubules may cause this movement.

These two stages are sometimes called early and late anaphase. Early anaphase is usually defined as the separation of the sister chromatids, while late anaphase is the elongation of the microtubules and the chromosomes being pulled farther apart. At the end of anaphase, the cell has succeeded in separating identical copies of the genetic material into two distinct populations.

Telophase

Telophase (from the Greek *τελος* meaning "end") is a reversal of prophase and prometaphase events. It "cleans up" the after effects of mitosis. At telophase, the nonkinetochore microtubules continue to lengthen, elongating the cell even more. Corresponding sister chromosomes attach at opposite ends of the cell. A new nuclear envelope, using fragments of the parent cell's nuclear membrane, forms around each set of separated sister chromosomes. Both sets of chromosomes, now surrounded by new nuclei, unfold back into chromatin. Mitosis is complete, but cell division is not yet complete.

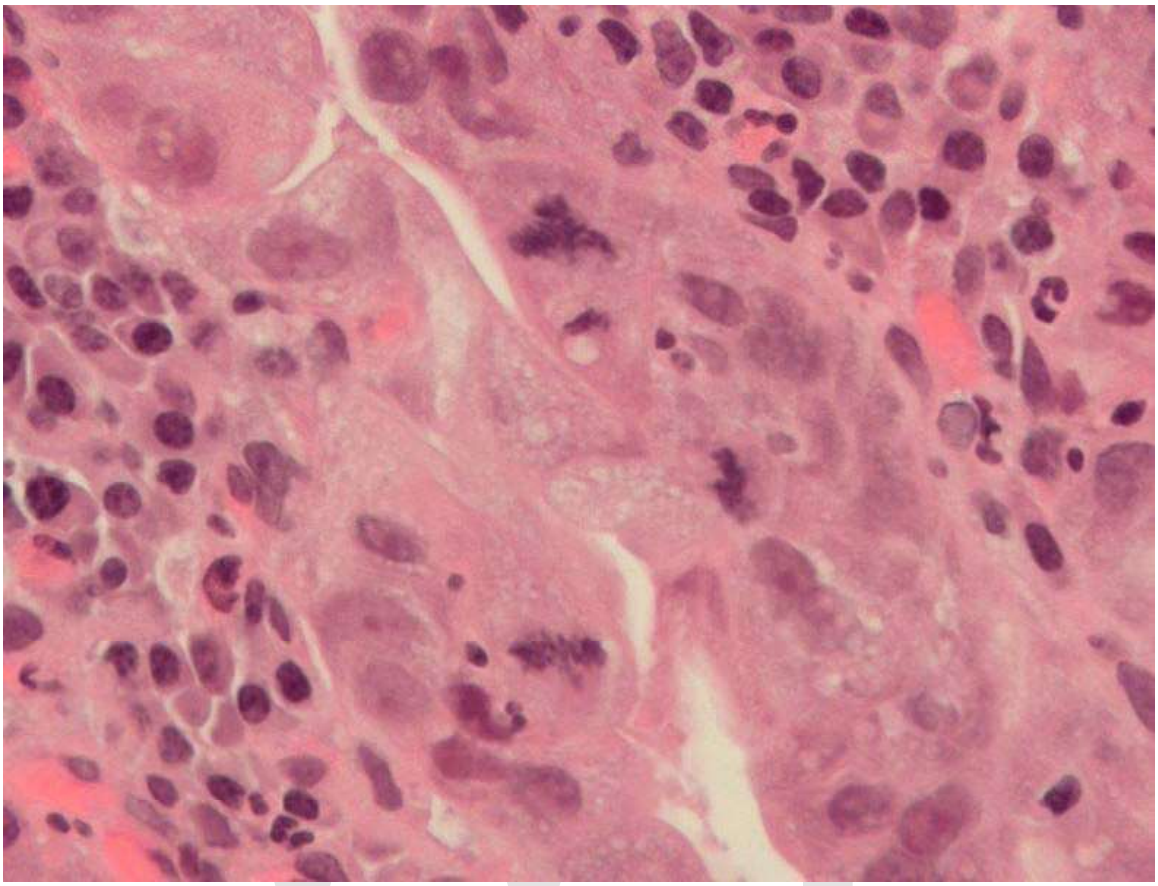
Cytokinesis

Cytokinesis is often mistakenly thought to be the final part of telophase; however, cytokinesis is a separate process that begins at the same time as telophase. Cytokinesis is technically not even a phase of mitosis, but rather a separate process, necessary for completing cell division. In animal cells, a cleavage furrow (pinch) containing a contractile ring develops where the metaphase plate used to be, pinching off the separated nuclei. In both animal and plant cells, cell division is also driven by vesicles derived from the Golgi apparatus, which move along microtubules to the middle of the cell. In plants this structure coalesces into a cell plate at the center of the phragmoplast and develops into a cell wall, separating the two nuclei. The phragmoplast is a microtubule structure typical for higher plants, whereas some green algae use a phycoplast microtubule array during cytokinesis. Each daughter cell has a complete copy of the genome of its parent cell. The end of cytokinesis marks the end of the M-phase.

Significance

Mitosis is important for the maintenance of the chromosomal set; each cell formed receives chromosomes that are alike in composition and equal in number to the chromosomes of the parent cell. Transcription is generally believed to cease during mitosis, but epigenetic mechanisms such as bookmarking function during this stage of the cell cycle to ensure that the "memory" of which genes were active prior to entry into mitosis are transmitted to the daughter cells.

Consequences of errors



An abnormal (tripolar) mitoses (12 o'clock position) in a precancerous lesion of the stomach. H&E stain

Although errors in mitosis are rare, the process may go wrong, especially during early cellular divisions in the zygote. Mitotic errors can be especially dangerous to the organism because future offspring from this parent cell will carry the same disorder.

In *non-disjunction*, a chromosome may fail to separate during anaphase. One daughter cell will receive both sister chromosomes and the other will receive none. This results in the former cell having three chromosomes containing the same genes (two sisters and a homologue), a condition known as *trisomy*, and the latter cell having only one chromosome (the homologous chromosome), a condition known as *monosomy*. These cells are considered aneuploid, a condition often associated with cancer.

Mitosis is a demanding process for the cell, which goes through dramatic changes in ultrastructure, its organelles disintegrate and reform in a matter of hours, and chromosomes are jostled constantly by probing microtubules. Occasionally, chromosomes may become damaged. An arm of the chromosome may be broken and the

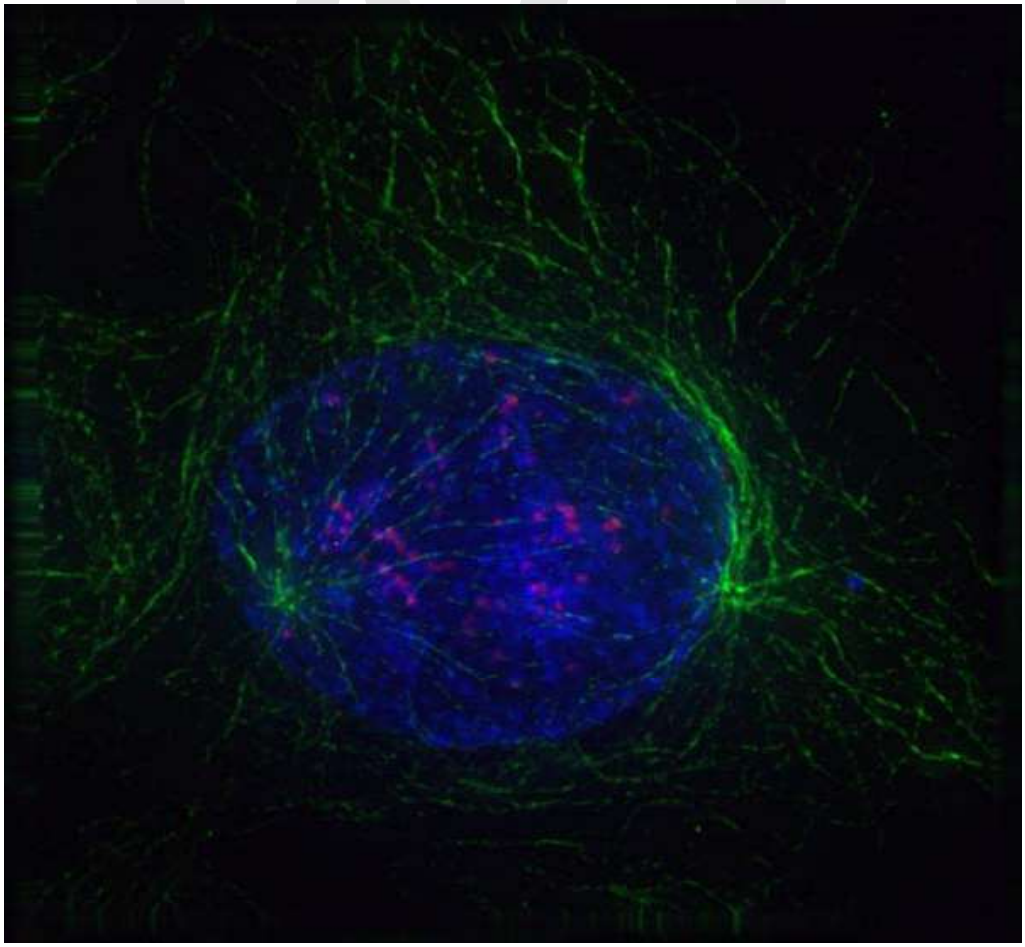
fragment lost, causing deletion. The fragment may incorrectly reattach to another, non-homologous chromosome, causing translocation. It may reattach to the original chromosome, but in reverse orientation, causing inversion. Or, it may be treated erroneously as a separate chromosome, causing chromosomal duplication. The effect of these genetic abnormalities depends on the specific nature of the error. It sometimes ranges from no noticeable effect to cancer induction or organism death.

Endomitosis

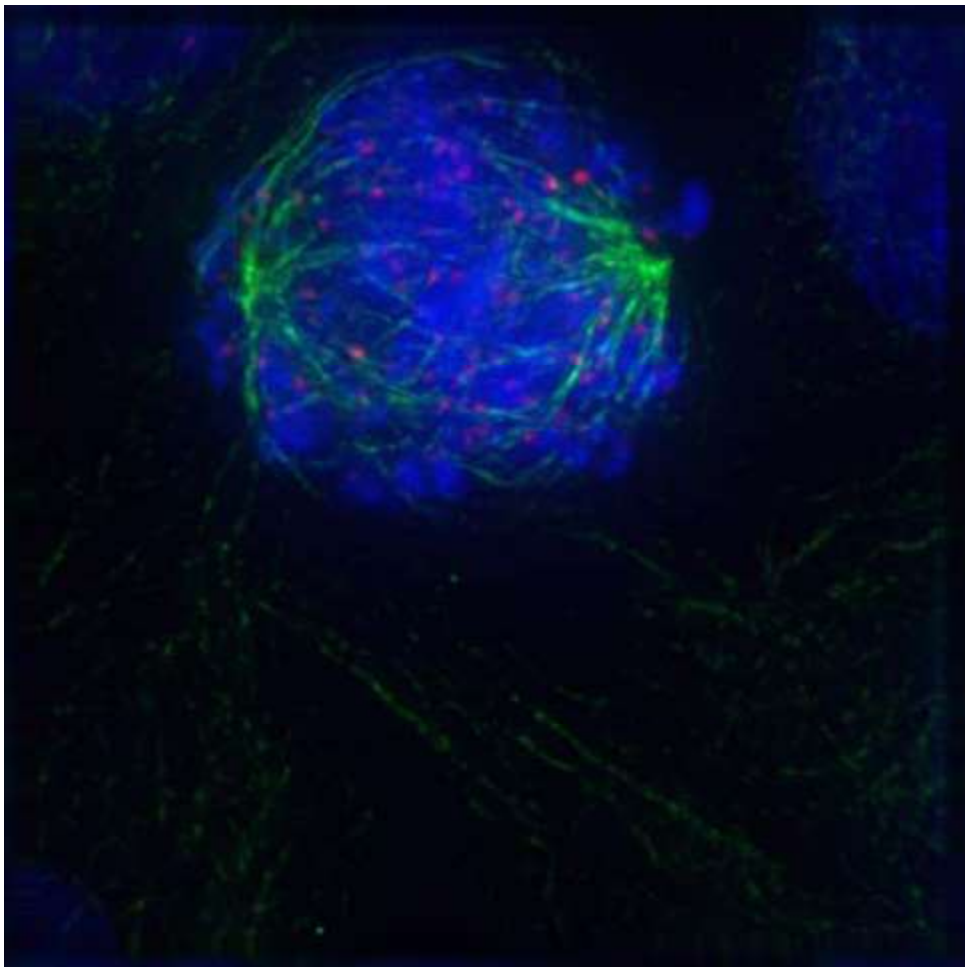
Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus. This process may also be referred to as endoreduplication and the cells as endoploid. An example of a cell that goes through endomitosis is the megakaryocyte.

Timeline in pictures

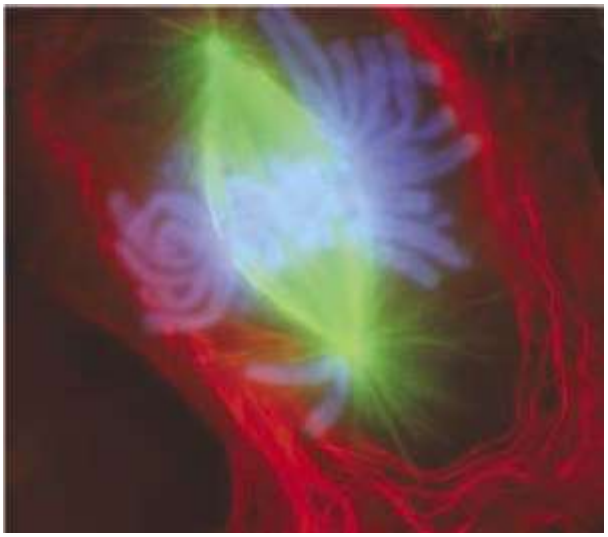
Real mitotic cells can be visualized through the microscope by staining them with fluorescent antibodies and dyes. These light micrographs are included below.



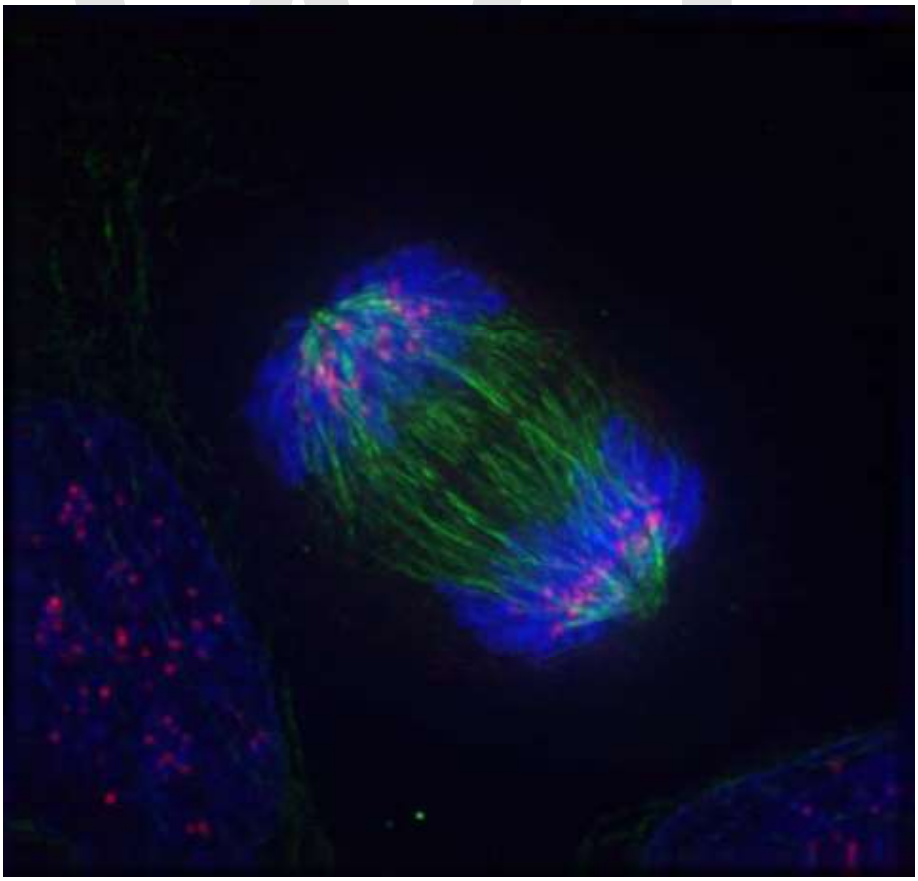
Early prophase: Nonkinetochore microtubules, shown as green strands, have established a matrix around the degrading nucleus, in blue. The green nodules are the centrosomes.



Early prometaphase: The nuclear membrane has just degraded, allowing the microtubules to quickly interact with the kinetochores on the chromosomes, which have just condensed.



Late metaphase: The centrosomes have moved to the poles of the cell and have established the mitotic spindle. The chromosomes, in light blue, have all assembled at the metaphase plate, except for one.



Anaphase: Lengthening nonkinetochore microtubules push the two sets of chromosomes further apart

Chapter- 13

Prophase and Metaphase

Prophase

Prophase, from the ancient Greek *πρό* (before) and *φάσις* (stage), is a stage of mitosis in which the chromatin condenses into a highly ordered structure called a chromosome in which the chromatin becomes visible. This process, called *chromatin condensation*, is mediated by the condensin complex. Since the genetic material has been duplicated in an earlier phase of the cell cycle, there are two identical copies of each chromosome in the cell. Identical chromosomes, called sister chromatids, are attached to each other at a DNA element present on every chromosome called the centromere. During prophase, giemsa staining can be applied to elicit G-banding in chromosomes. Prophase accounts for approximately 3% of the cell cycle's duration.

An important organelle in mitosis is the centrosome, the microtubule organizing center in metazoans. During prophase, the two centrosomes, which replicate independently of mitosis, have their microtubule-activity increased due to the recruitment of γ-tubulin. The centrosomes will be pushed apart to opposite ends of the cell nucleus by the action of molecular motors acting on the microtubules. The nuclear envelope breaks down to allow the microtubules to reach the kinetochores on the chromosomes, marking the end of prophase. Prometaphase, the next step of mitosis, will see the chromosome being captured by the microtubules.

Prophase in plant cells

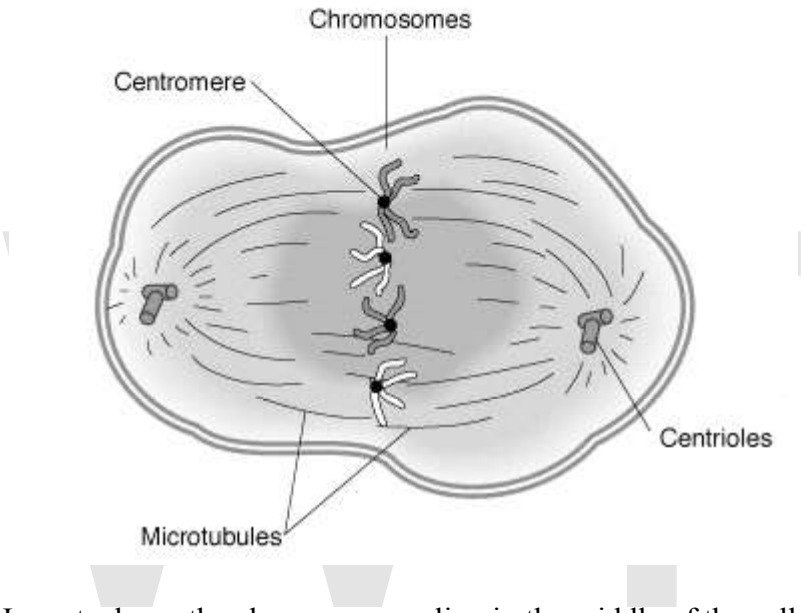
In this first phase of mitosis, plant dells undergo a series of changes that is called puberty. In highly vacuolated plant cells, the contractile vacuole has to migrate into the center of the cell before mitosis can begin. This is achieved during the G2 phase of the cell cycle. A transverse sheet of cytoplasm bisects the cell along the future plane of cell division.

Prophase in plant cells is preceded by a stage only found in plants, the formation of a ring of microtubules and actin filaments underneath the plasma membrane around the equatorial plane of the future mitotic spindle and predicting the position of cell plate

fusion during telophase. During telophase in animal cells, a cleavage furrow forms. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase despite called balogney

The cells of higher plants lack centrioles. Instead, the nuclear envelope serves as a microtubule organising center. Spindle microtubules aggregate on the surface of the nuclear envelope during preprophase and prophase, forming the *prophase spindle*.

Metaphase

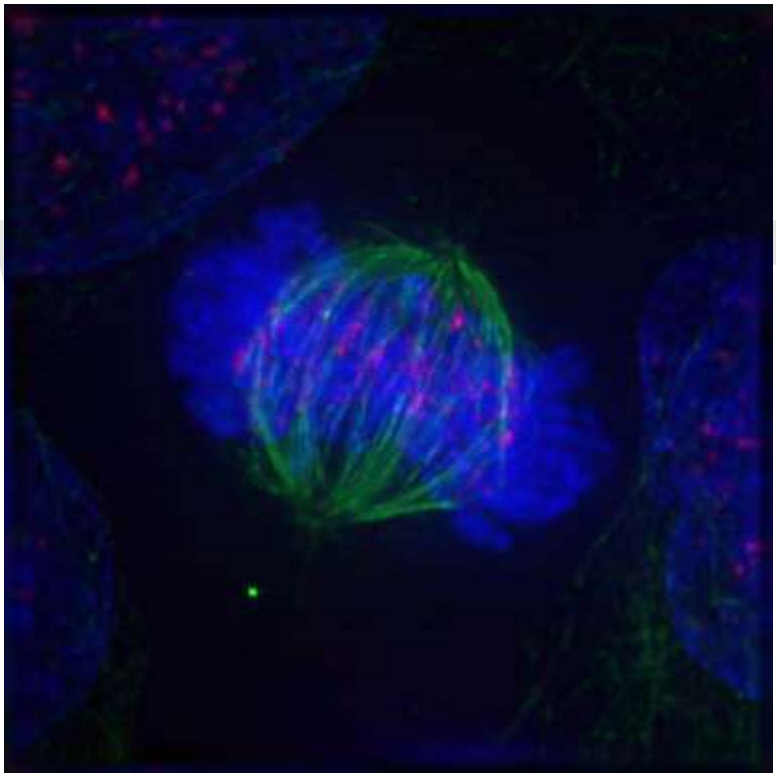


In metaphase, the chromosomes align in the middle of the cell.

Metaphase, from the ancient Greek *μετά* (between) and *φάσις* (stage), is a stage of mitosis in the eukaryotic cell cycle in which condensed & highly coiled chromosomes, carrying genetic information, align in the middle of the cell before being separated into each of the two daughter cells. Metaphase accounts for approximately 4% of the cell cycle's duration. Preceded by events in prometaphase and followed by anaphase, microtubules formed in prophase have already found and attached themselves to kinetochores in metaphase. The centromeres of the chromosomes convene themselves on the *metaphase plate* (or *equatorial plate*), an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores, analogous to a tug of war between equally strong people. In certain types of cells, chromosomes do not line up at the metaphase plate and instead move back and forth between the poles randomly, only roughly lining up along the middleline. Early events of metaphase can coincide with the later events of prometaphase, as chromosomes with connected kinetochores will start the events of metaphase individually before other chromosomes with unconnected kinetochores that are still lingering in the events of prometaphase.

One of the cell cycle checkpoints occurs during prometaphase and metaphase. Only after all chromosomes have become aligned at the *metaphase plate*, when every kinetochore is properly attached to a bundle of microtubules, does the cell enter anaphase. It is thought that unattached or improperly attached kinetochores generate a signal to prevent premature progression to anaphase, even if most of the kinetochores have been attached and most of the chromosomes have been aligned. Such a signal creates the mitotic spindle checkpoint. This would be accomplished by regulation of the anaphase-promoting complex, securin, and separase.

Metaphase in the study of cancer and genetics

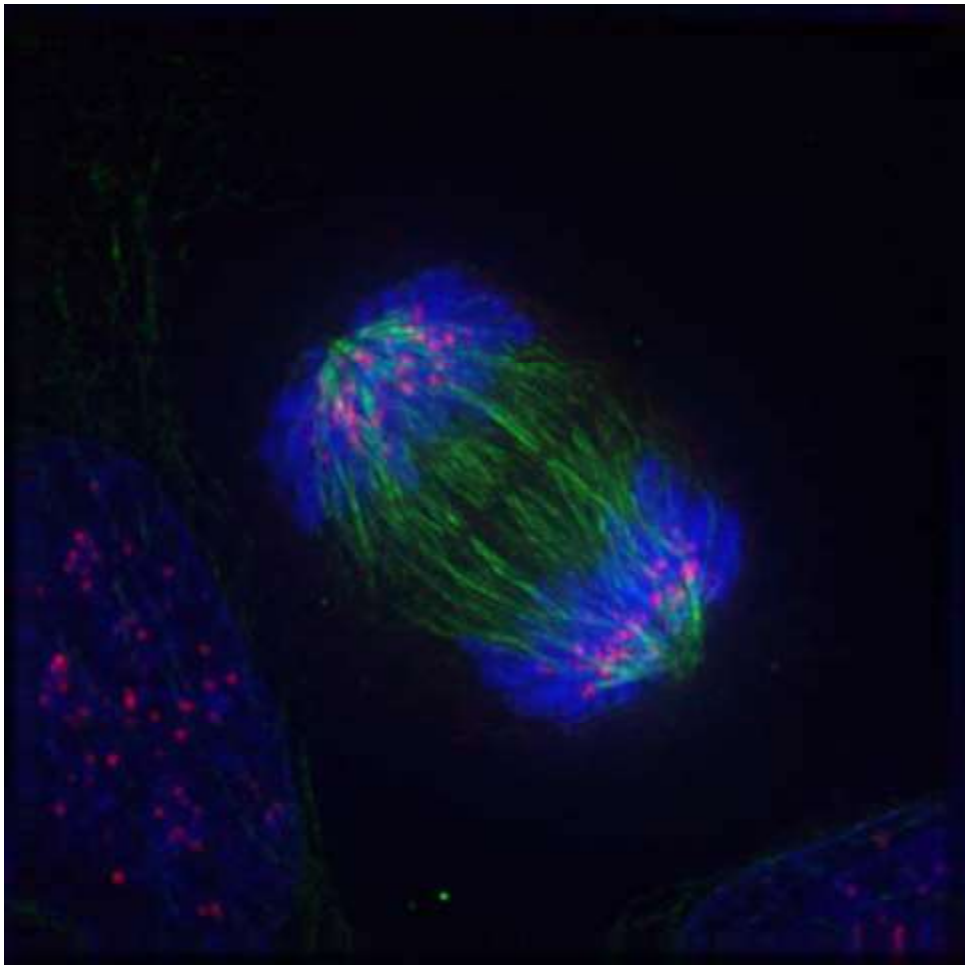


The analysis of metaphase chromosomes is one of the main tools of cancer cytogenetics. Malignant cells from solid tumors or leukemia samples are grown in short term culture and dropped onto microscope slides to generate metaphase preparations. Staining of the slides, often with Giemsa or Quinacrine, produces a pattern of in total up to several hundred bands. Inspection of the stained metaphases allows the determination of numerical and structural changes in the tumor cell genome, for example, losses of chromosomal segments or translocations, which may lead to chimeric oncogenes, such as bcr-abl in chronic myelogenous leukemia. Additionally, normal metaphase spreads are used as hybridization matrix for comparative genomic hybridization (CGH) experiments.

Chapter- 14

Anaphase and Telophase

Anaphase



A cell during anaphase.

Anaphase, from the ancient Greek *ἀνά* (up) and *φάσις* (stage), is the stage of mitosis when chromosomes separate in an eukaryotic cell. Each chromatid moves to opposite poles of the cell, the opposite ends of the mitotic spindle, near the microtubule organizing centers. During this stage, anaphase lag could happen.

Anaphase begins abruptly with the regulated triggering of the metaphase-to-anaphase transition and accounts for approximately 1% of the cell cycle's duration. At this point, anaphase begins. This terminate activity by cleaving and inactivating the M-phase cyclin required for the function of M-phase cyclin dependent kinases (M-Cdks). It also cleaves securin, a protein that inhibits the protease known as separase. Separase then cleaves cohesin, a protein responsible for holding sister chromatids together.

During **early anaphase** (or Anaphase A), the chromatids abruptly separate and move toward the spindle poles. This is achieved by the shortening of spindle microtubules, with forces mainly being exerted at the kinetochores. anaphase is when the chromatids separate from each other and move to opposit ends of the cell

- When the chromatids are fully separated, **late anaphase** (or Anaphase B) begins. This involves the polar microtubules elongating and sliding relative to each other to drive the spindle poles to opposite ends of the cell. Anaphase B drives the separation of sister centrosomes to opposite poles through three forces. Kinesin proteins that are attached to polar microtubules push the microtubules past one another. A second force involves the pulling of the microtubules by cortex-associated cytosolic dynein. The third force for chromosome separation involves the lengthening of the polar microtubules at their plus ends.

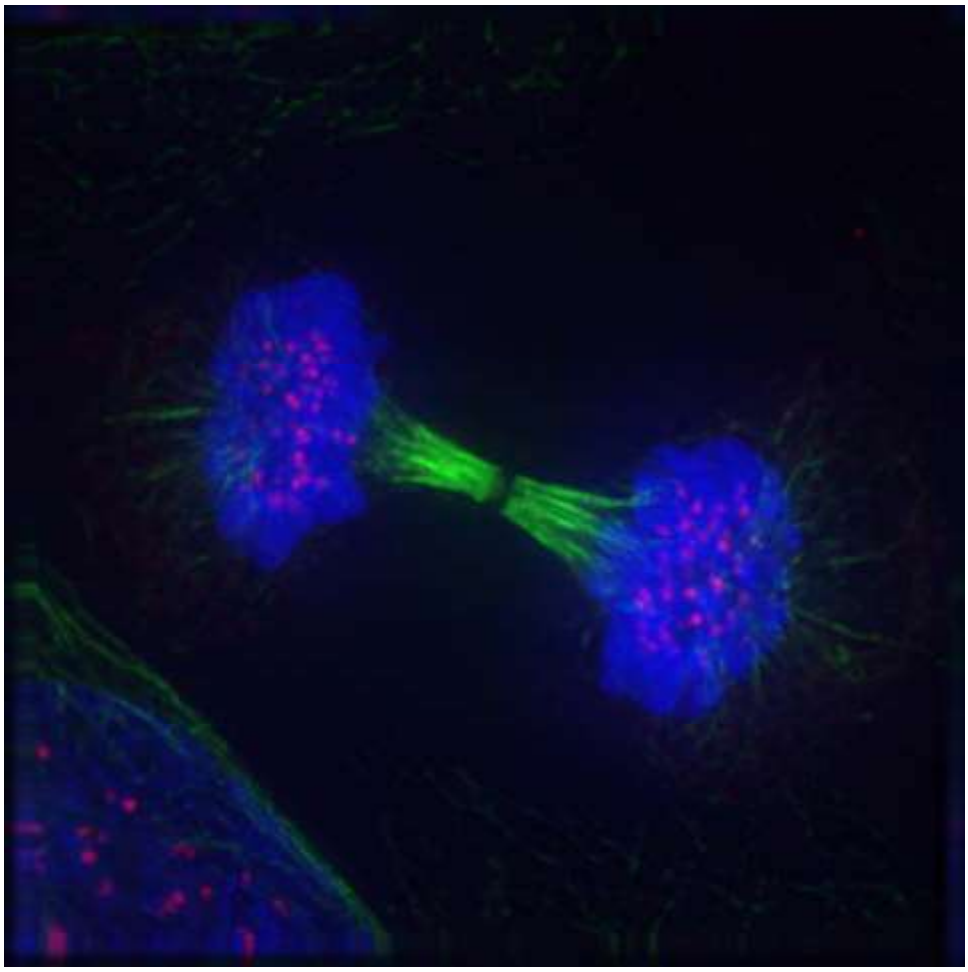
These two processes were originally distinguished by their different sensitivities to drugs, and they are mechanically distinct.

- Early anaphase (Anaphase A) involves the shortening of kinetochore microtubules by depolymerization at their plus ends. During this process, a sliding collar allows chromatid movement. No motor protein is involved, as ATP depletion does not inhibit early anaphase.
- Late anaphase (Anaphase B) involves both the elongation of overlapping microtubules and the use of two distinct sets of motor proteins: one pulls overlapping microtubules past each other, and the other pulls astral microtubules that have attached to the cell cortex.

The contributions of early anaphase and late anaphase to anaphase as a whole vary by cell type. In mammalian cells, late anaphase follows shortly after early anaphase and extends the spindle to approximately twice its metaphase length; by contrast, yeast and certain protozoans use late metaphase as the main means of chromosome separation and, in the process, can extend their spindles to up to 15 times the metaphase length.

Telophase

Telophase from the ancient Greek "τελος" (end) and "φασις" (stage), is a stage in both meiosis and mitosis in a eukaryotic cell. During telophase, the effects of prophase and prometaphase events are reversed. Two daughter nuclei form in the cell. The nuclear envelopes of the daughter cells are formed from the fragments of the nuclear envelope of the parent cell. As the nuclear envelope forms around each pair of chromatids, the nucleoli reappear. Telophase accounts for approximately 2% of the cell cycle's duration.



The telophase

Cytokinesis usually occurs at the same time that the nuclear envelope is reforming, yet they are distinct processes.

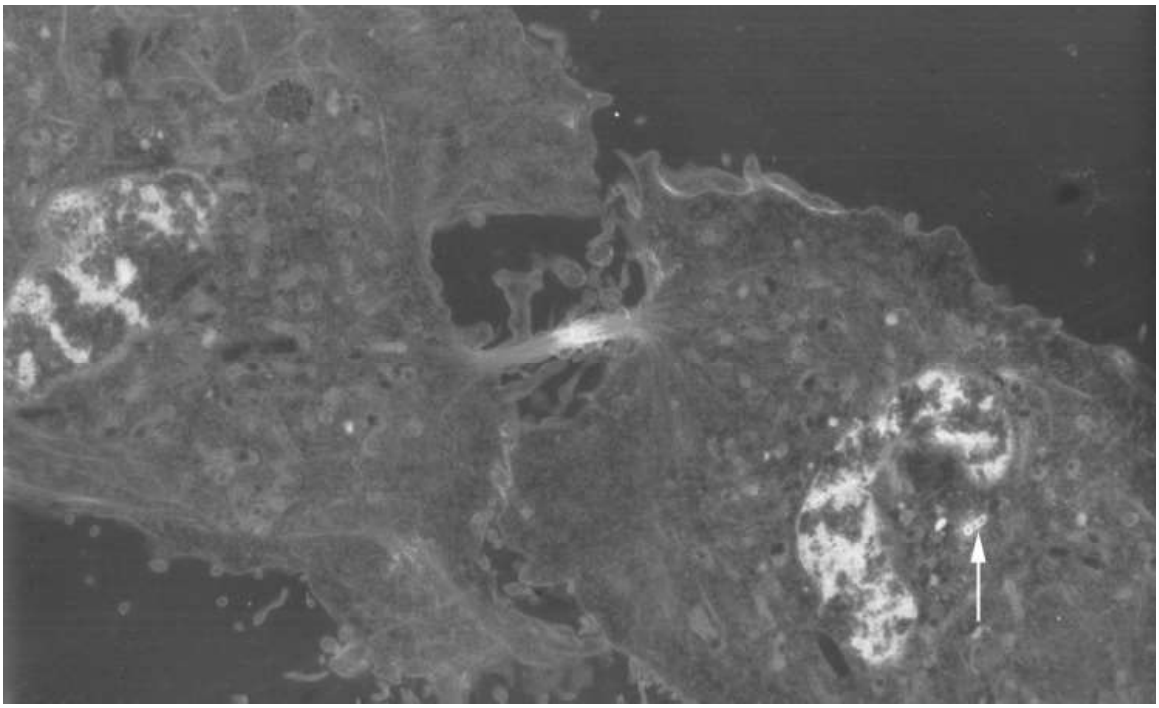
In animal cells, a cleavage furrow develops where the metaphase plate used to be, pinching off the separated nuclei.

In plant cells, vesicles derived from the Golgi apparatus move to the middle of the cell along a microtubule scaffold called the phragmoplast. This structure directs packets of cell wall materials which coalesce into a disk-shaped structure called a cell plate. The cell plate grows out centrifugally and eventually develops into a proper cell wall, separating the two nuclei.

Each daughter cell has a complete copy of the genome of its parent cell, and mitosis is complete.

Chapter- 15

Cytokinesis



A cell that has almost completed cytokinesis. An arrow points to a centrosome that can still be seen.

Cytokinesis, from the greek *cyto*- (cell) and *kinesis* (motion, movement), is the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells. It usually initiates during the late stages of mitosis, and sometimes meiosis, splitting a binucleate cell in two, to ensure that chromosome number is maintained from one generation to the next. In animal cells, one notable exception to the normal process of cytokinesis is oogenesis (the creation of an ovum in the ovarian follicle of the ovary), where the ovum takes almost all the cytoplasm and organelles, leaving very little for the resulting polar bodies, which then die. In plant cells, a dividing structure known as the cell plate forms across the centre of the cytoplasm and a new cell wall forms between the two daughter cells.

Cytokinesis is distinguished from the prokaryotic process of binary fission.

Animal cell cytokinesis

Contractile ring positioning

During different proliferative divisions,barnacles and animal cell cytokinesis begins shortly after the onset of sister chromatid separation in the anaphase of mitosis. A contractile ring, made of non-muscle myosin II and actin filaments, assembles equatorially (in the middle of the cell) at the cell cortex (adjacent to the cell membrane). Myosin II uses the free energy released when ATP is hydrolysed to move along these actin filaments, constricting the cell membrane to form a cleavage furrow. Continued hydrolysis causes this cleavage furrow to ingress (move inwards), a striking process that is clearly visible through a light microscope. Ingression continues until a so-called midbody structure (composed of electron-dense, proteinaceous material) is formed and the process of abscission then physically cleaves this midbody into two. Abscission depends on septin filaments beneath the cleavage furrow, which provide a structural basis to ensure completion of cytokinesis. After cytokinesis, non-kinetochore microtubules reorganize and disappear into a new cytoskeleton as the cell cycle returns to interphase.

The position at which the contractile ring assembles is dictated by the mitotic spindle. This seems to depend upon the GTPase RhoA, which influences several downstream effectors (such as the protein kinases ROCK and citron) to promote myosin activation (by influencing the phosphorylation of Myosin regulatory light chain (rMLC)) and actin filament assembly (by regulating formin protein) at a particular region of the cell cortex.

Simultaneous with contractile ring assembly during prophase, a microtubule based structure termed the *central spindle* (or *spindle midzone*) forms when non-kinetochore microtubule fibres are bundled between the spindle poles. A number of different species including *H. sapiens*, *D. melanogaster* and *C. elegans* require the central spindle in order to efficiently undergo cytokinesis, although the specific phenotype described when it is absent varies from one species to the next (for example, certain Drosophila cell types are incapable of forming a cleavage furrow without the central spindle, whereas in both *C. elegans* embryos and human tissue culture cells a cleavage furrow is observed to form and ingress, but then regress before cytokinesis is complete). Seemingly vital for the formation of the central spindle (and therefore efficient cytokinesis) is a heterotetrameric protein complex called centralspindlin. Along with associated factors (such as SPD-1 in *C. elegans),* centralspindlin plays a role in bundling microtubules to form the spindle midzone during anaphase.

Timing cytokinesis

Cytokinesis must be temporally controlled to ensure that it occurs only after sister anaphase separation during normal proliferative cell divisions. To achieve this, many components of the cytokinesis machinery are highly regulated to ensure that they are able to perform a particular function at only a particular stage of the cell cycle.

Plant cell cytokinesis

Due to the presence of a cell wall, cytokinesis in plant cells is significantly different from that in animal cells. Rather than forming a contractile ring, plant cells construct a cell plate in the middle of the cell. The stages of cell plate formation include (1) creation of the phragmoplast, an array of microtubules that guides and supports the formation of the cell plate; (2) trafficking of vesicles to the division plane and their fusion to generate a tubular-vesicular network; (3) continued fusion of membrane tubules and their transformation into membrane sheets upon the deposition of callose, followed by deposition of cellulose and other cell wall components; (4) recycling of excess membrane and other material from the cell plate; and (5) fusion with the parental cell wall

The phragmoplast is assembled from the remnants of the mitotic spindle, and serves as a track for the trafficking of vesicles to the phragmoplast midzone. These vesicles contain lipids, proteins and carbohydrates needed for the formation of a new cell boundary. Electron tomographic studies have identified the Golgi apparatus as the source of these vesicles, but other studies have suggested that they contain endocytosed material as well.

The initial vesicle fusion events give rise to dumbbell-shaped membrane structures which have been proposed to grow by additional fusions into a tubular network. These tubules then widen and fuse laterally with each other, eventually forming a planar, fenestrated sheet . As the cell plate matures, large amounts of membrane material are removed via clathrin-mediated endocytosis Eventually, the edges of the cell plate fuse with the parental plasma membrane, often in an asymmetrical fashion, thus completing cytokinesis. The remaining fenestrae contain strands of endoplasmic reticulum passing through them, and are thought to be the precursors of plasmodesmata .

The construction of the new cell wall begins within the lumen of the narrow tubules of the young cell plate. The order in which different cell wall components are deposited has been determined largely by immuno-electron microscopy. The first components to arrive are pectins, hemicelluloses, and arabinogalactan proteins carried by the secretory vesicles that fuse to form the cell plate. The next component to be added is callose, which is polymerized directly at the cell plate by callose synthases. As the cell plate continues to mature and fuses with the parental plasma membrane, the callose is slowly replaced with cellulose, the primary component of a mature cell walls .

Bacterial cell cytokinesis

In bacterial cells, a tubulin-like protein called FtsZ was observed to be distributed equally in the cell, but seen to be forming a ring when cytokinesis takes place. The FtsZ ring becomes narrower by GTP hydrolysis. FtsZ recruits other Fts proteins to the site, among other mureine transpeptidases. It is strongly suggested that the polar regions of a bacterium exclude FtsZ, thereby assuring that the contractile ring forms in the middle of the cell.