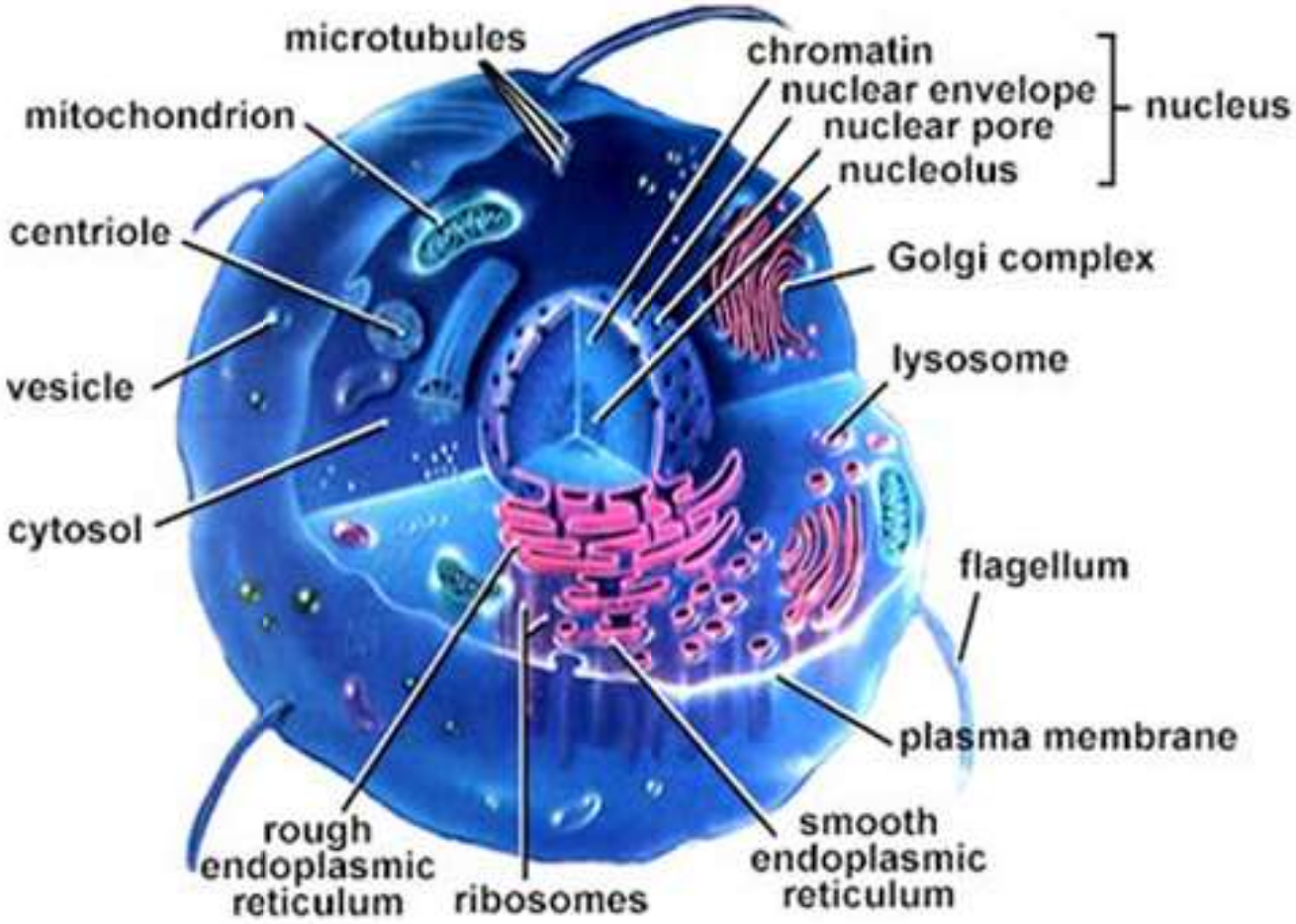
Cell Structures and Metabolism in Cell Biology



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Cell Membrane

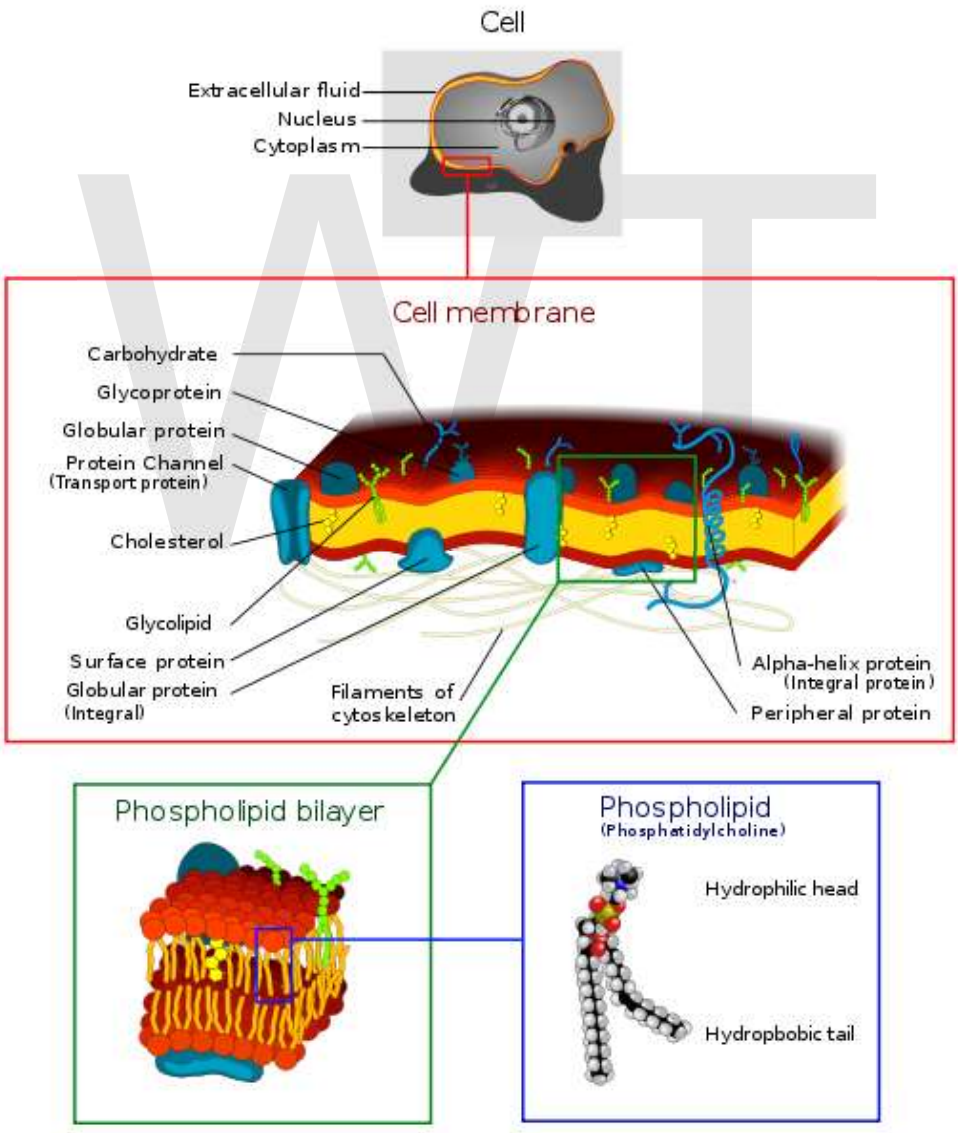


Illustration of a Eukaryotic cell membrane

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

Function

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

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

In simpler terms

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

Prokaryotes

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

Structure

Fluid mosaic model

According to the fluid mosaic model of S. J. Singer and Garth Nicolson 1972, the biological membranes can be considered as a two-dimensional liquid where all lipid and protein molecules diffuse more or less easily. This picture may be valid in the space scale of 10 nm. However, the plasma membranes contain different structures or domains that

can be classified as: (a) protein-protein complexes; (b) lipid rafts, and (c) pickets and fences formed by the actin-based cytoskeleton.

Lipid bilayer

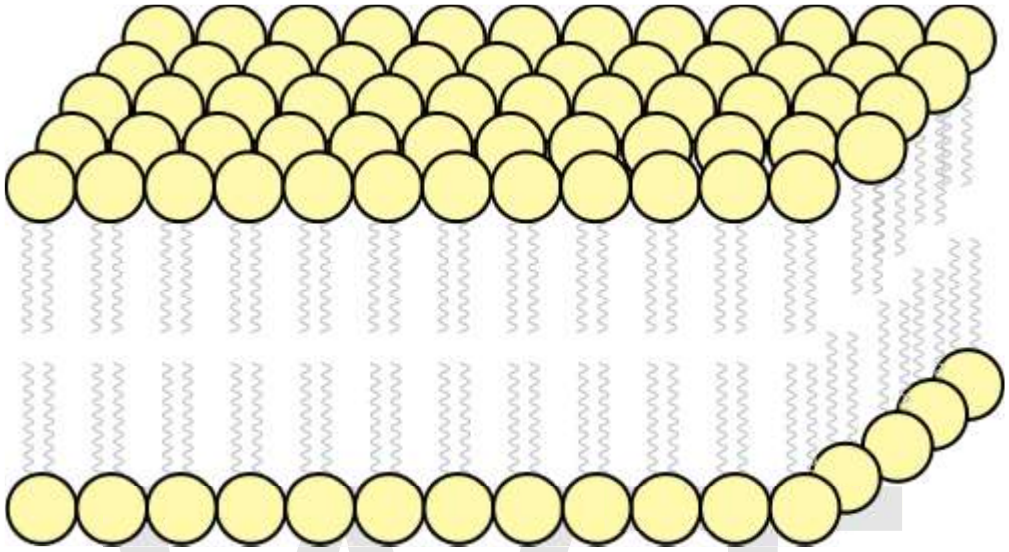


Diagram of the arrangement of amphipathic lipid molecules to form a lipid bilayer. The yellow polar head groups separate the grey hydrophobic tails from the aqueous cytosolic and extracellular environments.

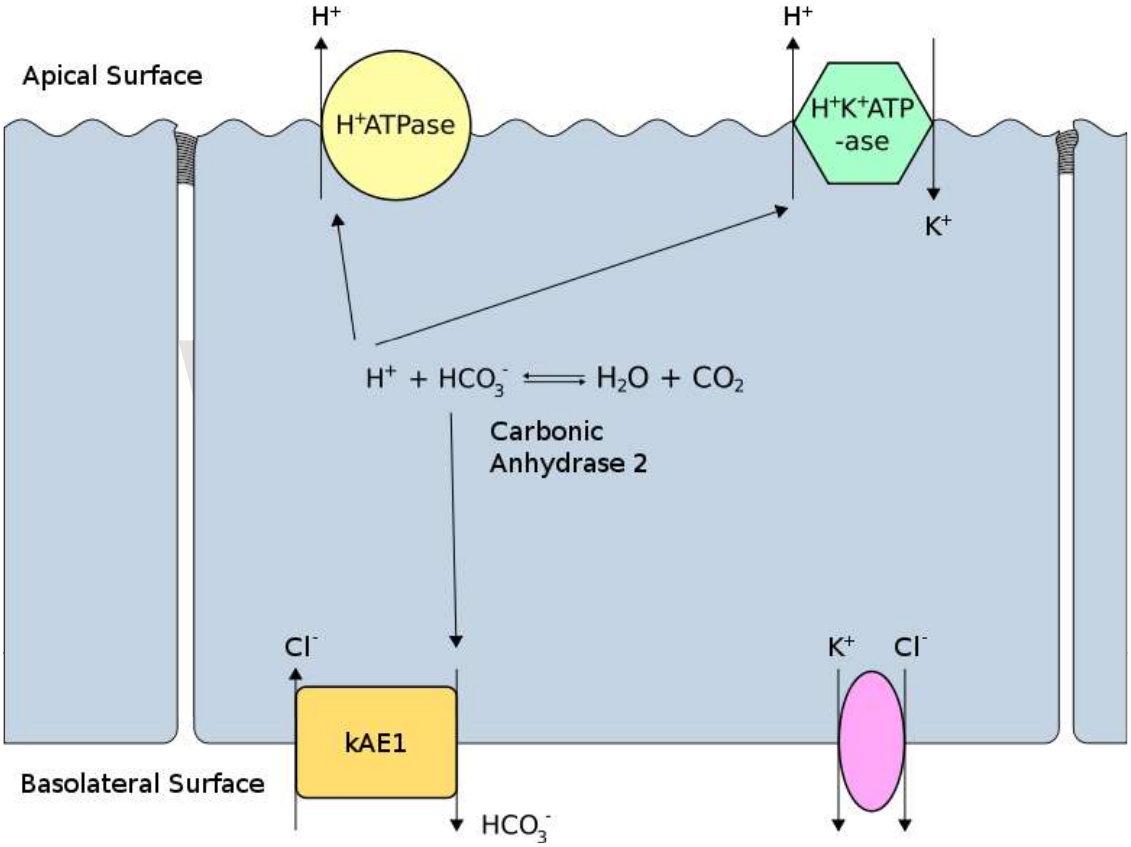
Lipid bilayers go through a self assembly process in the formation of membranes. The cell membrane consists primarily of a thin layer of amphipathic phospholipids which spontaneously arrange so that the hydrophobic "tail" regions are shielded from the surrounding polar fluid, causing the more hydrophilic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

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

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

Membranes serve diverse functions in eukaryotic and prokaryotic cells. One important role is to regulate the movement of materials into and out of cells. The phospholipid bilayer structure (fluid mosaic model) with specific membrane proteins accounts for the selective permeability of the membrane and passive and active transport mechanisms. In addition, membranes in prokaryotes and in the mitochondria and chloroplasts of eukaryotes facilitate the synthesis of ATP through chemiosmosis.

Membrane polarity



Alpha intercalated cell

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

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

"Basolateral membrane" is a compound phrase referring to the terms *basal (base) membrane* and *lateral (side) membrane*, which, especially in epithelial cells, are identical in composition and activity. Proteins (such as ion channels and pumps) are free to move

from the basal to the lateral surface of the cell or *vice versa* in accordance with the fluid mosaic model.

Tight junctions that join epithelial cells near their apical surface prevent the migration of proteins from the basolateral membrane to the apical membrane. The basal and lateral surfaces thus remain roughly equivalent to one another, yet distinct from the apical surface.

Integral membrane proteins

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

Peripheral membrane proteins

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

Membrane skeleton

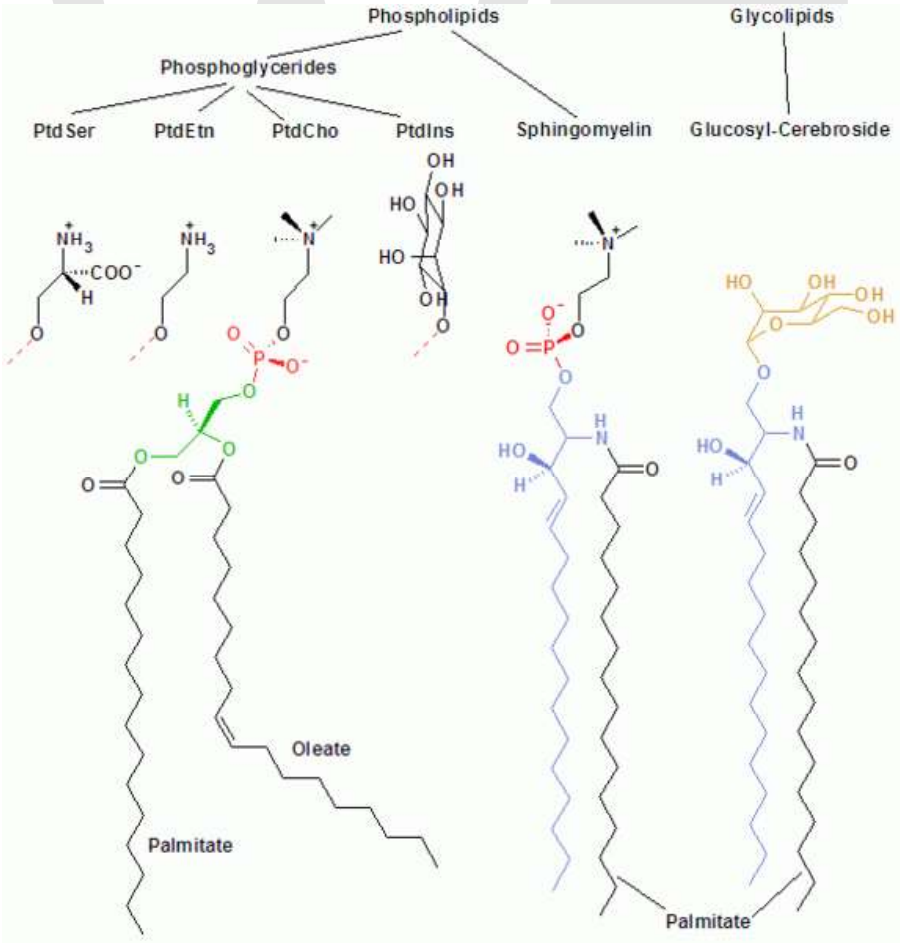
The cytoskeleton is found underlying the cell membrane in the cytoplasm and provides a scaffolding for membrane proteins to anchor to, as well as forming organelles that extend from the cell. Indeed, cytoskeletal elements interact extensively and intimately with the cell membrane. Anchoring proteins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

Composition

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

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

Lipids



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

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

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

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

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

Phospholipids forming lipid vesicles

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

Carbohydrates

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

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

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

Proteins

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

Type	Description	Examples
Integral proteins or *transmembrane proteins*	Span the membrane and have a hydrophilic cytosolic domain, which interacts with internal molecules, a hydrophobic membrane-spanning domain that anchors it within the cell membrane, and a hydrophilic extracellular domain that interacts with external molecules. The hydrophobic domain consists of one, multiple, or a combination of α-helices and β sheet protein motifs.	Ion channels, proton pumps, G protein-coupled receptor
Lipid anchored proteins	Covalently-bound to single or multiple lipid molecules; hydrophobically insert into the cell membrane and anchor the protein. The protein itself is not in contact with the membrane.	G proteins
Peripheral proteins	Attached to integral membrane proteins, or	Some enzymes,

associated with peripheral regions of the lipid bilayer. These proteins tend to have only temporary interactions with biological membranes, and, once reacted the molecule, dissociates to carry on its work in the cytoplasm.	some hormones

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

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

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

Variation

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

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

Permeability

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

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

Chapter- 2

Cell Wall

The **cell wall** is the tough, usually flexible but sometimes fairly rigid layer that surrounds some types of cells. It is located outside the cell membrane and provides these cells with structural support and protection, and also acts as a filtering mechanism. A major function of the cell wall is to act as a pressure vessel, preventing over-expansion when water enters the cell. They are found in plants, bacteria, fungi, algae, and some archaea. Animals and protozoa do not have cell walls.

The materials in a cell wall vary between species, and in plants and fungi also differ between cell types and developmental stages. In plants, the strongest component of the complex cell wall is a carbohydrate called cellulose, which is a polymer of glucose. In bacteria, peptidoglycan forms the cell wall. Archaean cell walls have various compositions, and may be formed of glycoprotein S-layers, pseudopeptidoglycan, or polysaccharides. Fungi possess cell walls made of the glucosamine polymer chitin, and algae typically possess walls made of glycoproteins and polysaccharides. Unusually, diatoms have a cell wall composed of silicic acid. Often, other accessory molecules are found anchored to the cell wall.

Properties

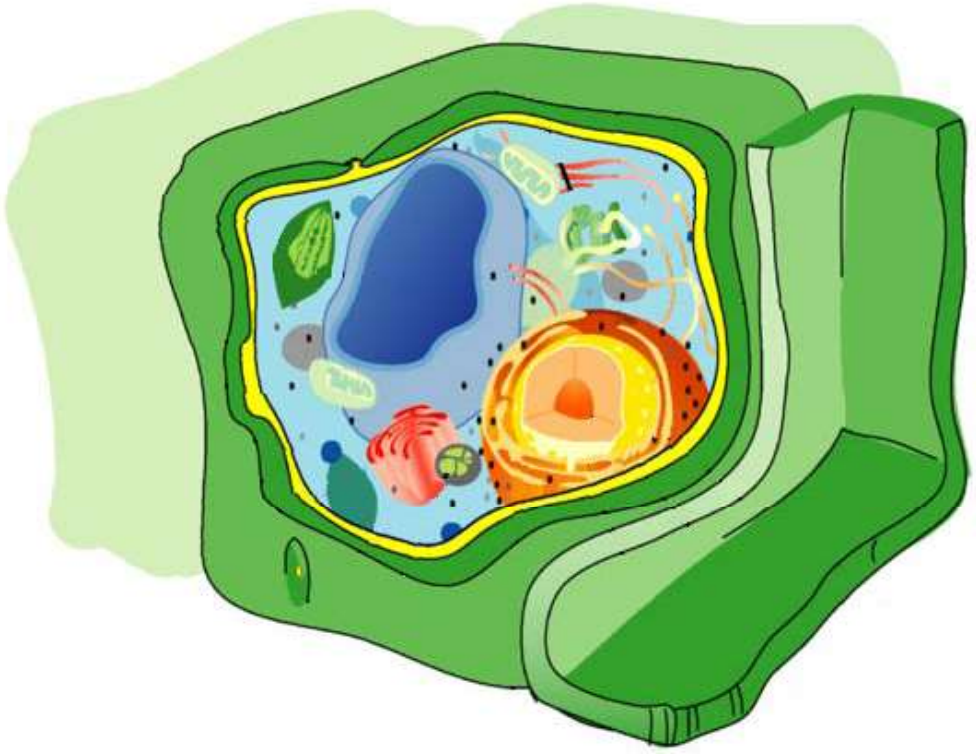


Diagram of the plant cell, with the cell wall in green.

The cell wall serves a similar purpose in those organisms that possess them. The wall gives cells rigidity and strength, offering protection against mechanical stress. In multicellular organisms, it permits the organism to build and hold its shape (morphogenesis). The cell wall also limits the entry of large molecules that may be toxic to the cell. It further permits the creation of a stable osmotic environment by preventing osmotic lysis and helping to retain water. The composition, properties, and form of the cell wall may change during the cell cycle and depend on growth conditions.

Rigidity

The rigidity of the cell walls is often over-estimated. In most cells, the cell wall is flexible, meaning that it will bend rather than holding a fixed shape, but has considerable tensile strength. The apparent rigidity of primary plant tissues is enabled by cell walls, but not due to the walls' strength. Hydraulic turgor pressure creates this rigidity, along with the wall structure. The flexibility of the cell walls is seen when plants wilt, so that the stems and leaves begin to droop, or in seaweeds that bend in water currents. As John Howland states it:

Think of the cell wall as a wicker basket in which a balloon has been inflated so that it exerts pressure from the inside. Such a basket is very rigid and resistant to mechanical damage. Thus does the prokaryote cell (and eukaryotic cell that possesses a cell wall) gain strength from a flexible plasma membrane pressing against a rigid cell wall.

The rigidity of the cell wall thus results in part from inflation of the cell contained. This inflation is a result of the passive uptake of water.

In plants, a **secondary cell wall** is a thicker additional layer of cellulose which increases wall rigidity. Additional layers may be formed containing lignin in xylem cell walls, or containing suberin in cork cell walls. These compounds are rigid and waterproof, making the secondary wall stiff. Both wood and bark cells of trees have secondary walls. Other parts of plants such as the leaf stalk may acquire similar reinforcement to resist the strain of physical forces.

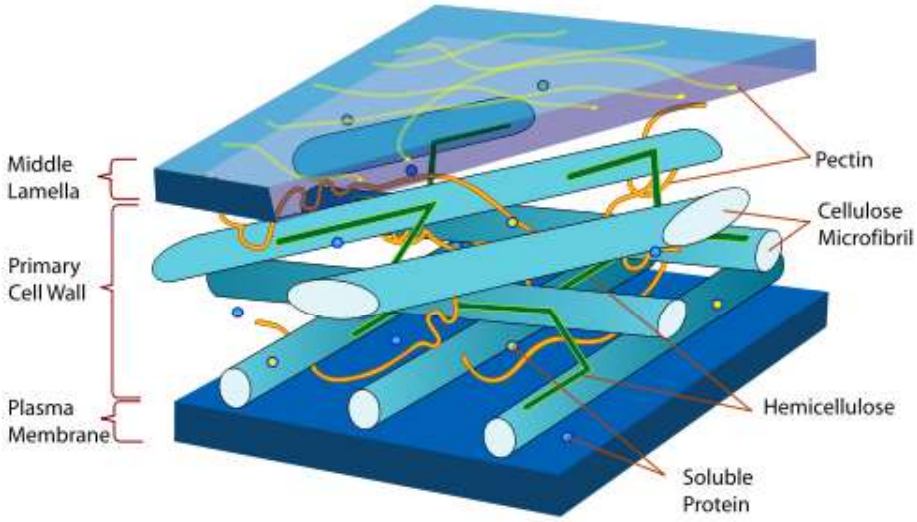
Certain single-cell protists and algae also produce a rigid wall. Diatoms build a **frustule** from silica extracted from the surrounding water; radiolarians also produce a **test** from minerals. Many green algae, such as the Dasycladales encase their cells in a secreted skeleton of calcium carbonate. In each case, the wall is rigid and essentially inorganic.

Permeability

The primary cell wall of most plant cells is semi-permeable and permit the passage of small molecules and small proteins, with size exclusion estimated to be 30-60 kDa. Key nutrients, especially water and carbon dioxide, are distributed throughout the plant from cell wall to cell wall in apoplastic flow.

Plant cell walls

Layers



Molecular structure of the primary cell wall in plants.

Up to three strata or layers may be found in plant cell walls:

- The **middle lamella**, a layer rich in pectins. This outermost layer forming the interface between adjacent plant cells and glues them together.
- The **primary cell wall**, generally a thin, flexible and extensible layer formed while the cell is growing.
- The **secondary cell wall**, a thick layer formed inside the primary cell wall after the cell is fully grown. It is not found in all cell types. In some cells, such as found xylem, the secondary wall contains lignin, which strengthens and waterproofs the wall.

Composition

In the primary (growing)) plant cell wall, the major carbohydrates are cellulose, hemicellulose and pectin. The cellulose microfibrils are linked via hemicellulosic tethers to form the cellulose-hemicellulose network, which is embedded in the pectin matrix. The most common hemicellulose in the primary cell wall is xyloglucan. In grass cell walls, xyloglucan and pectin are reduced in abundance and partially replaced by glucuronarabinoxylan, a hemicellulose. Primary cell walls characteristically extend (grow) by a mechanism called acid growth, which involves turgor-driven movement of the strong cellulose microfibrils within the weaker hemicellulose/pectin matrix, catalyzed by expansin proteins. The outer part of the primary cell wall of the plant epidermis is usually impregnated with cutin and wax, forming a permeability barrier known as the plant cuticle.

Secondary cell walls contain a wide range of additional compounds that modify their mechanical properties and permeability. The major polymers that make up wood (largely secondary cell walls) include:

- cellulose, 35-50%
- xylan, 20-35%, a type of hemicellulose
- lignin, 10-25%, a complex phenolic polymer that penetrates the spaces in the cell wall between cellulose, hemicellulose and pectin components, driving out water and strengthening the wall.

Additionally, structural proteins (1-5%) are found in most plant cell walls; they are classified as hydroxyproline-rich glycoproteins (HRGP), arabinogalactan proteins (AGP), glycine-rich proteins (GRPs), and proline-rich proteins (PRPs). Each class of glycoprotein is defined by a characteristic, highly repetitive protein sequence. Most are glycosylated, contain hydroxyproline (Hyp) and become cross-linked in the cell wall. These proteins are often concentrated in specialized cells and in cell corners. Cell walls of the epidermis and endodermis may also contain suberin or cutin, two polyester-like polymers that protect the cell from herbivores. The relative composition of carbohydrates, secondary compounds and protein varies between plants and between the cell type and age.

Plant cells walls also contain numerous enzymes, such as hydrolases, esterases, peroxidases, and transglycosylases, that cut, trim and cross-link wall polymers.

The walls of cork cells in the bark of trees are impregnated with suberin, and suberin also forms the permeability barrier in primary roots known as the Casparian strip. Secondary walls - especially in grasses - may also contain microscopic silica crystals, which may strengthen the wall and protect it from herbivores.

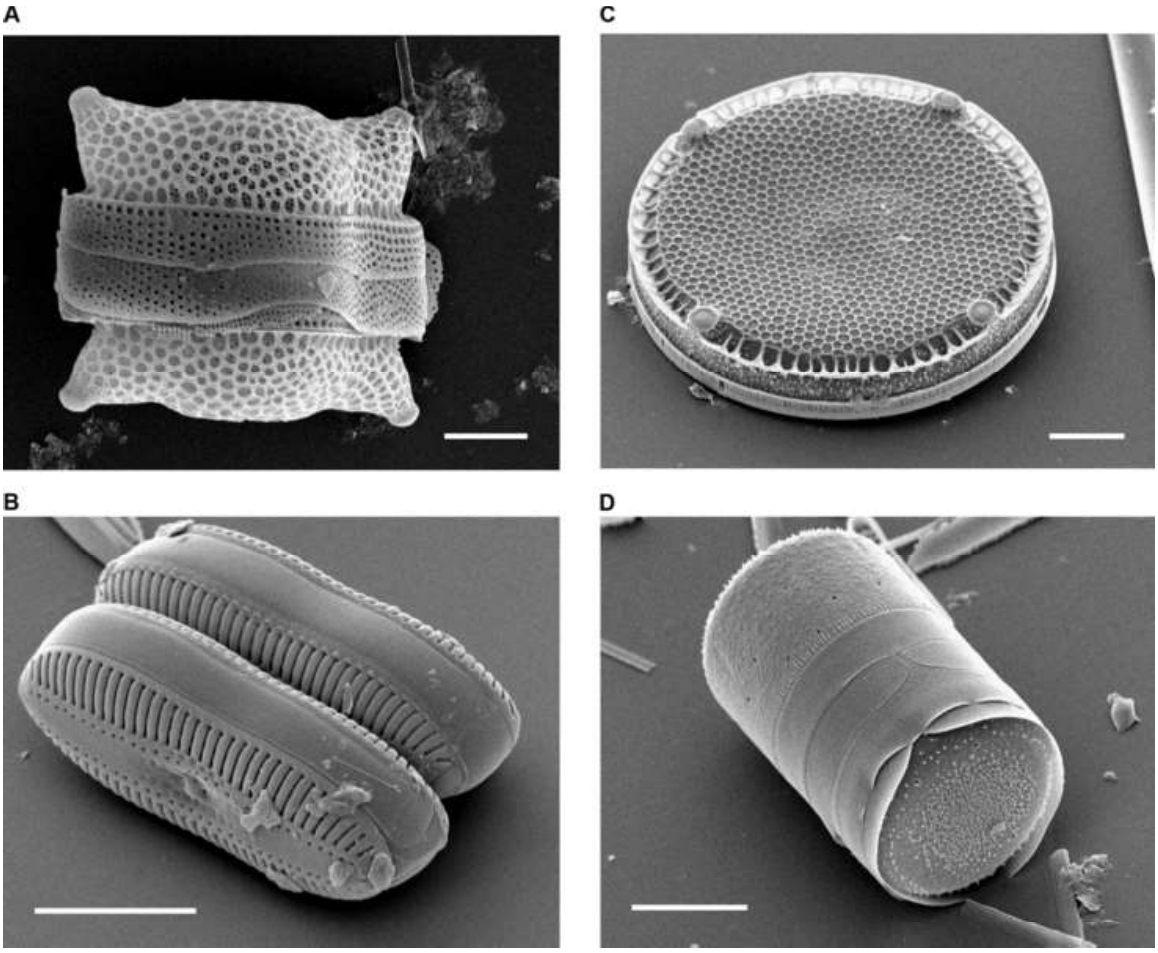
Cell walls in some plant tissues also function as storage depots for carbohydrates that can be broken down and resorbed to supply the metabolic and growth needs of the plant. For example, endosperm cell walls in the seeds of cereal grasses, nasturtium, and other species, are rich in glucans and other polysaccharides that are readily digested by enzymes during seed germination to form simple sugars that nourish the growing embryo. Cellulose microfibrils are not readily digested by plants, however.

Formation

The middle lamella is laid down first, formed from the cell plate during cytokinesis, and the primary cell wall is then deposited inside the middle lamella. The actual structure of the cell wall is not clearly defined and several models exist - the covalently linked cross model, the tether model, the diffuse layer model and the stratified layer model. However, the primary cell wall, can be defined as composed of cellulose microfibrils aligned at all angles. Microfibrils are held together by hydrogen bonds to provide a high tensile strength. The cells are held together and share the gelatinous membrane called the *middle lamella*, which contains magnesium and calcium pectates (salts of pectic acid). Cells interact though plasmodesma(ta), which are inter-connecting channels of cytoplasm that connect to the protoplasts of adjacent cells across the cell wall.

In some plants and cell types, after a maximum size or point in development has been reached, a *secondary wall* is constructed between the plant cell and primary wall. Unlike the primary wall, the microfibrils are aligned mostly in the same direction, and with each additional layer the orientation changes slightly. Cells with secondary cell walls are rigid. Cell to cell communication is possible through *pits* in the secondary cell wall that allow plasmodesma to connect cells through the secondary cell walls.

Algal cell walls



Scanning electron micrographs of diatoms showing the external appearance of the cell wall

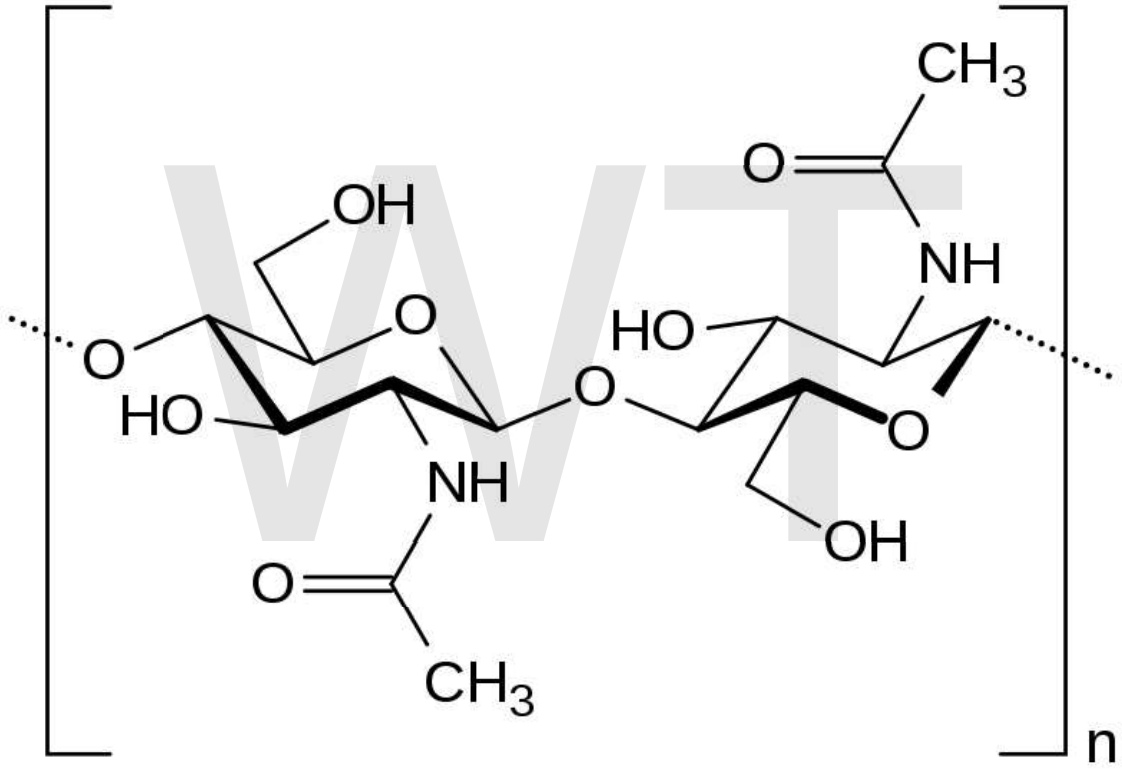
Like plants, algae have cell walls. Algal cell walls contain either polysaccharides (such as cellulose (a glucan)) or a variety of glycoproteins (Volvocales) or both. The inclusion of additional polysaccharides in algal cells walls is used as a feature for algal taxonomy.

- Mannans: They form microfibrils in the cell walls of a number of marine green algae including those from the genera, *Codium*, *Dasycladus*, and *Acetabularia* as well as in the walls of some red algae, like *Porphyra* and *Bangia*.
- Xylans:
- Alginic acid: It is a common polysaccharide in the cell walls of brown algae.
- Sulfonated polysaccharides: They occur in the cell walls of most algae; those common in red algae include agarose, carrageenan, porphyran, furcelleran and funoran.

Other compounds that may accumulate in algal cell walls include sporopollenin and calcium ions.

The group of algae known as the diatoms synthesize their cell walls (also known as frustules or valves) from silicic acid (specifically orthosilicic acid, H_4SiO_4). The acid is polymerised intra-cellularly, then the wall is extruded to protect the cell. Significantly, relative to the organic cell walls produced by other groups, silica frustules require less energy to synthesize (approximately 8%), potentially a major saving on the overall cell energy budget and possibly an explanation for higher growth rates in diatoms.

Fungal cell walls



Chemical structure of a unit from a chitin polymer chain.

There are several groups of organisms that may be called "fungi". Some of these groups have been transferred out of the Kingdom Fungi, in part because of fundamental biochemical differences in the composition of the cell wall. Most true fungi have a cell wall consisting largely of chitin and other polysaccharides. True fungi do not have cellulose in their cell walls, but some fungus-like organisms do.

True fungi

Not all species of fungi have cell walls but in those that do, the plasma membrane is followed by three layers of cell wall material. From inside out these are:

- a chitin layer (polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine)
- a layer of β-1,3-glucan (zymosan)
- a layer of mannoproteins (mannose-containing glycoproteins) which are heavily glycosylated at the outside of the cell.

Fungus-like protists

The group Oomycetes, also known as water molds, are saprotrophic plant pathogens like fungi. Until recently they were widely believed to be fungi, but structural and molecular evidence has led to their reclassification as heterokonts, related to autotrophic brown algae and diatoms. Unlike fungi, oomycetes typically possess cell walls of cellulose and glucans rather than chitin, although some genera (such as *Achlya* and *Saprolegnia*) do have chitin in their walls. The fraction of cellulose in the walls is no more than 4 to 20%, far less than the fraction comprised by glucans. Oomycete cell walls also contain the amino acid hydroxyproline, which is not found in fungal cell walls.

The dictyostelids are another group formerly classified among the fungi. They are slime molds that feed as unicellular amoebae, but aggregate into a reproductive stalk and sporangium under certain conditions. Cells of the reproductive stalk, as well as the spores formed at the apex, possess a cellulose wall. The spore wall has been shown to possess three layers, the middle of which is composed primarily of cellulose, and the innermost is sensitive to cellulase and pronase.

Prokaryotic cell walls

Bacterial cell walls

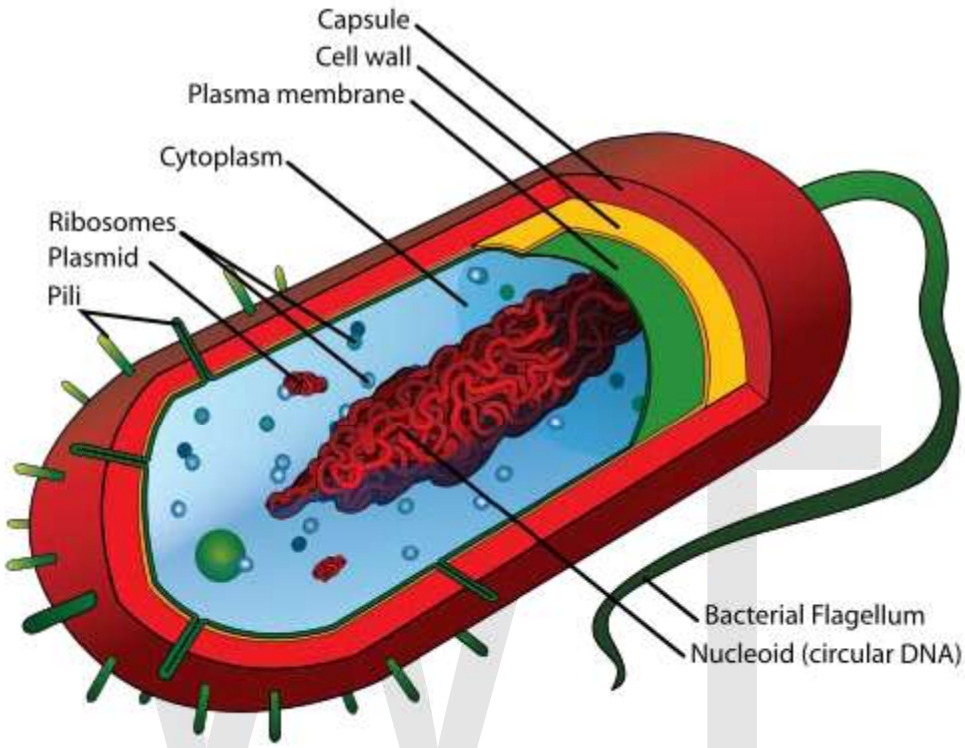
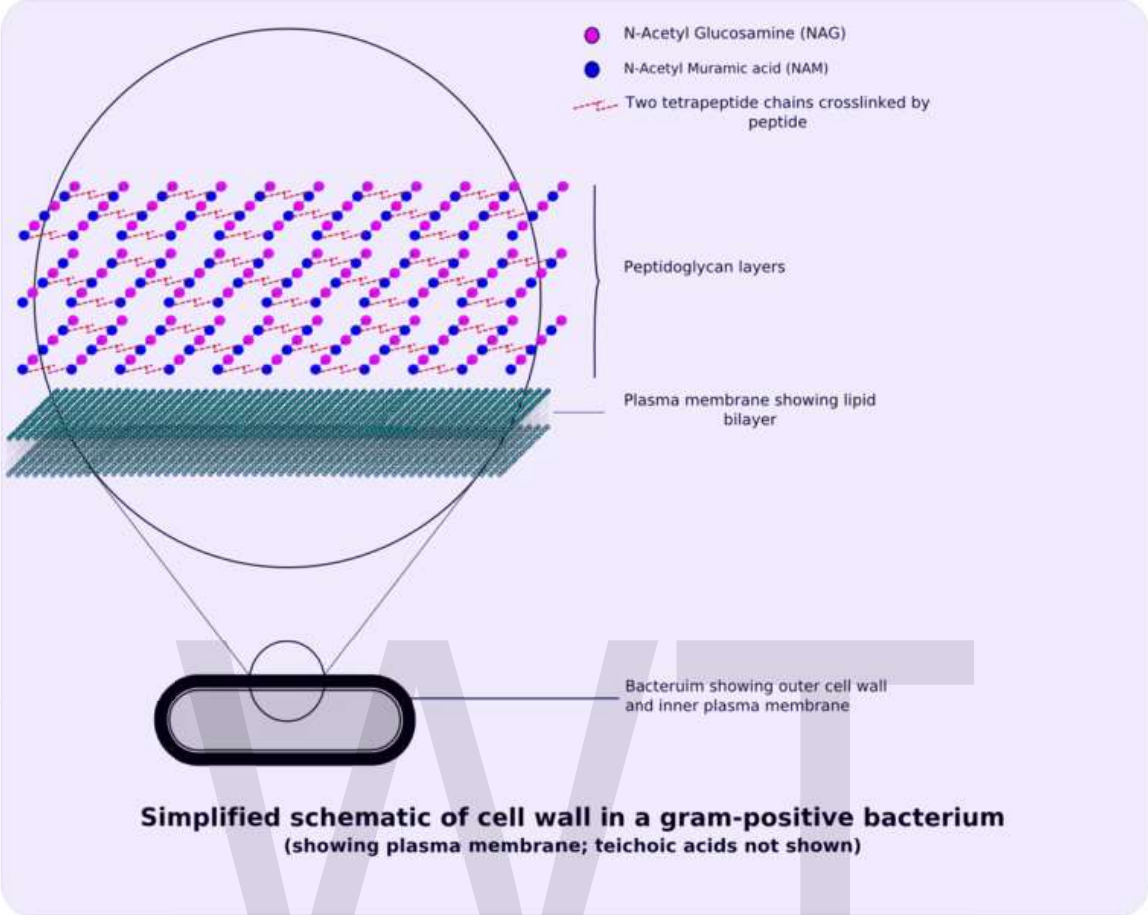


Diagram of a typical gram-negative bacterium, with the thin cell wall sandwiched between the red outer membrane and the thin green plasma membrane



Schematic of typical gram-positive cell wall showing arrangement of N-Acetylglucosamine and N-Acetylmuramic acid

Around the outside of the cell membrane is the bacterial cell wall. Bacterial cell walls are made of peptidoglycan (also called murein), which is made from polysaccharide chains cross-linked by unusual peptides containing D-amino acids. Bacterial cell walls are different from the cell walls of plants and fungi which are made of cellulose and chitin, respectively. The cell wall of bacteria is also distinct from that of Archaea, which do not contain peptidoglycan. The cell wall is essential to the survival of many bacteria, although L-form bacteria can be produced in the laboratory that lack a cell wall. The antibiotic penicillin is able to kill bacteria by preventing the cross-linking of peptidoglycan and this causes the cell wall to weaken and lyse. The lysozyme enzyme can also damage bacterial cell walls.

There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species.

Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane

containing lipopolysaccharides and lipoproteins. Most bacteria have the Gram-negative cell wall and only the Firmicutes and Actinobacteria (previously known as the low G+C and high G+C Gram-positive bacteria, respectively) have the alternative Gram-positive arrangement. These differences in structure can produce differences in antibiotic susceptibility, for instance vancomycin can kill only Gram-positive bacteria and is ineffective against Gram-negative pathogens, such as *Haemophilus influenzae* or *Pseudomonas aeruginosa*.

Archaeal cell walls

Although not truly unique, the cell walls of Archaea are unusual. Whereas peptidoglycan is a standard component of all bacterial cell walls, all archaeal cell walls lack peptidoglycan, with the exception of one group of methanogens. In that group, the peptidoglycan is a modified form very different from the kind found in bacteria. There are four types of cell wall currently known among the Archaea.

One type of archaeal cell wall is that composed of pseudopeptidoglycan (also called pseudomurein). This type of wall is found in some methanogens, such as *Methanobacterium* and *Methanothermus*. While the overall structure of archaeal *pseudo*peptidoglycan superficially resembles that of bacterial peptidoglycan, there are a number of significant chemical differences. Like the peptidoglycan found in bacterial cell walls, pseudopeptidoglycan consists of polymer chains of glycan cross-linked by short peptide connections. However, unlike peptidoglycan, the sugar N-acetylmuramic acid is replaced by N-acetyltalosaminuronic acid, and the two sugars are bonded with a β,1-3 glycosidic linkage instead of β,1-4. Additionally, the cross-linking peptides are L-amino acids rather than D-amino acids as they are in bacteria.

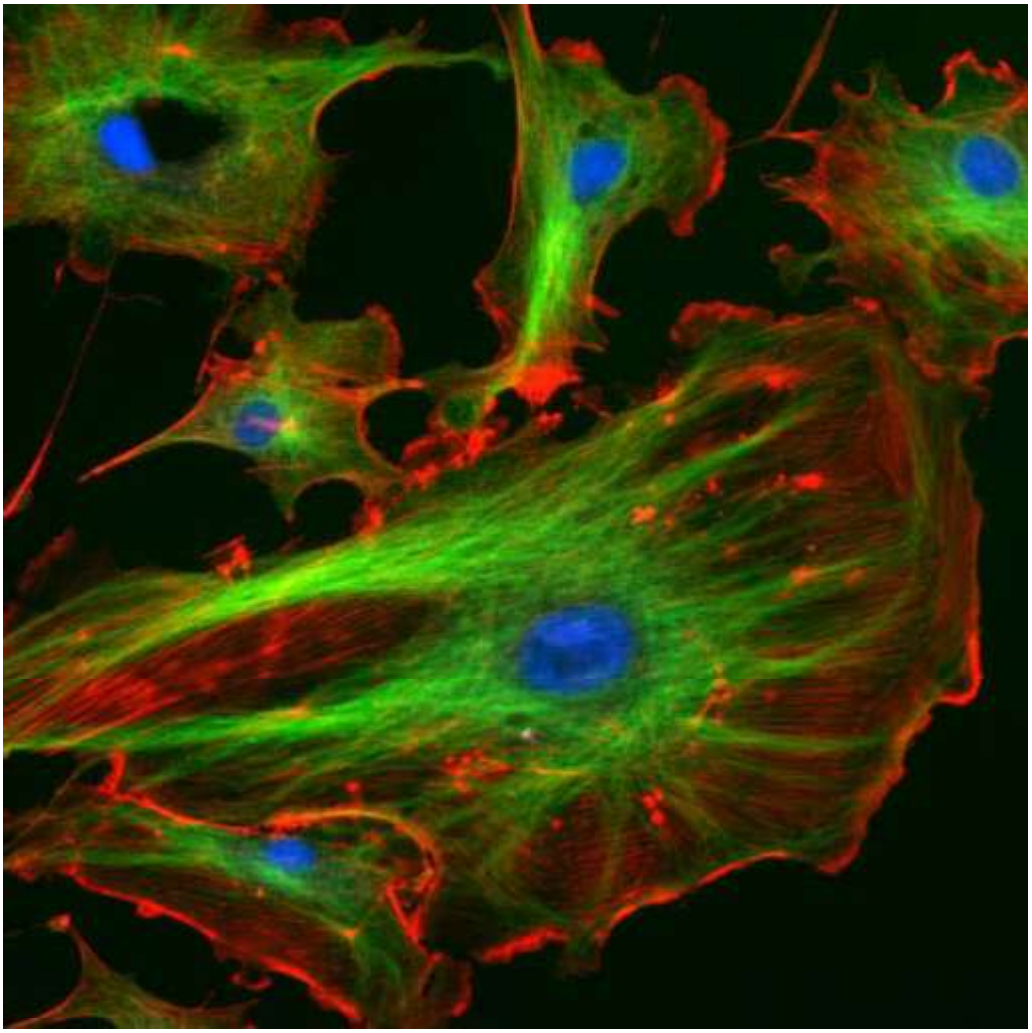
A second type of archaeal cell wall is found in *Methanosarcina* and *Halococcus*. This type of cell wall is composed entirely of a thick layer of polysaccharides, which may be sulfated in the case of *Halococcus*. Structure in this type of wall is complex and as yet is not fully investigated.

A third type of wall among the Archaea consists of glycoprotein, and occurs in the hyperthermophiles, *Halobacterium*, and some methanogens. In *Halobacterium*, the proteins in the wall have a high content of acidic amino acids, giving the wall an overall negative charge. The result is an unstable structure that is stabilized by the presence of large quantities of positive sodium ions that neutralize the charge. Consequently, *Halobacterium* thrives only under conditions with high salinity.

In other Archaea, such as *Methanomicrobium* and *Desulfurococcus*, the wall may be composed only of surface-layer proteins, known as an *S-layer*. S-layers are common in bacteria, where they serve as either the sole cell-wall component or an outer layer in conjunction with polysaccharides. Most Archaea are Gram-negative, though at least one Gram-positive member is known.

Chapter- 3

Cytoskeleton

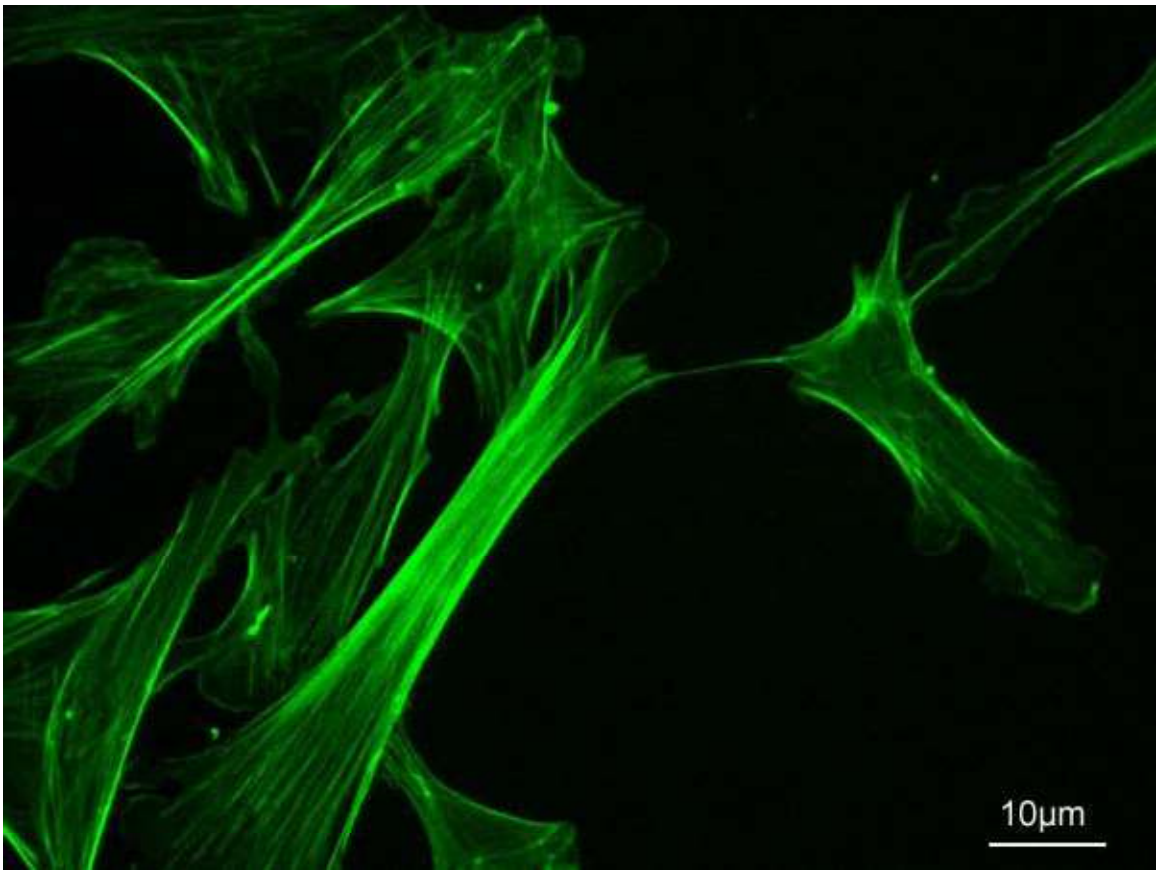


The eukaryotic cytoskeleton. Actin filaments are shown in red, microtubules in green, and the nuclei are in blue.

The **cytoskeleton** (also CSK) is a cellular "scaffolding" or "skeleton" contained within the cytoplasm and is made out of protein. The cytoskeleton is present in all cells; it was

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

The eukaryotic cytoskeleton



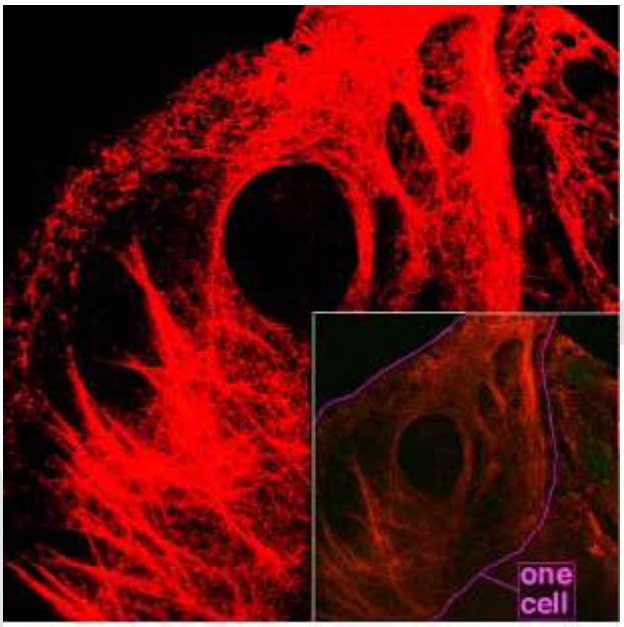
Actin cytoskeleton of mouse embryo fibroblasts, stained with phalloidin.

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

Microfilaments

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

Intermediate filaments



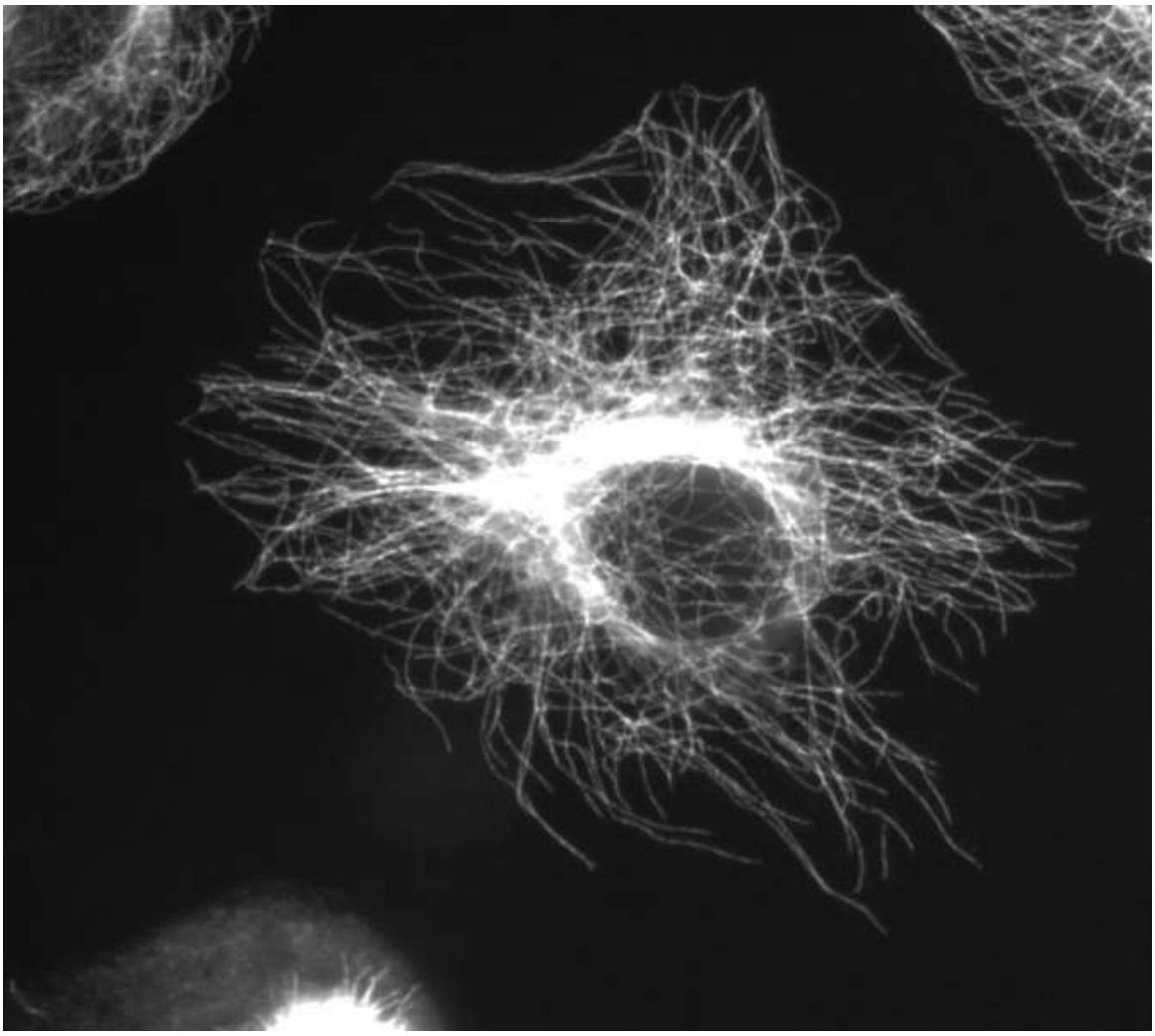
Microscopy of keratin filaments inside cells.

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

Different intermediate filaments are:

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

Microtubules



Microtubules in a gel fixated cell.

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

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

They play key roles in:

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

Comparison

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

The prokaryotic cytoskeleton

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

FtsZ

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

MreB and ParM

Prokaryotic actin-like proteins, such as MreB, are involved in the maintenance of cell shape. All non-spherical bacteria have genes encoding actin-like proteins, and these proteins form a helical network beneath the cell membrane that guides the proteins involved in cell wall biosynthesis.

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

Crescentin

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

History

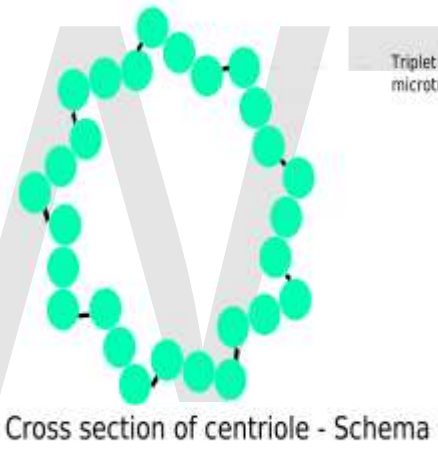
Microtrabeculae

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

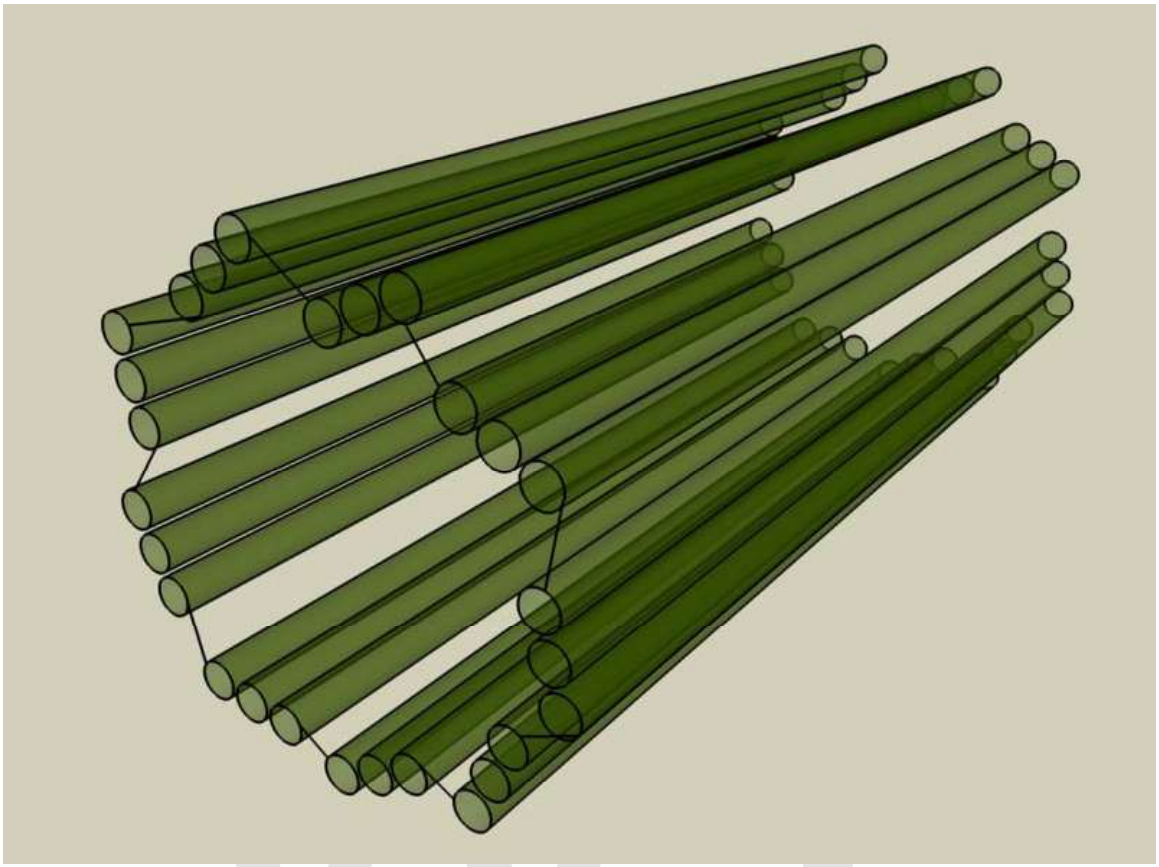
Chapter- 4

Centriole and Spindle Apparatus

Centriole



Schematic of centriole showing microtubule triplets



Three-dimensional view of a centriole

A **centriole** is a barrel-shaped cell structure found in most animal eukaryotic cells, though absent in higher plants and most fungi. The walls of each centriole are usually composed of nine triplets of microtubules (protein of the cytoskeleton). Deviations from this structure include *Drosophila melanogaster* embryos, with nine doublets, and *Caenorhabditis elegans* sperm cells and early embryos, with nine singlets. An associated pair of centrioles, arranged perpendicularly and surrounded by an amorphous mass of dense material (the pericentriolar material) constitutes the compound structure known as the centrosome.

Cell division

Centrioles are involved in the organization of the mitotic spindle and in the completion of cytokinesis. Centrioles were previously thought to be required for the formation of a mitotic spindle in animal cells. However, more recent experiments have demonstrated that cells whose centrioles have been removed via laser ablation can still progress through the G_1 stage of interphase before centrioles can be synthesized later in a de novo fashion. Additionally, mutant flies lacking centrioles can develop almost normally, although the adult flies lack flagella and cilia, a lack that underscores the requirement of centrioles for the formation of these organelles.

Cellular organization

Centrioles are a very important part of centrosomes, which are involved in organizing microtubules in the cytoplasm. The position of the centriole determines the position of the nucleus and plays a crucial role in the spatial arrangement of the cell.

Ciliogenesis

In organisms with flagella and cilia, the position of these organelles is determined by the mother centriole, which becomes the basal body. An inability of cells to use centrioles to make functional cilia and flagella has been linked to a number of genetic and developmental diseases. In particular, the inability of centrioles to properly migrate prior to ciliary assembly has recently been linked to Meckel-Gruber syndrome.

Animal development

Proper orientation of cilia via centriole positioning toward the posterior of embryonic node cells is critical for establishing left–right asymmetry during mammalian development.

Centriole duplication

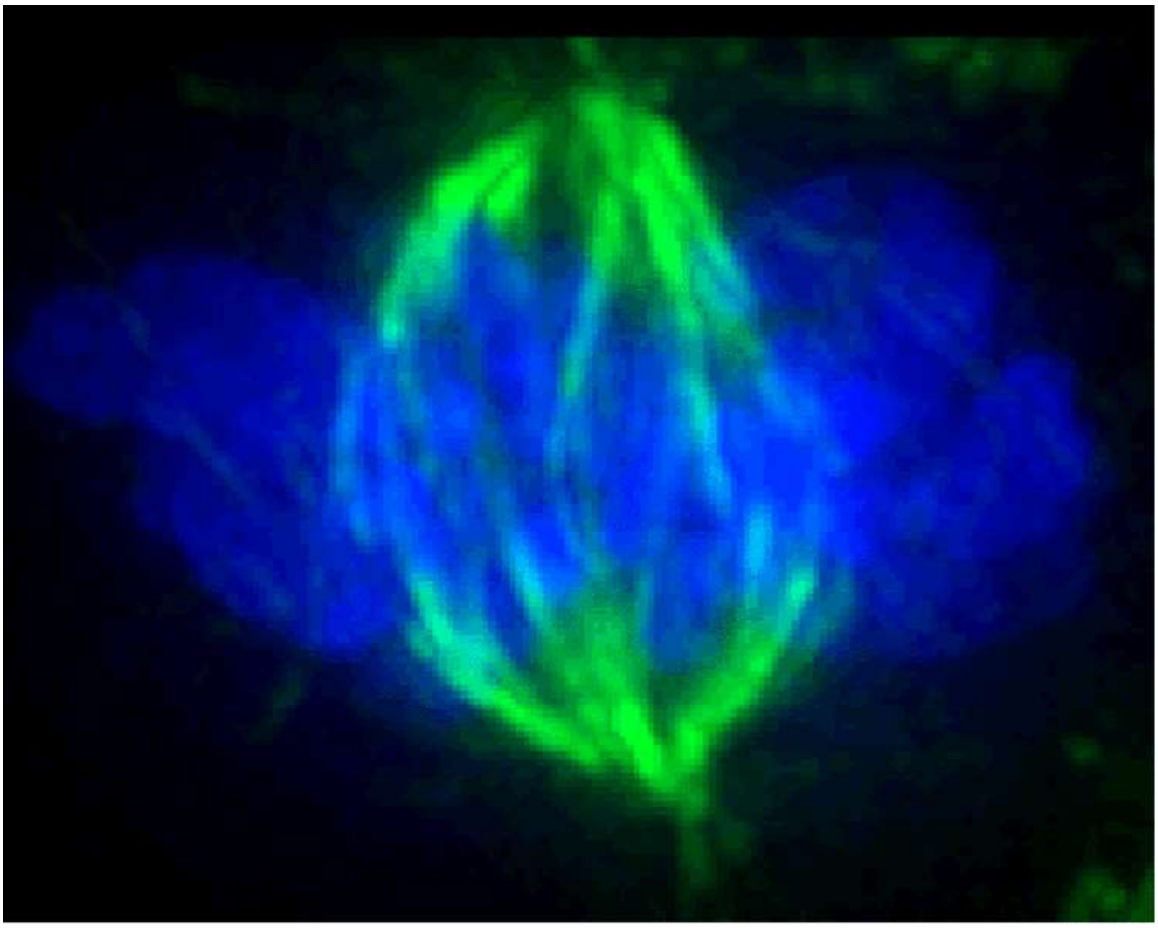
Cells in G_0 and G_1 usually contain two complete centrioles. The older of the two centrioles in a pair is termed the *mother centriole*, whereas the younger is termed the *daughter centriole*. During the cell division cycle, a new centriole grows from the side of each of the existing "mother" centrioles. After centriole duplication, the two pairs of centrioles remain attached to each other in an orthogonal configuration until mitosis, when the mother and daughter centrioles separate in a manner dependent upon the enzyme separase.

The two centrioles in the centrosome are connected to each other by unidentified proteins. The mother centriole has radiating appendages at the distal end of its long axis and is attached to the daughter centriole at the other proximal end. Each daughter cell formed after cell division will inherit one of these pairs (one older and one newer centriole). Duplication of centrioles starts at the time of the G_1/S transition and ends before the onset of mitosis.

Origin

The last common ancestor of all eukaryotes was a ciliated cell with centrioles. Some lineages of eukaryotes do not have centrioles anymore, for example land plants. It is unclear if the last common ancestor had one or two cilia. Important genes required for centriole duplication, like centrins, are only found in eukaryotes and neither in prokaryotes or archea.

Spindle apparatus



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

In cell biology, the **spindle fibers** are the structure that separates the chromosomes into the daughter cells during cell division. It is part of the cytoskeleton in eukaryotic cells. Depending on the type of cell division, it is also referred to as the **mitotic spindle** during mitosis and the **meiotic spindle** during meiosis.

Shape and components

The cellular spindle apparatus includes the spindle microtubules, associated proteins, and any centrosomes or asters present at the spindle poles. The spindle apparatus is vaguely ellipsoid in shape and tapers at the ends. In the wide middle portion, known as the spindle midzone, antiparallel microtubules are bundled by kinesins. At the pointed ends, known as spindle poles, microtubules are nucleated by the centrosomes in most animal cells. *Acentrosomal* or *anastral* spindles lack centromere or asters at the spindle poles, respectively, and occur for example during gametogenesis in animals. In fungi, spindles

form between spindle pole bodies embedded in the nuclear envelope. Most plants lack centrosomes or spindle pole bodies and instead spindle microtubules are nucleated on their nuclear envelopes. The spindles take the cell's chromosomes through most stages of mitosis. They start to form in early metaphase and are connected to the centrioles at each end of the cell.

Regulation of spindle assembly

The mitotic kinase aurora A is required for proper spindle assembly and separation. There have been identified many proteins necessary for the mitotic spindle assembly. Lamin B is not essential protein for spindle assembly. It is only a component of the spindle matrix helping microtubule assembly, since proper mitotic spindle can be formed without it.

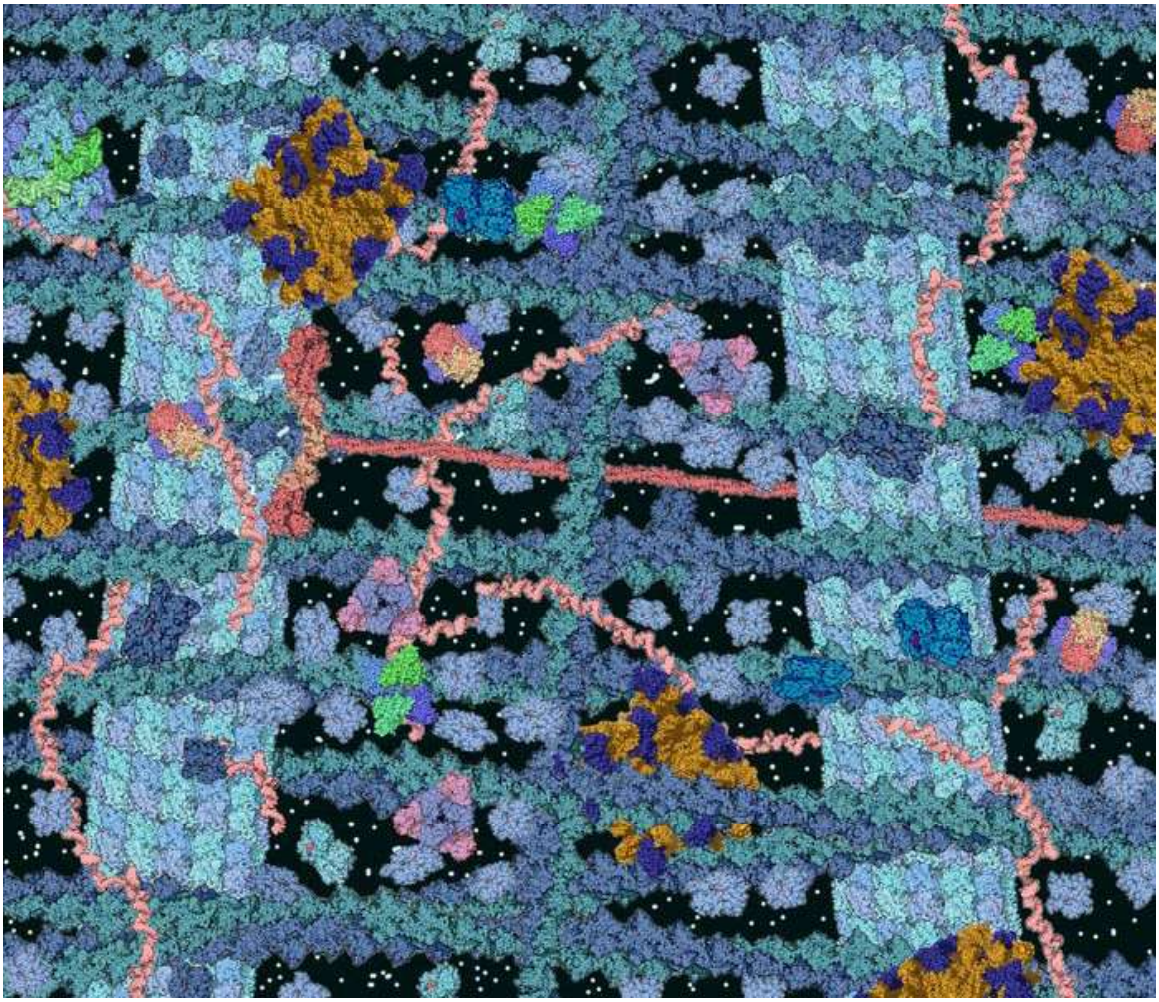
Polo-like kinase, also known as PLK, especially PLK1 has important roles in the spindle maintenance by regulating microtubule dynamics.

Mitotic spindle assembly checkpoint

The completion of spindle formation is a crucial transition point in the cell cycle called the spindle assembly checkpoint. If some chromosomes are not properly attached to the mitotic spindle by the time of this checkpoint, the onset of anaphase will be delayed. Failure of this spindle assembly checkpoint can result in aneuploidy and may be involved in aging and the formation of cancer. Abnormal mitotic spindles can produce tripolar mitosis. These are clearly abnormal cases and, if present, are considered definitive evidence that a tumor is malignant rather than benign. Such abnormalities are therefore often searched for in histological assays by pathologists when evaluating the potential malignancy of a tumor mass.

Chapter- 5

Cytosol



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

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

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

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

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

Definition

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

Properties and composition

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

Water

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

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

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

Ions

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

Typical ion concentrations in mammalian cytosol and blood.

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

Amino acids in proteins	138	9
Magnesium	0.8	1.5
Calcium	<0.0002	1.8

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

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

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

Macromolecules

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

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

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

Organization

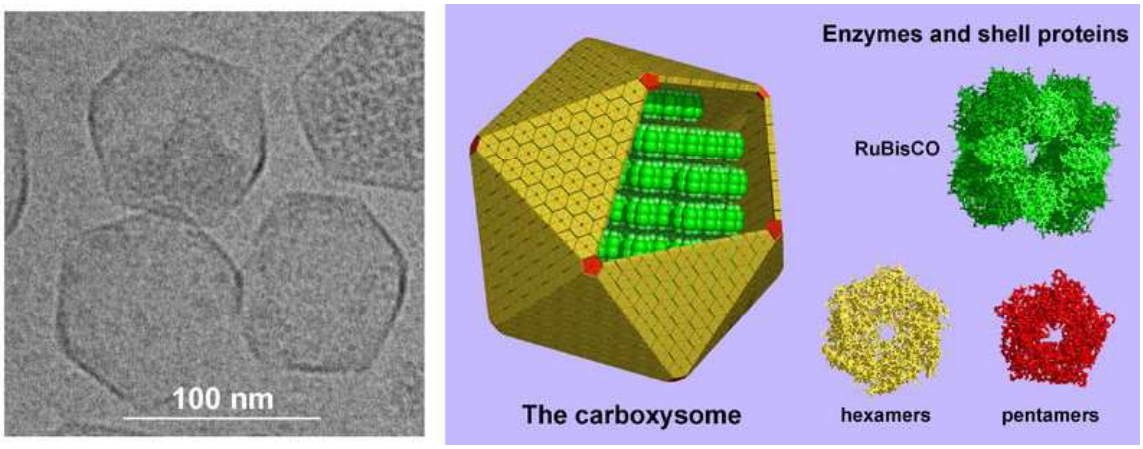
Although the components of the cytosol are not separated into regions by cell membranes, these components do not always mix randomly and several levels of organization can localize specific molecules to defined sites within the cytosol.

Concentration gradients

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

Protein complexes

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



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

Protein compartments

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

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

Cytoskeletal sieving

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

Function

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

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

Chapter- 6

Chromosome

A **chromosome** is an organized structure of DNA and protein that is found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Chromosomes also contain DNA-bound proteins, which serve to package the DNA and control its functions. The word *chromosome* comes from the Greek χρῶμα (*chroma*, colour) and σῶμα (*soma*, body) due to their property of being very strongly stained by particular dyes.

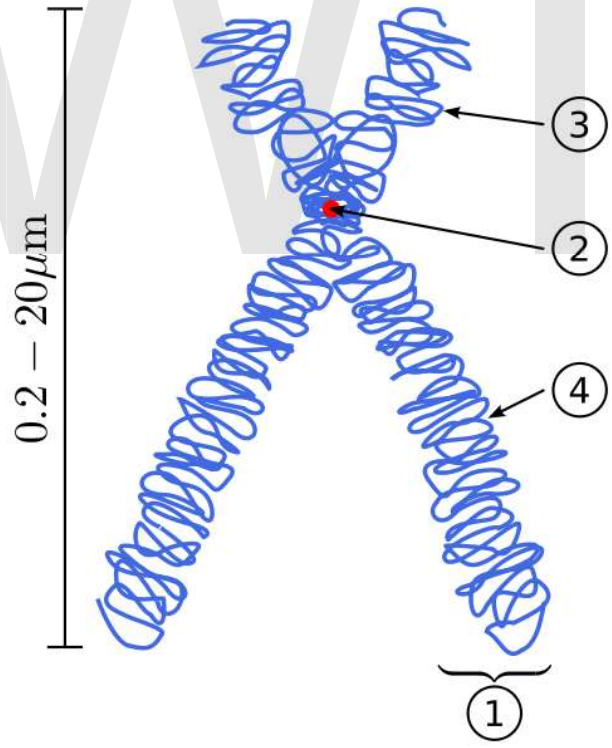


Diagram of a replicated and condensed metaphase eukaryotic chromosome. (1) Chromatid – one of the two identical parts of the chromosome after S phase. (2) Centromere – the point where the two chromatids touch, and where the microtubules attach. (3) Short arm. (4) Long arm.

Chromosomes vary widely between different organisms. The DNA molecule may be circular or linear, and can be composed of 10,000 to 1,000,000,000 nucleotides in a long chain. Typically eukaryotic cells (cells with nuclei) have large linear chromosomes and prokaryotic cells (cells without defined nuclei) have smaller circular chromosomes, although there are many exceptions to this rule. Furthermore, cells may contain more than one type of chromosome; for example, mitochondria in most eukaryotes and chloroplasts in plants have their own small chromosomes.

In eukaryotes, nuclear chromosomes are packaged by proteins into a condensed structure called chromatin. This allows the very long DNA molecules to fit into the cell nucleus. The structure of chromosomes and chromatin varies through the cell cycle. Chromosomes are the essential unit for cellular division and must be replicated, divided, and passed successfully to their daughter cells so as to ensure the genetic diversity and survival of their progeny. Chromosomes may exist as either duplicated or unduplicated—unduplicated chromosomes are single linear strands, whereas duplicated chromosomes (copied during synthesis phase) contain two copies joined by a centromere.

Compaction of the duplicated chromosomes during mitosis and meiosis results in the classic four-arm structure (pictured to the right). Chromosomal recombination plays a vital role in genetic diversity. If these structures are manipulated incorrectly, through processes known as chromosomal instability and translocation, the cell may undergo mitotic catastrophe and die, or it may unexpectedly evade apoptosis leading to the progression of cancer.

In practice "chromosome" is a rather loosely defined term. In prokaryotes and viruses, the term genophore is more appropriate when no chromatin is present. However, a large body of work uses the term chromosome regardless of chromatin content. In prokaryotes DNA is usually arranged as a circle, which is tightly coiled in on itself, sometimes accompanied by one or more smaller, circular DNA molecules called plasmids. These small circular genomes are also found in mitochondria and chloroplasts, reflecting their bacterial origins. The simplest genophores are found in viruses: these DNA or RNA molecules are short linear or circular genophores that often lack structural proteins.

History

Chromosomes as vectors of heredity

In a series of experiments, Theodor Boveri gave the definitive demonstration that chromosomes are the vectors of heredity. His two principles were based upon the *continuity* of chromosomes and the *individuality* of chromosomes. It is the second of these principles that was so original. Boveri was able to test the proposal put forward by Wilhelm Roux, that each chromosome carries a different genetic load, and showed that Roux was right. Upon the rediscovery of Mendel, Boveri was able to point out the connection between the rules of inheritance and the behaviour of the chromosomes. It is interesting to see that Boveri influenced two generations of American cytologists:

Edmund Beecher Wilson, Walter Sutton and Theophilus Painter were all influenced by Boveri (Wilson and Painter actually worked with him).

In his famous textbook *The Cell*, Wilson linked Boveri and Sutton together by the Boveri-Sutton theory. Mayr remarks that the theory was hotly contested by some famous geneticists: William Bateson, Wilhelm Johannsen, Richard Goldschmidt and T.H. Morgan, all of a rather dogmatic turn-of-mind. Eventually complete proof came from chromosome maps in Morgan's own lab.

Chromosomes in eukaryotes

Eukaryotes (cells with nuclei such as those found in plants, yeast, and animals) possess multiple large linear chromosomes contained in the cell's nucleus. Each chromosome has one centromere, with one or two arms projecting from the centromere, although, under most circumstances, these arms are not visible as such. In addition, most eukaryotes have a small circular mitochondrial genome, and some eukaryotes may have additional small circular or linear cytoplasmic chromosomes.

In the nuclear chromosomes of eukaryotes, the uncondensed DNA exists in a semi-ordered structure, where it is wrapped around histones (structural proteins), forming a composite material called chromatin.

Chromatin

Chromatin is the complex of DNA and protein found in the eukaryotic nucleus, which packages chromosomes. The structure of chromatin varies significantly between different stages of the cell cycle, according to the requirements of the DNA.

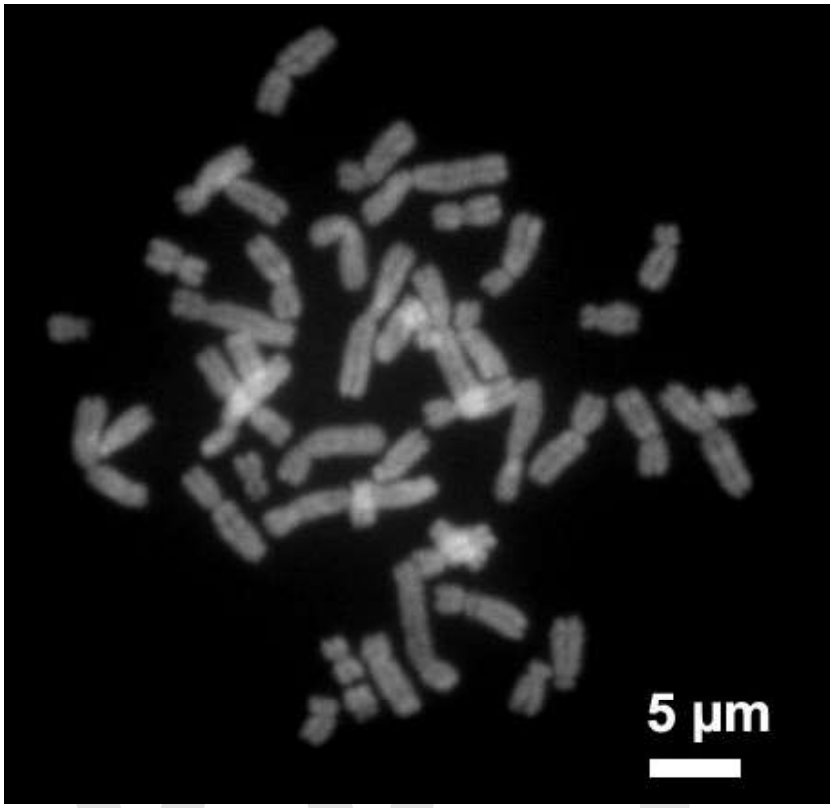
Interphase chromatin

During interphase (the period of the cell cycle where the cell is not dividing), two types of chromatin can be distinguished:

- Euchromatin, which consists of DNA that is active, e.g., being expressed as protein.
- Heterochromatin, which consists of mostly inactive DNA. It seems to serve structural purposes during the chromosomal stages. Heterochromatin can be further distinguished into two types:
    - *Constitutive heterochromatin*, which is never expressed. It is located around the centromere and usually contains repetitive sequences.
    - *Facultative heterochromatin*, which is sometimes expressed.

Individual chromosomes cannot be distinguished at this stage – they appear in the nucleus as a homogeneous tangled mix of DNA and protein.

Metaphase chromatin and division



Human chromosomes during metaphase.

In the early stages of mitosis or meiosis (cell division), the chromatin strands become more and more condensed. They cease to function as accessible genetic material (transcription stops) and become a compact transportable form. This compact form makes the individual chromosomes visible, and they form the classic four arm structure, a pair of sister chromatids attached to each other at the centromere. The shorter arms are called *p arms* (from the French *petit*, small) and the longer arms are called *q arms* (*q* follows *p* in the Latin alphabet). This is the only natural context in which individual chromosomes are visible with an optical microscope.

During divisions, long microtubules attach to the centromere and the two opposite ends of the cell. The microtubules then pull the chromatids apart, so that each daughter cell inherits one set of chromatids. Once the cells have divided, the chromatids are uncoiled and can function again as chromatin. In spite of their appearance, chromosomes are structurally highly condensed, which enables these giant DNA structures to be contained within a cell nucleus (Fig. 2).

The self-assembled microtubules form the spindle, which attaches to chromosomes at specialized structures called kinetochores, one of which is present on each sister chromatid. A special DNA base sequence in the region of the kinetochores provides, along with special proteins, longer-lasting attachment in this region.

Chromosomes in prokaryotes

The prokaryotes – bacteria and archaea – typically have a single circular chromosome, but many variations do exist. Most bacteria have a single circular chromosome that can range in size from only 160,000 base pairs in the endosymbiotic bacterium *Candidatus Carsonella ruddii*, to 12,200,000 base pairs in the soil-dwelling bacterium *Sorangium cellulosum*. Spirochaetes of the genus *Borrelia* are a notable exception to this arrangement, with bacteria such as *Borrelia burgdorferi*, the cause of Lyme disease, containing a single linear chromosome.

Structure in sequences

Prokaryotic chromosomes have less sequence-based structure than eukaryotes. Bacteria typically have a single point (the origin of replication) from which replication starts, whereas some archaea contain multiple replication origins. The genes in prokaryotes are often organized in operons, and do not usually contain introns, unlike eukaryotes.

DNA packaging

Prokaryotes do not possess nuclei. Instead, their DNA is organized into a structure called the nucleoid. The nucleoid is a distinct structure and occupies a defined region of the bacterial cell. This structure is, however, dynamic and is maintained and remodeled by the actions of a range of histone-like proteins, which associate with the bacterial chromosome. In archaea, the DNA in chromosomes is even more organized, with the DNA packaged within structures similar to eukaryotic nucleosomes.

Bacterial chromosomes tend to be tethered to the plasma membrane of the bacteria. In molecular biology application, this allows for its isolation from plasmid DNA by centrifugation of lysed bacteria and pelleting of the membranes (and the attached DNA).

Prokaryotic chromosomes and plasmids are, like eukaryotic DNA, generally supercoiled. The DNA must first be released into its relaxed state for access for transcription, regulation, and replication.

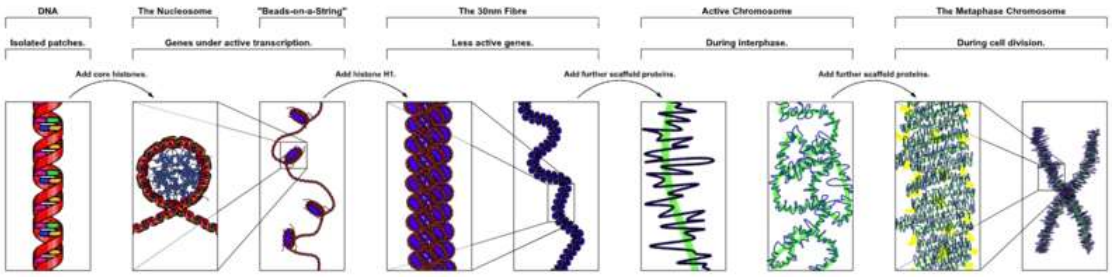


Fig. 2: The major structures in DNA compaction; DNA, the nucleosome, the 10nm "beads-on-a-string" fibre, the 30nm fibre and the metaphase chromosome.

Number of chromosomes in various organisms

Eukaryotes

These tables give the total number of chromosomes (including sex chromosomes) in a cell nucleus. For example, human cells are diploid and have 22 different types of autosome, each present as two copies, and two sex chromosomes. This gives 46 chromosomes in total. Other organisms have more than two copies of their chromosomes, such as bread wheat, which is *hexaploid* and has six copies of seven different chromosomes – 42 chromosomes in total.

Chromosome numbers in some plants

Plant Species	#
Arabidopsis thaliana (diploid)	10
Rye (diploid)	14
Maize (diploid or palaeotetraploid)	20
Einkorn wheat (diploid)	14
Durum wheat (tetraploid)	28
Bread wheat (hexaploid)	42
Cultivated tobacco (tetraploid)	48

Adder's Tongue Fern (diploid) }

Chromosome numbers (2n) in some animals

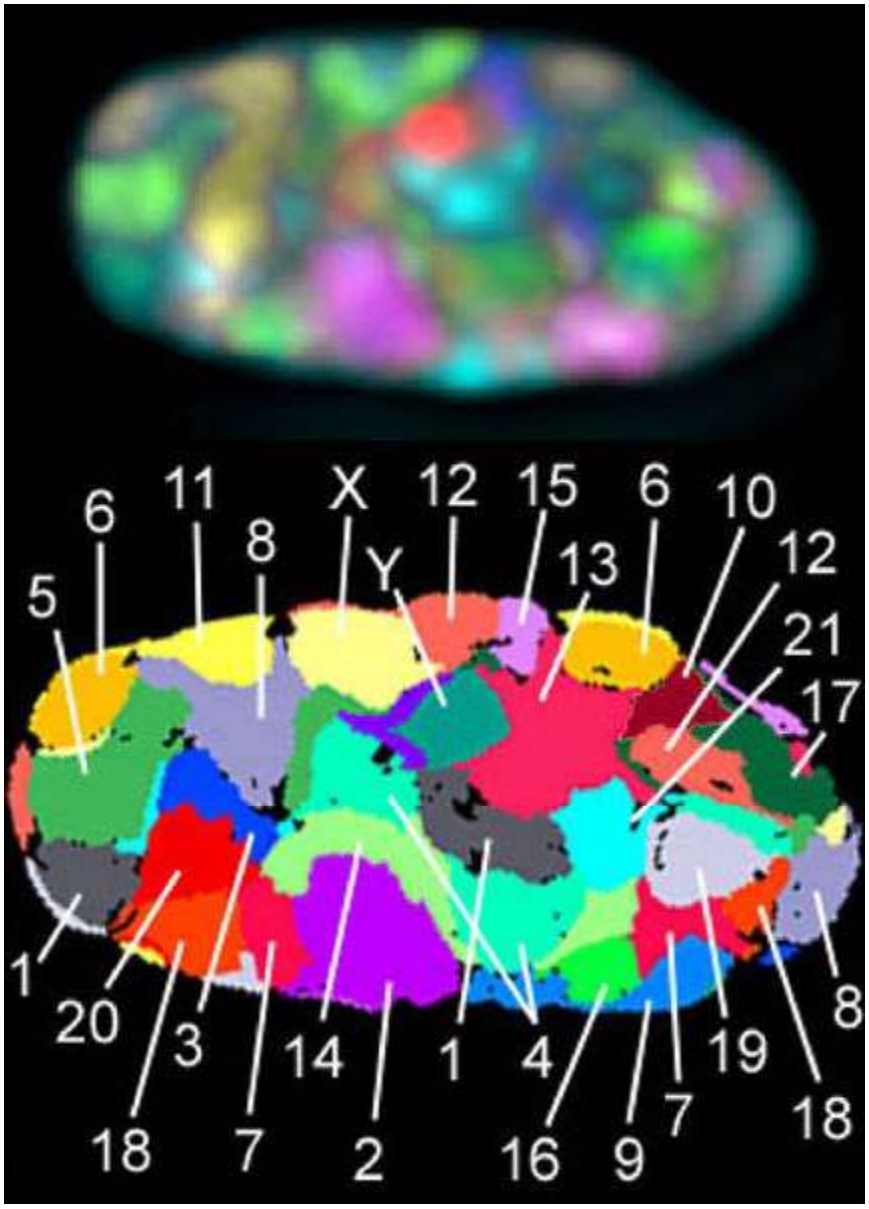
Species	#	Species	#
Common fruit fly	8	Guinea Pig	64
Guppy (*poecilia reticulata*)	46	Garden snail	54
Earthworm (*Octodrilus complanatus*)	36	Tibetan fox	36
Domestic cat	38	Domestic pig	38
Laboratory mouse	40	Laboratory rat	42
Rabbit (*Oryctolagus cuniculus*)	44	Syrian hamster	44
Hares	48	Human	46
Gorillas, Chimpanzees	48	Domestic sheep	54
Elephants	56	Cow	60

Donkey	62	Horse	64
Dog	78	Kingfisher	132
Goldfish	100-104	Silkworm	56

Chromosome numbers in other organisms

Species	Large Chromosomes	Intermediate Chromosomes	Microchromosomes
Trypanosoma brucei	11	6	~100
Domestic Pigeon (*Columba livia domestics*)	18	-	59-63
Chicken	8	2 sex chromosomes	60

Normal members of a particular eukaryotic species all have the same number of nuclear chromosomes. Other eukaryotic chromosomes, i.e., mitochondrial and plasmid-like small chromosomes, are much more variable in number, and there may be thousands of copies per cell.



The 23 human chromosome territories during prometaphase in fibroblast cells.

Asexually reproducing species have one set of chromosomes, which are the same in all body cells. However, asexual species can be either haploid or diploid.

Sexually reproducing species have somatic cells (body cells), which are diploid [2n] having two sets of chromosomes, one from the mother and one from the father. Gametes, reproductive cells, are haploid [n]: They have one set of chromosomes. Gametes are produced by meiosis of a diploid germ line cell. During meiosis, the matching chromosomes of father and mother can exchange small parts of themselves (crossover), and thus create new chromosomes that are not inherited solely from either parent. When a male and a female gamete merge (fertilization), a new diploid organism is formed.

Some animal and plant species are polyploid [Xn]: They have more than two sets of homologous chromosomes. Plants important in agriculture such as tobacco or wheat are often polyploid, compared to their ancestral species. Wheat has a haploid number of seven chromosomes, still seen in some cultivars as well as the wild progenitors. The more-common pasta and bread wheats are polyploid, having 28 (tetraploid) and 42 (hexaploid) chromosomes, compared to the 14 (diploid) chromosomes in the wild wheat.

Prokaryotes

Prokaryote species generally have one copy of each major chromosome, but most cells can easily survive with multiple copies. For example, *Buchnera*, a symbiont of aphids has multiple copies of its chromosome, ranging from 10–400 copies per cell. However, in some large bacteria, such as *Epulopiscium fishelsoni* up to 100,000 copies of the chromosome can be present. Plasmids and plasmid-like small chromosomes are, as in eukaryotes, very variable in copy number. The number of plasmids in the cell is almost entirely determined by the rate of division of the plasmid – fast division causes high copy number, and vice versa.

Karyotype

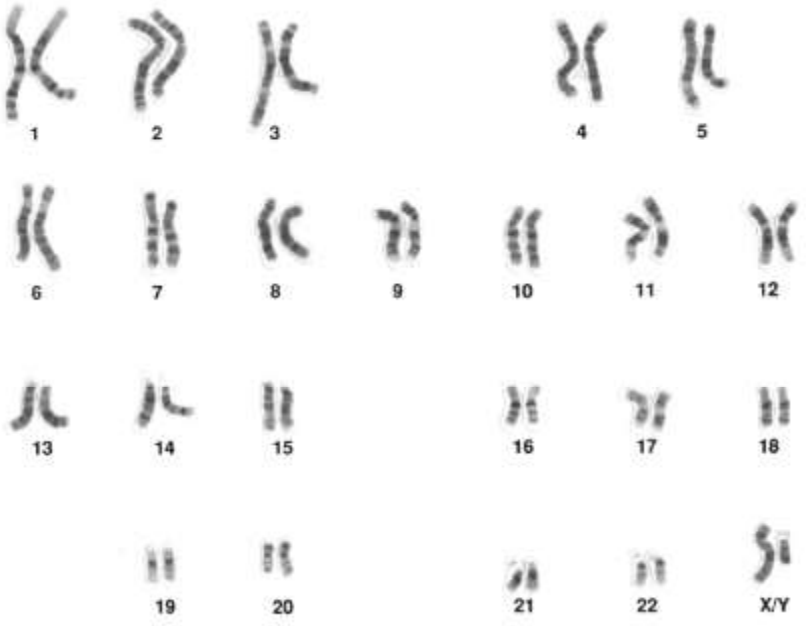


Figure 3: Karyogram of a human male

In general, the **karyotype** is the characteristic chromosome complement of a eukaryote species. The preparation and study of karyotypes is part of cytogenetics.

Although the replication and transcription of DNA is highly standardized in eukaryotes, *the same cannot be said for their karyotypes*, which are often highly variable. There may

be variation between species in chromosome number and in detailed organization. In some cases, there is significant variation within species. Often there is:

1. variation between the two sexes
2. variation between the germ-line and soma (between gametes and the rest of the body)
3. variation between members of a population, due to balanced genetic polymorphism
4. geographical variation between races
5. mosaics or otherwise abnormal individuals.

Also, variation in karyotype may occur during development from the fertilised egg.

The technique of determining the karyotype is usually called *karyotyping*. Cells can be locked part-way through division (in metaphase) in vitro (in a reaction vial) with colchicine. These cells are then stained, photographed, and arranged into a *karyogram*, with the set of chromosomes arranged, autosomes in order of length, and sex chromosomes (here X/Y) at the end: Fig. 3.

Like many sexually reproducing species, humans have special gonosomes (sex chromosomes, in contrast to autosomes). These are XX in females and XY in males.

Historical note

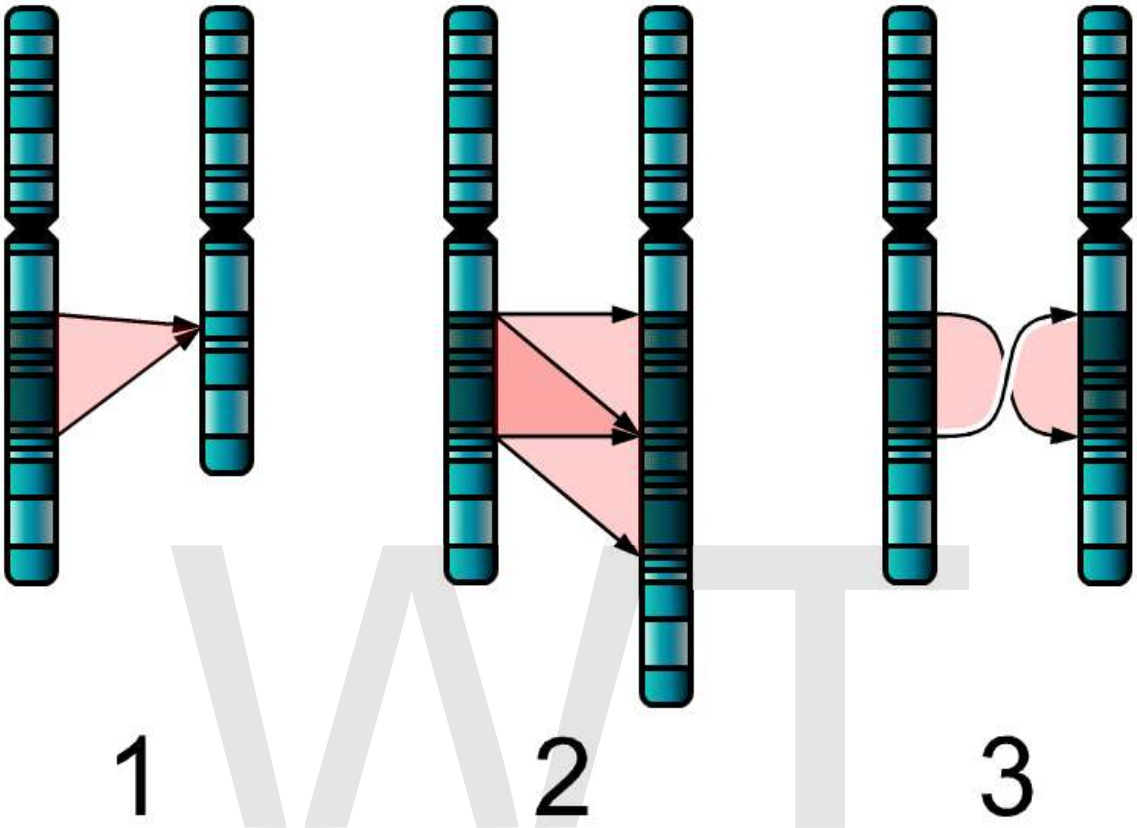
Investigation into the human karyotype took many years to settle the most basic question. How many chromosomes does a normal diploid human cell contain? In 1912, Hans von Winiwarter reported 47 chromosomes in spermatogonia and 48 in oogonia, concluding an XX/XO sex determination mechanism. Painter in 1922 was not certain whether the diploid number of man is 46 or 48, at first favouring 46. He revised his opinion later from 46 to 48, and he correctly insisted on humans having an XX/XY system.

New techniques were needed to definitively solve the problem:

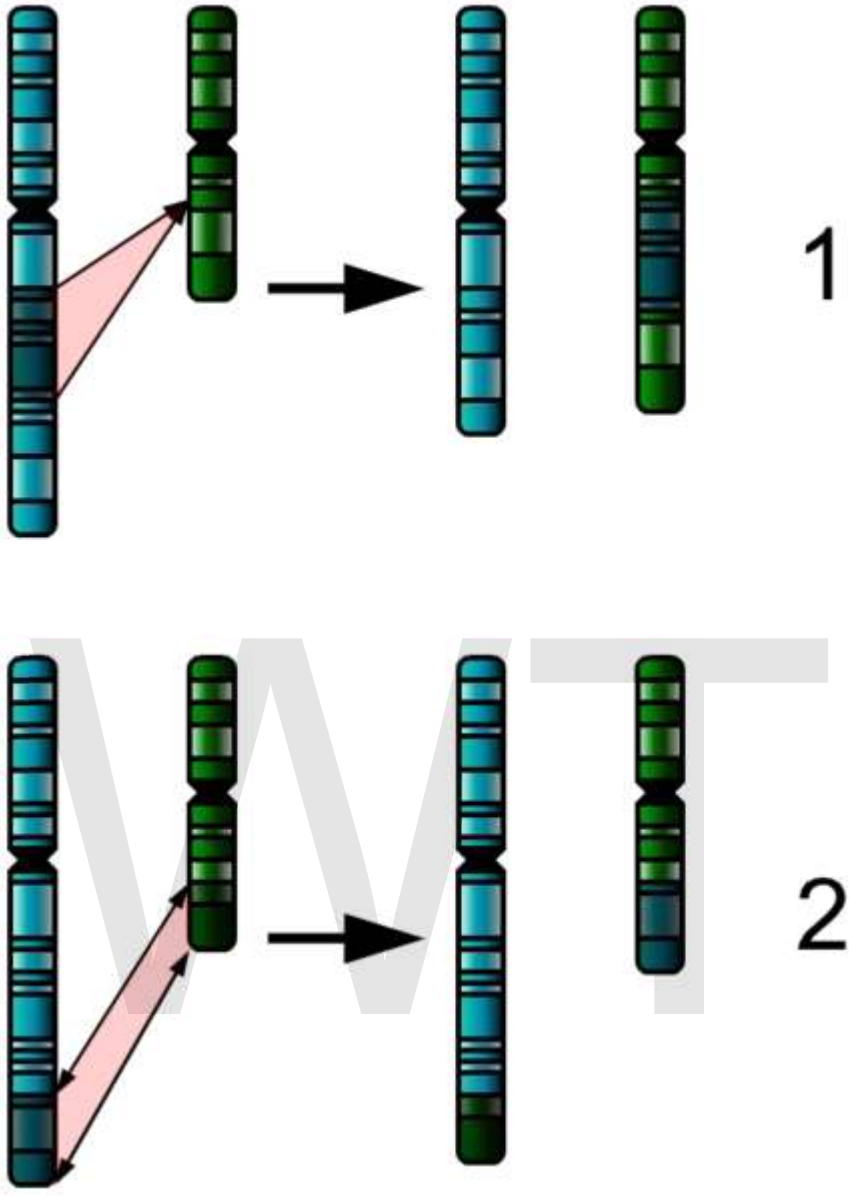
1. Using cells in culture
2. Pretreating cells in a hypotonic solution, which swells them and spreads the chromosomes
3. Arresting mitosis in metaphase by a solution of colchicine
4. Squashing the preparation on the slide forcing the chromosomes into a single plane
5. Cutting up a photomicrograph and arranging the result into an indisputable karyogram.

It took until the mid-1950s for it to become generally accepted that the human karyotype include only 46 chromosomes. Considering the techniques of Winiwarter and Painter, their results were quite remarkable. Chimpanzees (the closest living relatives to modern humans) have 48 chromosomes.

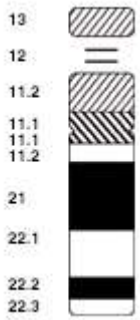
Chromosomal aberrations



The three major single chromosome mutations; deletion (1), duplication (2) and inversion (3).



The two major two-chromosome mutations; insertion (1) and translocation (2).



In Down syndrome, there are three copies of chromosome 21

Chromosomal aberrations are disruptions in the normal chromosomal content of a cell and are a major cause of genetic conditions in humans, such as Down syndrome. Some chromosome abnormalities do not cause disease in carriers, such as translocations, or chromosomal inversions, although they may lead to a higher chance of birthing a child with a chromosome disorder. Abnormal numbers of chromosomes or chromosome sets, aneuploidy, may be lethal or give rise to genetic disorders. Genetic counseling is offered for families that may carry a chromosome rearrangement.

The gain or loss of DNA from chromosomes can lead to a variety of genetic disorders. Human examples include:

- Cri du chat, which is caused by the deletion of part of the short arm of chromosome 5. "Cri du chat" means "cry of the cat" in French, and the condition was so-named because affected babies make high-pitched cries that sound like those of a cat. Affected individuals have wide-set eyes, a small head and jaw, moderate to severe mental health issues, and are very short.
- Down syndrome, usually is caused by an extra copy of chromosome 21 (trisomy 21). Characteristics include decreased muscle tone, stockier build, asymmetrical skull, slanting eyes and mild to moderate developmental disability.
- Edwards syndrome, which is the second-most-common trisomy; Down syndrome is the most common. It is a trisomy of chromosome 18. Symptoms include motor retardation, developmental disability and numerous congenital anomalies causing serious health problems. Ninety percent die in infancy; however, those that live past their first birthday usually are quite healthy thereafter. They have a characteristic clenched hands and overlapping fingers.
- Idic15, abbreviation for Isodicentric 15 on chromosome 15; also called the following names due to various researches, but they all mean the same; IDIC(15), Inverted duplication 15, extra Marker, Inv dup 15, partial tetrasomy 15
- Jacobsen syndrome, also called the terminal 11q deletion disorder. This is a very rare disorder. Those affected have normal intelligence or mild developmental disability, with poor expressive language skills. Most have a bleeding disorder called Paris-Trousseau syndrome.
- Klinefelter's syndrome (XXY). Men with Klinefelter syndrome are usually sterile, and tend to have longer arms and legs and to be taller than their peers. Boys with the syndrome are often shy and quiet, and have a higher incidence of speech delay and dyslexia. During puberty, without testosterone treatment, some of them may develop gynecomastia.
- Patau Syndrome, also called D-Syndrome or trisomy-13. Symptoms are somewhat similar to those of trisomy-18, but they do not have the characteristic hand shape.
- Small supernumerary marker chromosome. This means there is an extra, abnormal chromosome. Features depend on the origin of the extra genetic material. Cat-eye syndrome and isodicentric chromosome 15 syndrome (or Idic15) are both caused by a supernumerary marker chromosome, as is Pallister-Killian syndrome.

- Triple-X syndrome (XXX). XXX girls tend to be tall and thin. They have a higher incidence of dyslexia.
- Turner syndrome (X instead of XX or XY). In Turner syndrome, female sexual characteristics are present but underdeveloped. People with Turner syndrome often have a short stature, low hairline, abnormal eye features and bone development and a "caved-in" appearance to the chest.
- XYY syndrome. XYY boys are usually taller than their siblings. Like XXY boys and XXX girls, they are somewhat more likely to have learning difficulties.
- Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4. It is characterized by severe growth retardation and severe to profound mental health issues.

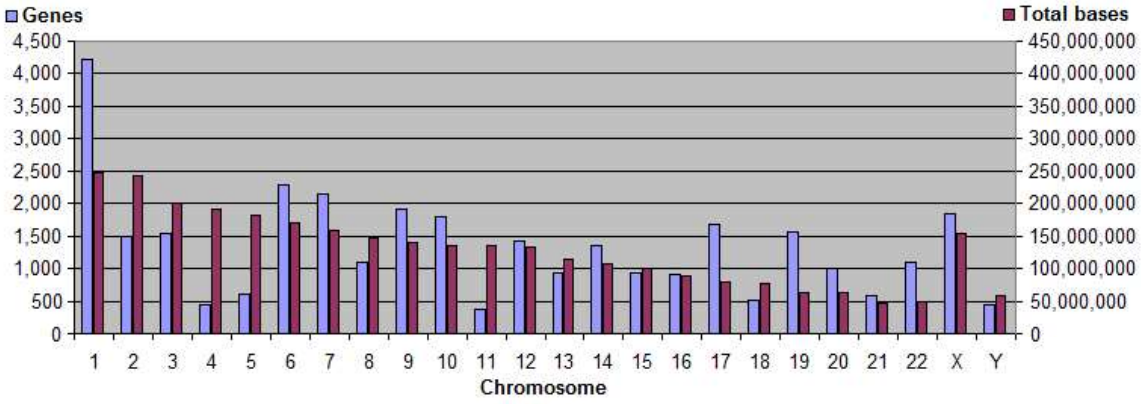
Chromosomal mutations produce changes in whole chromosomes (more than one gene) or in the number of chromosomes present.

- Deletion – loss of part of a chromosome
- Duplication – extra copies of a part of a chromosome
- Inversion – reverse the direction of a part of a chromosome
- Translocation – part of a chromosome breaks off and attaches to another chromosome

Most mutations are neutral – have little or no effect. Chromosomal aberrations are the changes in the structure of chromosomes. It has a great role in evolution. A detailed graphical display of all human chromosomes and the diseases annotated at the correct spot may be found at the Oak Ridge National Laboratory.

Human chromosomes

Chromosomes can be divided into two types—autosomes, and sex chromosomes. Certain genetic traits are linked to your sex, and are passed on through the sex chromosomes. The autosomes contain the rest of the genetic hereditary information. All act in the same way during cell division. Human cells have 23 pairs of large linear nuclear chromosomes, (22 pairs of autosomes and one pair of sex chromosomes) giving a total of 46 per cell. In addition to these, human cells have many hundreds of copies of the mitochondrial genome. Sequencing of the human genome has provided a great deal of information about each of the chromosomes. Below is a table compiling statistics for the chromosomes, based on the Sanger Institute's human genome information in the Vertebrate Genome Annotation (VEGA) database. Number of genes is an estimate as it is in part based on gene predictions. Total chromosome length is an estimate as well, based on the estimated size of unsequenced heterochromatin regions.

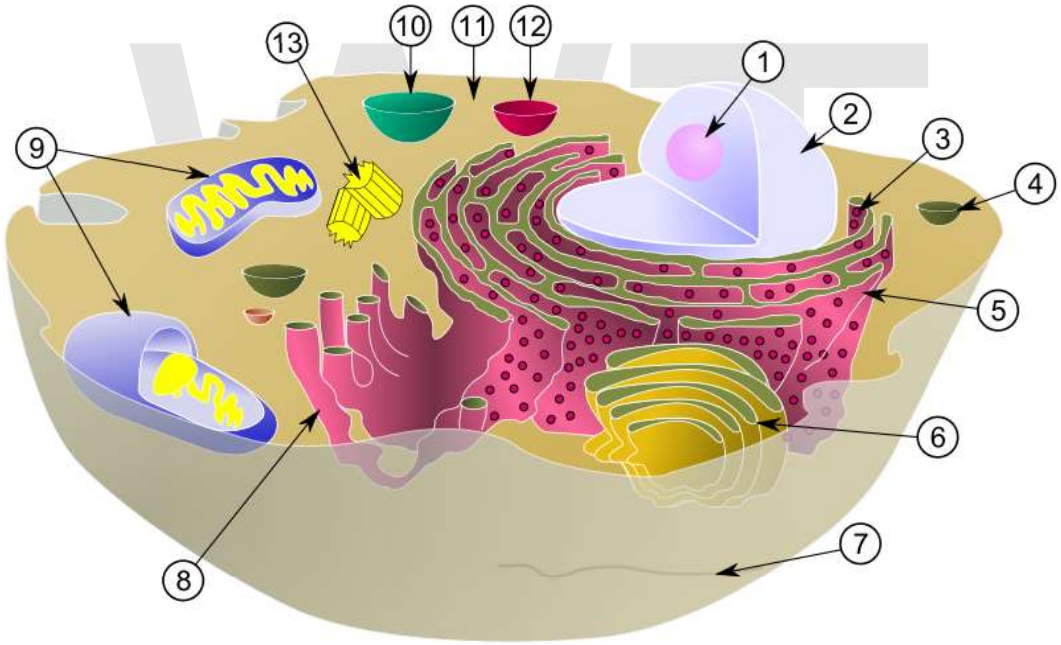


Chromosome	Genes	Total bases	Sequenced bases
1	4,220	247,199,719	224,999,719
2	1,491	242,751,149	237,712,649
3	1,550	199,446,827	194,704,827
4	446	191,263,063	187,297,063
5	609	180,837,866	177,702,766
6	2,281	170,896,993	167,273,993
7	2,135	158,821,424	154,952,424
8	1,106	146,274,826	142,612,826
9	1,920	140,442,298	120,312,298
10	1,793	135,374,737	131,624,737
11	379	134,452,384	131,130,853
12	1,430	132,289,534	130,303,534
13	924	114,127,980	95,559,980
14	1,347	106,360,585	88,290,585
15	921	100,338,915	81,341,915
16	909	88,822,254	78,884,754
17	1,672	78,654,742	77,800,220
18	519	76,117,153	74,656,155
19	1,555	63,806,651	55,785,651
20	1,008	62,435,965	59,505,254
21	578	46,944,323	34,171,998
22	1,092	49,528,953	34,893,953
X (sex chromosome)	1,846	154,913,754	151,058,754
Y (sex chromosome)	454	57,741,652	25,121,652
Total	**32,185**	**3,079,843,747**	**2,857,698,560**

Chapter- 7

Cytoplasm and Plasmid

Cytoplasm



Schematic showing the cytoplasm, with major components of a typical animal cell (organelles):
(1) nucleolus
(2) nucleus
(3) ribosomes (indicated by purple dots)
(4) vesicle
(5) rough endoplasmic reticulum (ER)
(6) Golgi apparatus
(7) cytoskeleton
(8) smooth ER
(9) mitochondria
(10) vacuole

(11) cytosol
(12) lysosome
(13) centrioles within centrosome

The **cytoplasm** is a thick liquid residing between the cell membrane holding organelles, except for the nucleus. All the contents of the cells of prokaryote organisms (which lack a cell nucleus) are contained within the cytoplasm. Within the cells of eukaryote organisms the contents of the cell nucleus are separated from the cytoplasm, and are then called the nucleoplasm.

In eukaryotic cells also, the cytoplasm contains organelles, such as mitochondria, which are filled with liquid that is kept separate from the rest of the cytoplasm by biological membranes. It is within the cytoplasm that most cellular activities occur, such as many metabolic pathways including glycolysis, and processes such as cell division. The inner, granular mass is called the endoplasm and the outer, clear and glassy layer is called the cell cortex or the ectoplasm.

The part of the cytoplasm that is not held within organelles is called the cytosol. The cytosol is a complex mixture of cytoskeleton filaments, dissolved molecules, and water that fills much of the volume of a cell. The cytosol is a gel, with a network of fibers dispersed through water. Due to this network of pores and high concentrations of dissolved macromolecules, such as proteins, an effect called macromolecular crowding occurs and the cytosol does not act as an ideal solution. This crowding effect alters how the components of the cytosol interact with each other.

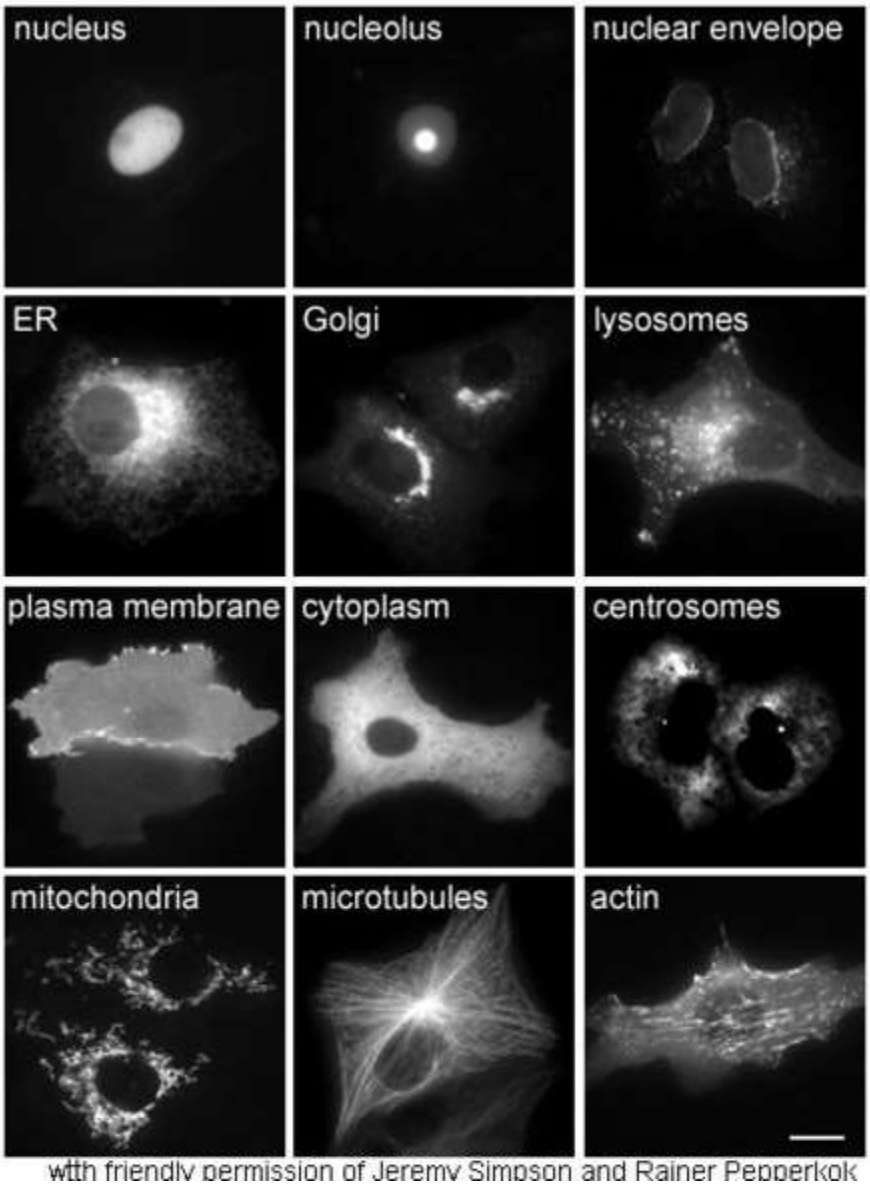
Movement of the calcium ion in and out of the cytoplasm is thought to be a signalling activity for metabolic processes.

Constituents

The cytoplasm has three major elements; the cytosol, organelles and inclusions.

Cytosol

The cytosol is the portion not within membrane-bound organelles. The cytosol is a translucent fluid in which the plasmic elements are suspended. Cytosol makes up about 70% of the cell volume and is composed of water, salts and organic molecules. The cytoplasm also contains the protein filaments that make up the cytoskeleton, as well as soluble proteins and small structures such as ribosomes, proteasomes, and the mysterious vault complexes. The inner, granular and more fluid portion of the cytoplasm is referred to as endoplasm.



with friendly permission of Jeremy Simpson and Rainer Pepperkok

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

Organelles

Organelles are membrane-bound "organs" inside the cell that have specific functions. Some major organelles that are suspended in the cytosol are the mitochondria, the endoplasmic reticulum, the Golgi apparatus, vacuoles, lysosomes, and in plant cells chloroplasts.

Cytoplasmic inclusions

The inclusions are small particles of insoluble substances suspended in the cytosol. A huge range of inclusions exist in different cell types, and range from crystals of calcium oxalate or silicon dioxide in plants, to granules of energy-storage materials such as starch, glycogen, or polyhydroxybutyrate. A particularly widespread example are lipid droplets, which are spherical droplets composed of lipids and proteins that are used in both prokaryotes and eukaryotes as a way of storing lipids such as fatty acids and sterols. Lipid droplets make up much of the volume of adipocytes, which are specialized lipid-storage cells, but they are also found in a range of other cell types.

Plasmid

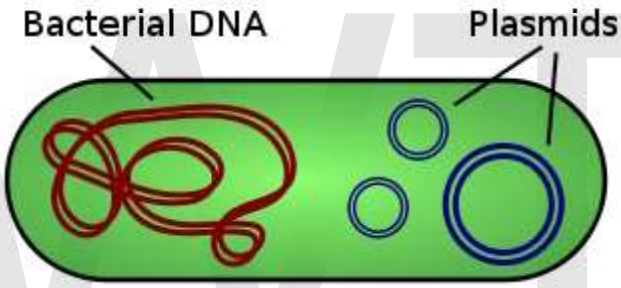


Figure 1: Illustration of a bacterium with plasmid enclosed showing chromosomal DNA and plasmids.

A **plasmid** is a DNA molecule that is separate from, and can replicate independently of, the chromosomal DNA. They are double stranded and, in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms (e.g., the *2-micrometre-ring* in *Saccharomyces cerevisiae*).

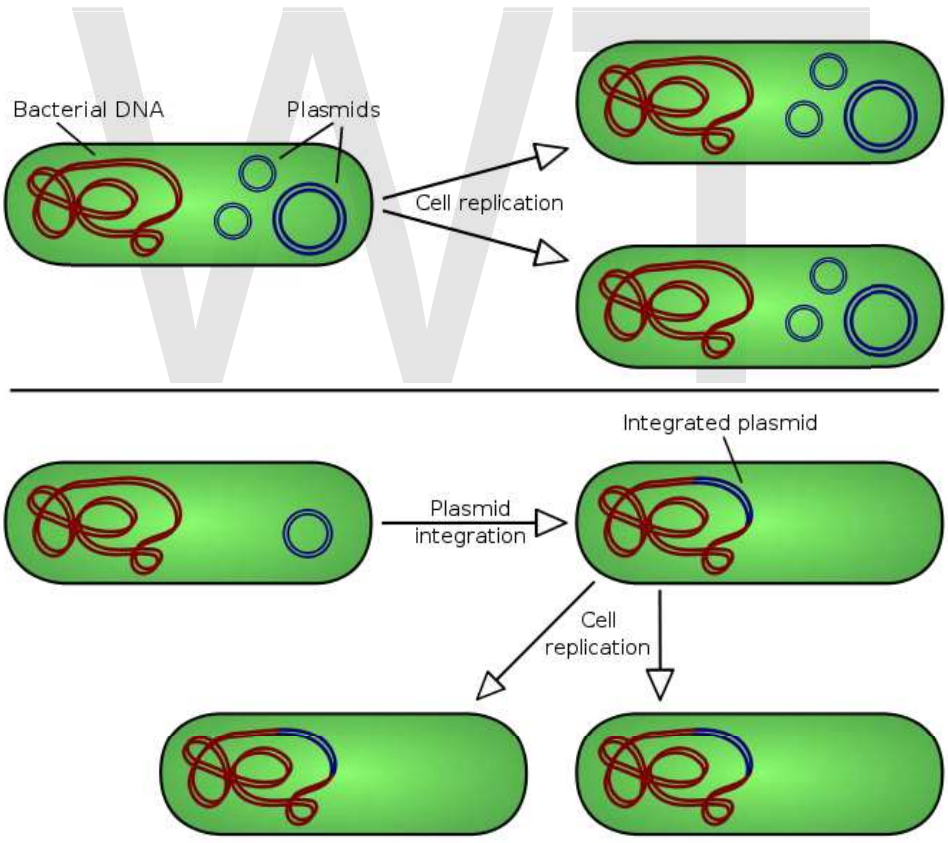
Plasmid size varies from 1 to over 1,000 kilobase pairs (kbp). The number of identical plasmids within a single cell can range anywhere from one to even thousands under some circumstances. Plasmids can be considered to be part of the mobilome, since they are often associated with conjugation, a mechanism of horizontal gene transfer.

The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952.

Plasmids are considered transferable genetic elements, or "replicons", capable of autonomous replication within a suitable host. Plasmids can be found in all three major domains, Archea, Bacteria and Eukarya. Similar to viruses, plasmids are not considered a form of "life" as it is currently defined. Unlike viruses, plasmids are "naked" DNA and do

not encode genes necessary to encase the genetic material for transfer to a new host, though some classes of plasmids encode the sex pilus necessary for their own transfer. Plasmid host-to-host transfer requires direct, mechanical transfer by conjugation or changes in host gene expression allowing the intentional uptake of the genetic element by transformation. Microbial transformation with plasmid DNA is neither parasitic nor symbiotic in nature, since each implies the presence of an independent species living in a commensal or detrimental state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances. Plasmids also can provide bacteria with an ability to fix elemental nitrogen or to degrade recalcitrant organic compounds which provide an advantage under conditions of nutrient deprivation.

Vectors



There are two types of plasmid integration into a host bacteria: Non-integrating plasmids replicate as with the top instance; whereas episomes, the lower example, integrate into the host chromosome.

Plasmids used in genetic engineering are called vectors. Plasmids serve as important tools in genetics and biotechnology labs, where they are commonly used to multiply (make many copies of) or *express* particular genes. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Next, the plasmids are inserted into bacteria by a process called *transformation*. Then, the bacteria are exposed to the particular antibiotics. Only bacteria which take up copies of the plasmid survive, since the plasmid makes them resistant. In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics. In this way the antibiotics act as a filter to select only the modified bacteria. Now these bacteria can be grown in large amounts, harvested and lysed (often using the alkaline lysis method) to isolate the plasmid of interest.

Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacteria produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for, for example, insulin or even antibiotics.

However, a plasmid can only contain inserts of about 1–10 kbp. To clone longer lengths of DNA, lambda phage with lysogeny genes deleted, cosmids, bacterial artificial chromosomes or yeast artificial chromosomes could be used.

Applications

Disease Models

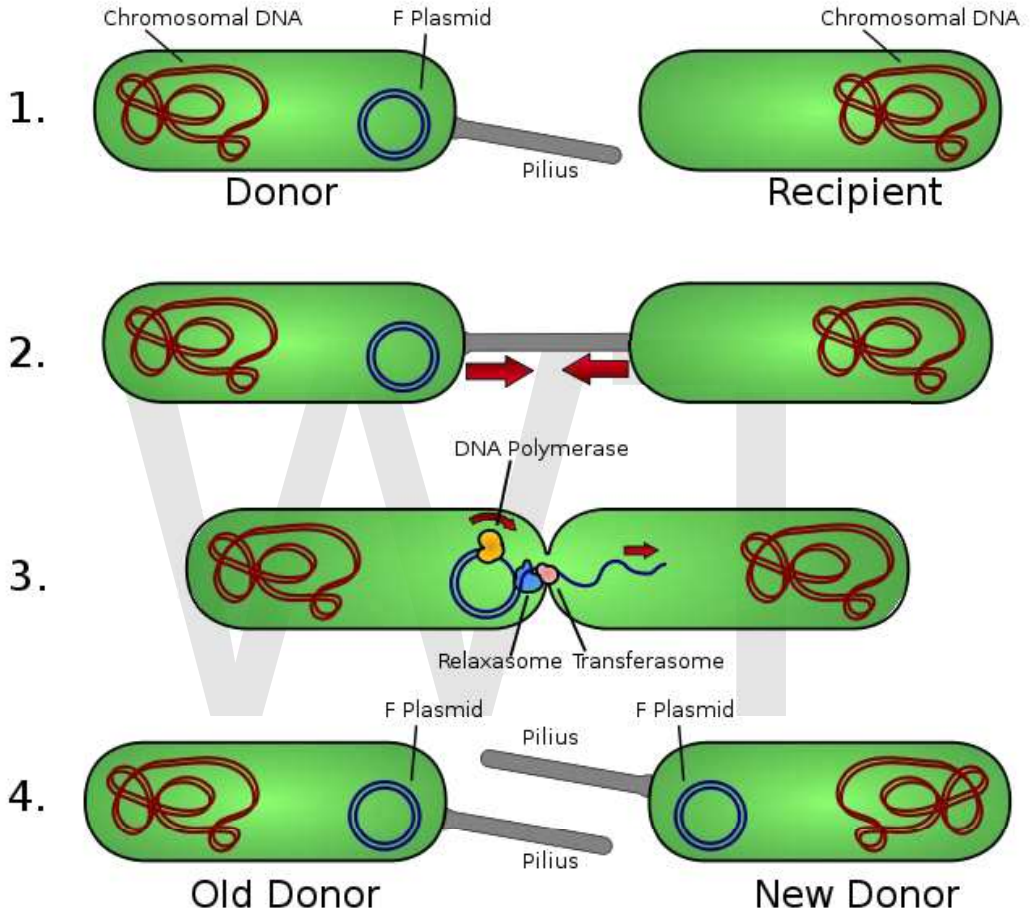
Plasmids were historically used to genetically engineer the embryonic stem cells of rats in order to create rat genetic disease models. The limited efficiency of plasmid based techniques precluded their use in the creation of more accurate human cell models. Fortunately, developments in Adeno-associated virus recombination techniques, and Zinc finger nucleases, have enabled the creation of a new generation of isogenic human disease models.

Gene therapy

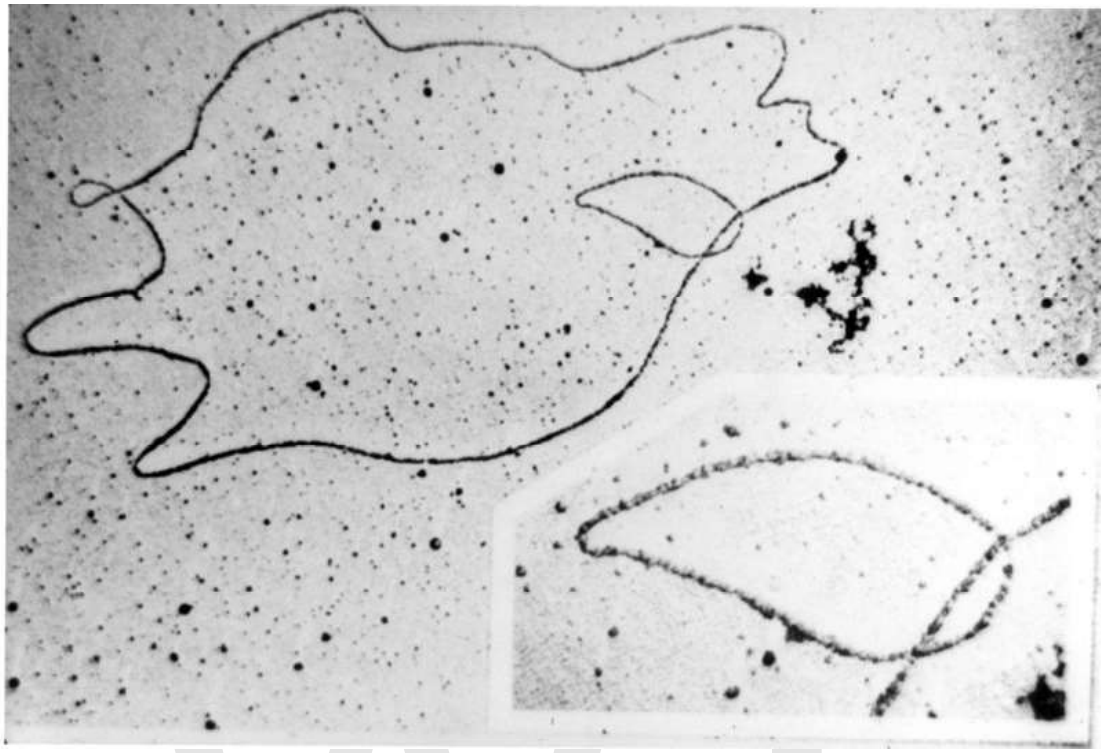
The success of some strategies of gene therapy depends on the efficient insertion of therapeutic genes at the appropriate chromosomal target sites within the human genome, without causing cell injury, oncogenic mutations (cancer) or an immune response. Plasmid vectors are one of many approaches that could be used for this purpose. Zinc finger nucleases (ZFNs) offer a way to cause a site-specific double strand break to the DNA genome and cause homologous recombination. This makes targeted gene correction a possibility in human cells. Plasmids encoding ZFN could be used to deliver a

therapeutic gene to a pre-selected chromosomal site with a frequency higher than that of random integration. Although the practicality of this approach to gene therapy has yet to be proven, some aspects of it could be less problematic than the alternative viral-based delivery of therapeutic genes.

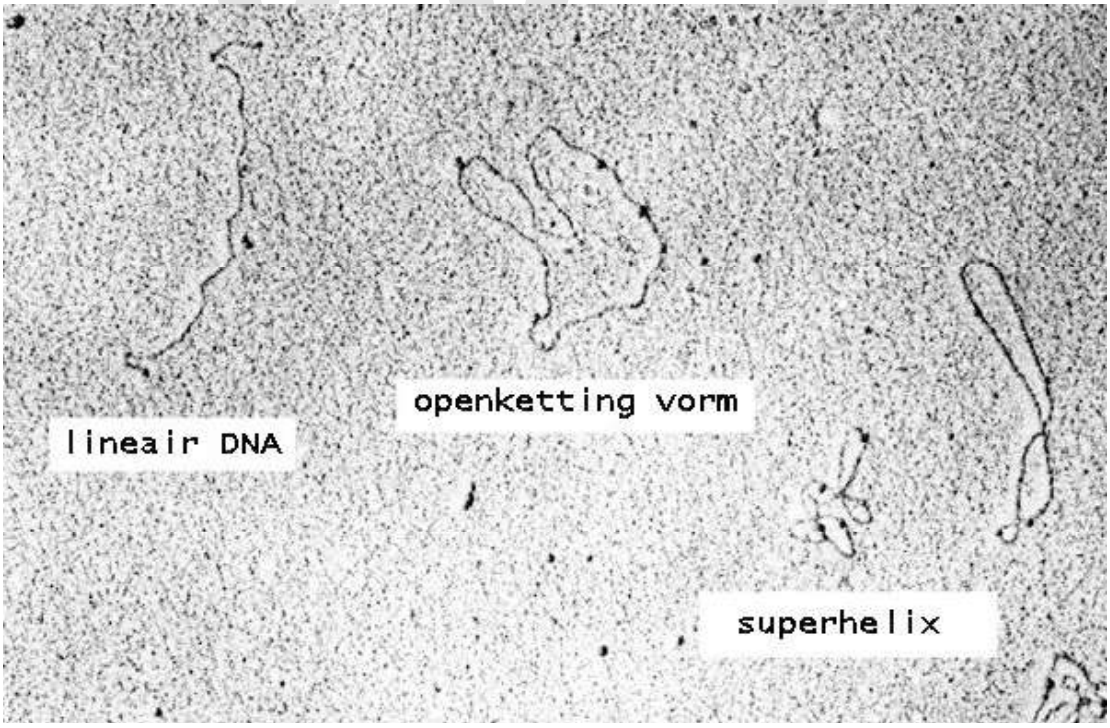
Types



Overview of bacterial conjugation



Electron micrograph of a DNA fiber bundle, presumably of a single bacterial chromosome loop.



Electron micrograph of a bacterial DNA plasmid (chromosome fragment).

One way of grouping plasmids is by their ability to transfer to other bacteria. *Conjugative* plasmids contain so-called *tra-genes*, which perform the complex process of *conjugation*, the transfer of plasmids to another bacterium (Fig. 4). *Non-conjugative* plasmids are incapable of initiating conjugation, hence they can only be transferred with the assistance of conjugative plasmids, by 'accident'. An intermediate class of plasmids are *mobilizable*, and carry only a subset of the genes required for transfer. They can 'parasitize' a conjugative plasmid, transferring at high frequency only in its presence. Plasmids are now being used to manipulate DNA and may possibly be a tool for curing many diseases.

It is possible for plasmids of different types to coexist in a single cell. Several different plasmids have been found in *E. coli*. But *related* plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation of vital plasmid functions. Therefore, plasmids can be assigned into *compatibility groups*.

Another way to classify plasmids is by function. There are five main classes:

- *Fertility-F-plasmids*, which contain tra-genes. They are capable of conjugation (transfer of genetic material between bacteria which are touching).
- *Resistance-(R)plasmids*, which contain genes that can build a resistance against antibiotics or poisons and help bacteria produce pili. Historically known as R-factors, before the nature of plasmids was understood.
- *Col-plasmids*, which contain genes that *code for* (determine the production of) bacteriocins, proteins that can kill other bacteria.
- *Degradative plasmids*, which enable the digestion of unusual substances, e.g., toluene or salicylic acid.
- *Virulence plasmids*, which turn the bacterium into a pathogen (one that causes disease).

Plasmids can belong to more than one of these functional groups.

Plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single-copy plasmids have systems which attempt to actively distribute a copy to both daughter cells.

Some plasmids or microbial hosts include an *addiction system* or "postsegregational killing system (PSK)", such as the hok/sok (host killing/suppressor of killing) system of plasmid R1 in Escherichia coli. This variant produces both a long-lived poison and a short-lived antidote. Several types of plasmid addiction systems (toxin/ antitoxin, metabolism-based, ORT systems) were described in the literature and used in biotechnical (fermentation) or biomedical (vaccine therapy) applications. Daughter cells that retain a copy of the plasmid survive, while a daughter cell that fails to inherit the plasmid dies or suffers a reduced growth-rate because of the lingering poison from the parent cell. Finally, the overall productivity could be enhanced.

Yeast Plasmids

Other types of plasmids, often related to yeast cloning vectors include:

- *Yeast integrative plasmid (YIp)*, yeast vectors that rely on integration into the host chromosome for survival and replication, and are usually used when studying the functionality of a solo gene or when the gene is toxic. Also connected with the gene URA3, that codes an enzyme related to the biosynthesis of pyrimidine nucleotides (T, C);
- *Yeast Replicative Plasmid (YRp)*, which transport a sequence of chromosomal DNA that includes an origin of replication. These plasmids are less stable, as they can "get lost" during the budding.

Plasmid DNA extraction

As alluded to above, plasmids are often used to purify a specific sequence, since they can easily be purified away from the rest of the genome. For their use as vectors, and for molecular cloning, plasmids often need to be isolated.

There are several methods to isolate plasmid DNA from bacteria, the archetypes of which are the **miniprep** and the **maxiprep**/**bulkprep**. The former can be used to quickly find out whether the plasmid is correct in any of several bacterial clones. The yield is a small amount of impure plasmid DNA, which is sufficient for analysis by restriction digest and for some cloning techniques.

In the latter, much larger volumes of bacterial suspension are grown from which a maxi-prep can be performed. Essentially this is a scaled-up miniprep followed by additional purification. This results in relatively large amounts (several micrograms) of very pure plasmid DNA.

In recent times many commercial kits have been created to perform plasmid extraction at various scales, purity and levels of automation. Commercial services can prepare plasmid DNA at quoted prices below $300/mg in milligram quantities and $15/mg in gram quantities (early 2007).

Conformations

Plasmid DNA may appear in one of five conformations, which (for a given size) run at different speeds in a gel during electrophoresis. The conformations are listed below in order of electrophoretic mobility (speed for a given applied voltage) from slowest to fastest:

- "Nicked Open-Circular" DNA has one strand cut.

- "Relaxed Circular" DNA is fully intact with both strands uncut, but has been enzymatically "relaxed" (supercoils removed). You can model this by letting a twisted extension cord unwind and relax and then plugging it into itself.
- "Linear" DNA has free ends, either because both strands have been cut, or because the DNA was linear *in vivo*. You can model this with an electrical extension cord that is not plugged into itself.
- "Supercoiled" (or "Covalently Closed-Circular") DNA is fully intact with both strands uncut, and with a twist built in, resulting in a compact form. You can model this by twisting an extension cord and then plugging it into itself.
- "Supercoiled Denatured" DNA is like **supercoiled DNA**, but has unpaired regions that make it slightly less compact; this can result from excessive alkalinity during plasmid preparation.

The rate of migration for small linear fragments is directly proportional to the voltage applied at low voltages. At higher voltages, larger fragments migrate at continually increasing yet different rates. Therefore the resolution of a gel decreases with increased voltage.

At a specified, low voltage, the migration rate of small linear DNA fragments is a function of their length. Large linear fragments (over 20kb or so) migrate at a certain fixed rate regardless of length. This is because the molecules 'resperate', with the bulk of the molecule following the leading end through the gel matrix. Restriction digests are frequently used to analyse purified plasmids. These enzymes specifically break the DNA at certain short sequences. The resulting linear fragments form 'bands' after gel electrophoresis. It is possible to purify certain fragments by cutting the bands out of the gel and dissolving the gel to release the DNA fragments.

Because of its tight conformation, supercoiled DNA migrates faster through a gel than linear or open-circular DNA.

Simulation of plasmids

The use of plasmids as a technique in molecular biology is supported by bioinformatics software. These programmes record the DNA sequence of plasmid vectors, help to predict cut sites of restriction enzymes, and to plan manipulations. Examples of software packages that handle plasmid maps are Geneious, Lasergene, GeneConstructionKit, ApE, and Vector NTI.

Chapter- 8

Metabolic Pathway

In biochemistry, **metabolic pathways** are series of chemical reactions occurring within a cell. In each pathway, a principal chemical is modified by a series of chemical reactions. Enzymes catalyze these reactions, and often require dietary minerals, vitamins, and other cofactors in order to function properly. Because of the many chemicals (a.k.a. "metabolites") that may be involved, metabolic pathways can be quite elaborate. In addition, numerous distinct pathways co-exist within a cell. This collection of pathways is called the metabolic network. Pathways are important to the maintenance of homeostasis within an organism. Catabolic (break-down) and Anabolic (synthesis) pathways often work interdependently to create new biomolecules as the final end-products.

A metabolic pathway involves the step-by-step modification of an initial molecule to form another product. The resulting product can be used in one of three ways:

- To be used immediately, as the end-product of a metabolic pathway
- To initiate another metabolic pathway, called a flux generating step
- To be stored by the cell

A molecule called a substrate enters a metabolic pathway depending on the needs of the cell and the availability of the substrate. An increase in concentration of anabolic and catabolic intermediates and/or end-products may influence the metabolic rate for that particular pathway.

Overview

Each metabolic pathway consists of a series of biochemical reactions that are connected by their intermediates: the products of one reaction are the substrates for subsequent reactions, and so on. Metabolic pathways are often considered to flow in one direction. Although all chemical reactions are technically reversible, conditions in the cell are often such that it is thermodynamically more favorable for flux to flow in one direction of a reaction. For example, one pathway may be responsible for the synthesis of a particular

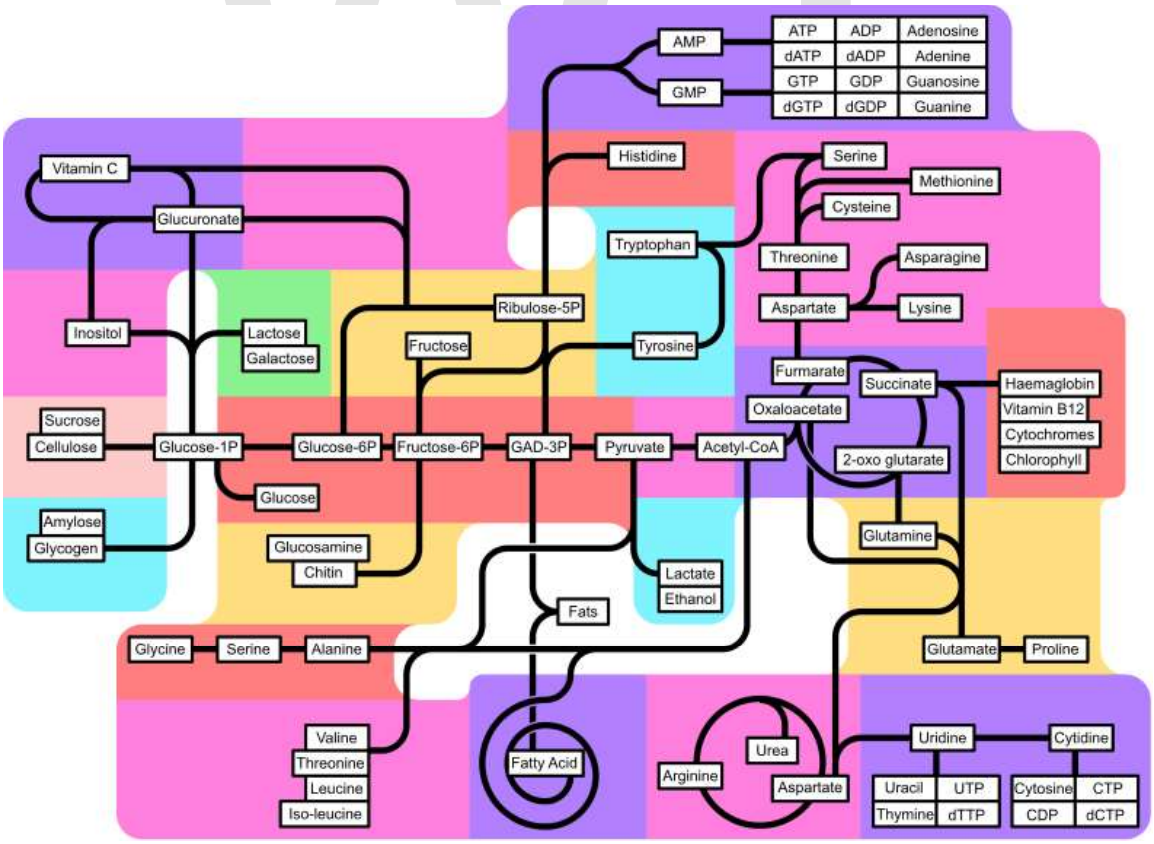
amino acid, but the breakdown of that amino acid may occur via a separate and distinct pathway. One example of an exception to this "rule" is the metabolism of glucose. Glycolysis results in the breakdown of glucose, but several reactions in the glycolysis pathway are reversible and participate in the re-synthesis of glucose (gluconeogenesis).

- Glycolysis was the first metabolic pathway discovered:

1. As glucose enters a cell, it is immediately phosphorylated by ATP to glucose 6-phosphate in the irreversible first step.
2. In times of excess lipid or protein energy sources, certain reactions in the glycolysis pathway may run in reverse in order to produce glucose 6-phosphate which is then used for storage as glycogen or starch.

- Metabolic pathways are often regulated by feedback inhibition.
- Some metabolic pathways flow in a 'cycle' wherein each component of the cycle is a substrate for the subsequent reaction in the cycle, such as in the Krebs Cycle.
- Anabolic and catabolic pathways in eukaryotes often occur independently of each other, separated either physically by compartmentalization within organelles or separated biochemically by the requirement of different enzymes and co-factors.

Major metabolic pathways



Cellular respiration

Several distinct but linked metabolic pathways are used by cells to transfer the energy released by breakdown of fuel molecules into ATP and other small molecules used for energy (e.g. GTP, NADPH, FADH).

These pathways occur within all living organisms in some form:

1. Glycolysis
2. Aerobic respiration and/or Anaerobic respiration
3. Citric acid cycle / Krebs cycle
4. Oxidative phosphorylation

Other pathways occurring in (most or) all living organisms include (but are not limited to):

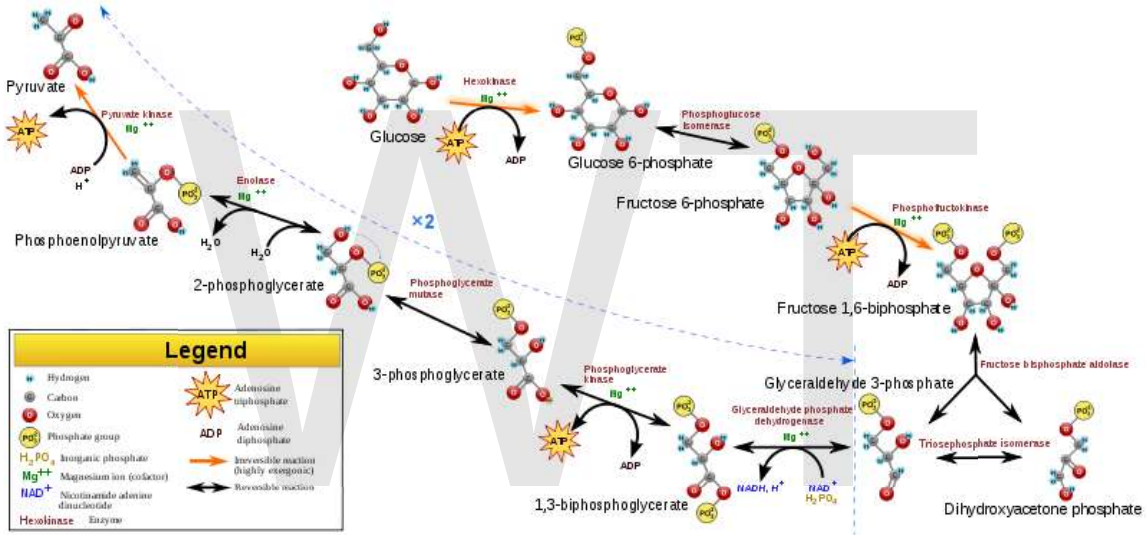
- Fatty acid oxidation (β-oxidation)
- Gluconeogenesis
- Amino acid metabolism
- Urea cycle / Nitrogen metabolism
- Nucleotide metabolism
- Glycogen synthesis / Glycogen storage
- Pentose phosphate pathway (hexose monophosphate shunt)
- Porphyrin synthesis (or heme synthesis) pathway
- Lipogenesis
- HMG-CoA reductase pathway

Synthesis of energetic compounds from non-living matter:

- Photosynthesis (plants, algae, cyanobacteria)
- Chemosynthesis (some bacteria)

Chapter- 9

Glycolysis



Glycolysis overview

Glycolysis (from *glycose*, an older term for glucose + *-lysis* degradation) is the metabolic pathway that converts glucose $C_6H_{12}O_6$, into pyruvate, $CH_3COCOO^- + H^+$. The free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide).

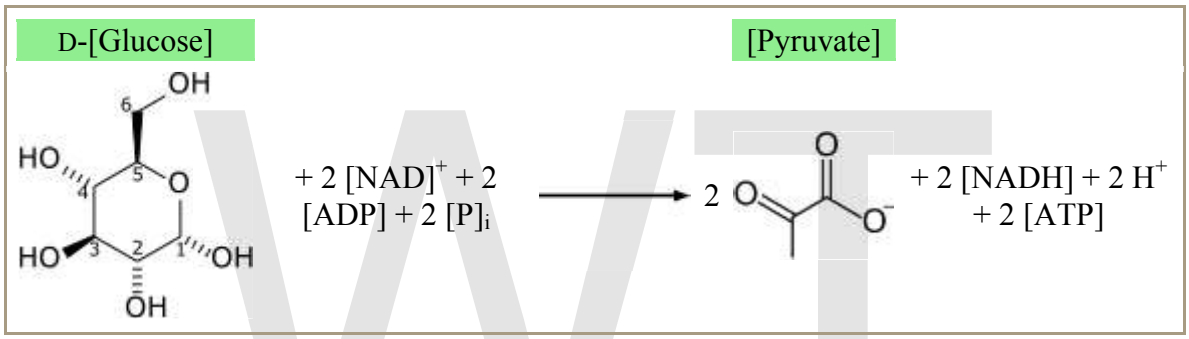
Glycolysis is a definite sequence of ten reactions involving ten intermediate compounds (one of the steps involves two intermediates). The intermediates provide entry points to glycolysis. For example, most monosaccharides, such as fructose, glucose, and galactose, can be converted to one of these intermediates. The intermediates may also be directly useful. For example, the intermediate dihydroxyacetone phosphate (DHAP) is a source of the glycerol that combines with fatty acids to form fat.

Glycolysis is thought to be the archetype of a universal metabolic pathway. It occurs, with variations, in nearly all organisms, both aerobic and anaerobic. The wide occurrence of glycolysis indicates that it is one of the most ancient known metabolic pathways.

The most common type of glycolysis is the *Embden-Meyerhof-Parnas pathway (EMP pathway)*, which was first discovered by Gustav Embden, Otto Meyerhof and Jakub Karol Parnas. Glycolysis also refers to other pathways, such as the *Entner–Doudoroff pathway* and various heterofermentative and homofermentative pathways. However, the discussion here will be limited to the Embden-Meyerhof pathway.

Overview

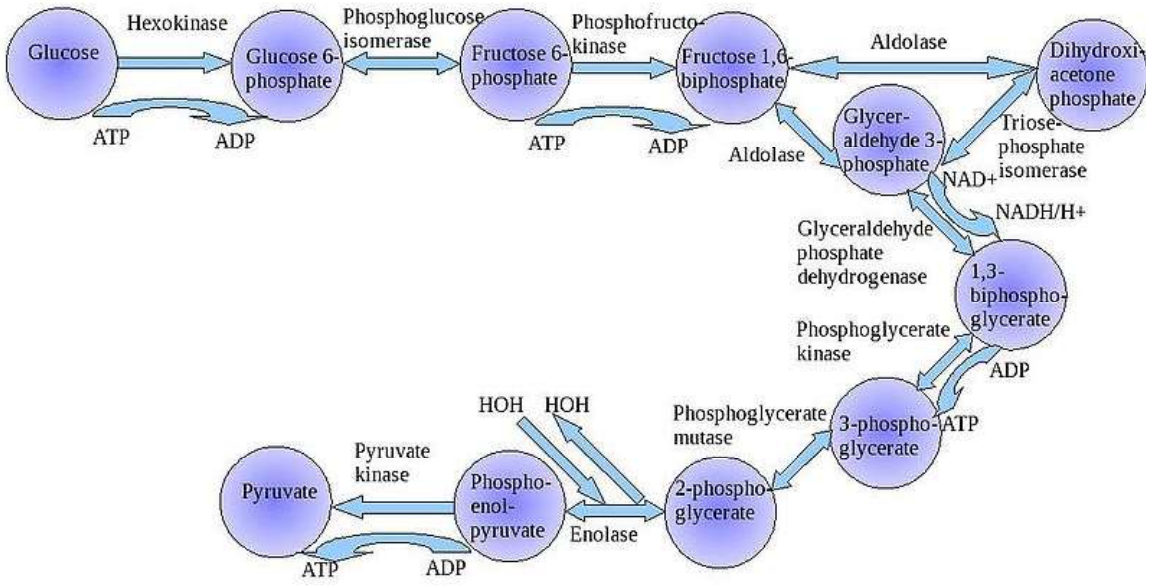
The overall reaction of glycolysis is:



The use of symbols in this equation makes it appear unbalanced with respect to oxygen atoms, hydrogen atoms, and charges. Atom balance is maintained by the two phosphate (P_i) groups:

- each exists in the form of a hydrogen phosphate anion (HPO_4^{2-}), dissociating to contribute 2 H^+ overall
- each liberates an oxygen atom when it binds to an ADP (adenosine diphosphate) molecule, contributing 2 O overall

Charges are balanced by the difference between ADP and ATP. In the cellular environment, all three hydroxy groups of ADP dissociate into $-O^-$ and H^+, giving ADP^{3-}, and this ion tends to exist in an ionic bond with Mg^{2+}, giving $ADPMg^-$. ATP behaves identically except that it has four hydroxy groups, giving $ATPMg^{2-}$. When these differences along with the true charges on the two phosphate groups are considered together, the net charges of -4 on each side are balanced.



Glycolysis

For simple anaerobic fermentations, the metabolism of one molecule of glucose to two molecules of pyruvate has a net yield of two molecules of ATP. Most cells will then carry out further reactions to 'repay' the used NAD^+ and produce a final product of ethanol or lactic acid. Many bacteria use inorganic compounds as hydrogen acceptors to regenerate the NAD^+.

Cells performing aerobic respiration synthesize much more ATP, but not as part of glycolysis. These further aerobic reactions use pyruvate and NADH + H^+ from glycolysis. Eukaryotic aerobic respiration produces approximately 34 additional molecules of ATP for each glucose molecule, however most of these are produced by a vastly different mechanism to the substrate-level phosphorylation in glycolysis.

The lower-energy production, per glucose, of anaerobic respiration relative to aerobic respiration, results in greater flux through the pathway under hypoxic (low-oxygen) conditions, unless alternative sources of anaerobically-oxidizable substrates, such as fatty acids, are found.

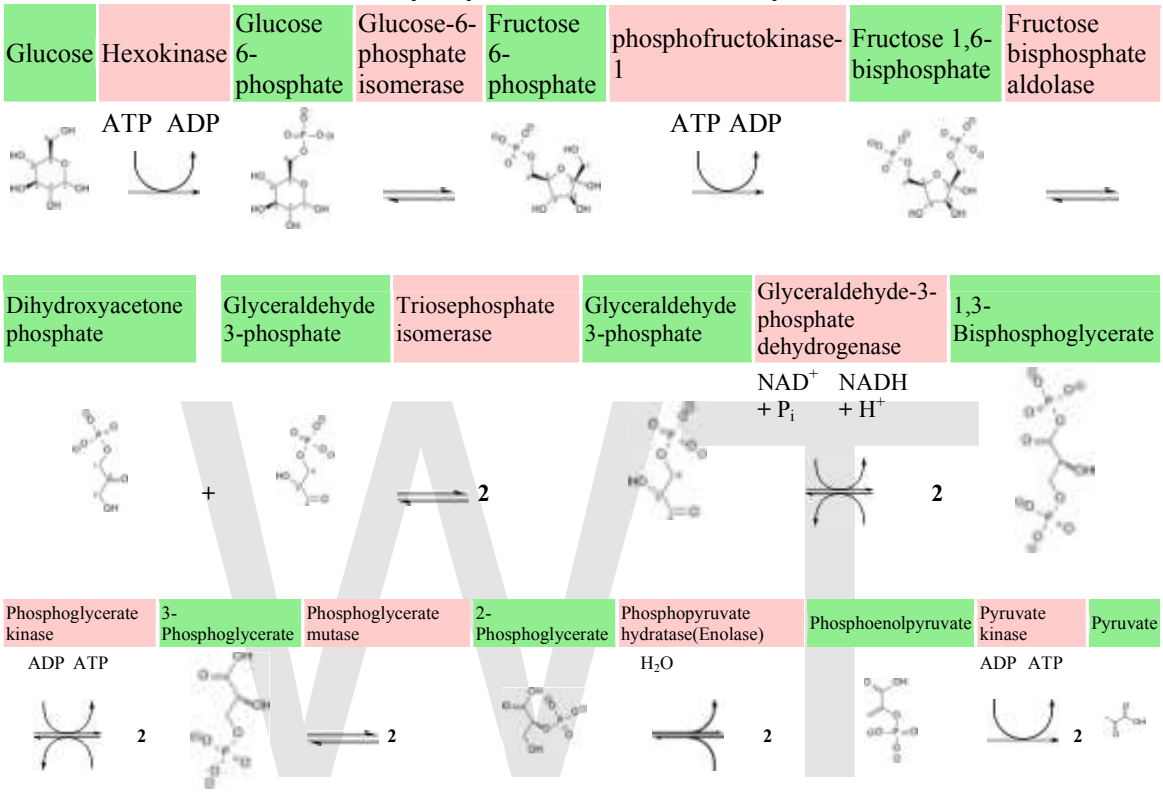
Elucidation of the pathway

In 1860, Louis Pasteur discovered that microorganisms are responsible for fermentation. In 1897, Eduard Buchner found that *extracts* of certain cells can cause fermentation. In 1905, Arthur Harden and William Youngalong with Nick Sheppard determined that a heat-sensitive high-molecular-weight subcellular fraction (the enzymes) and a heat-insensitive low-molecular-weight cytoplasm fraction (ADP, ATP and NAD^+ and other cofactors) are required together for fermentation to proceed. The details of the pathway were eventually determined by 1940, with a major input from Otto Meyerhof and some years later by Luis Leloir. The biggest difficulties in determining the intricacies of the

pathway were due to the very short lifetime and low steady-state concentrations of the intermediates of the fast glycolytic reactions.

Sequence of reactions

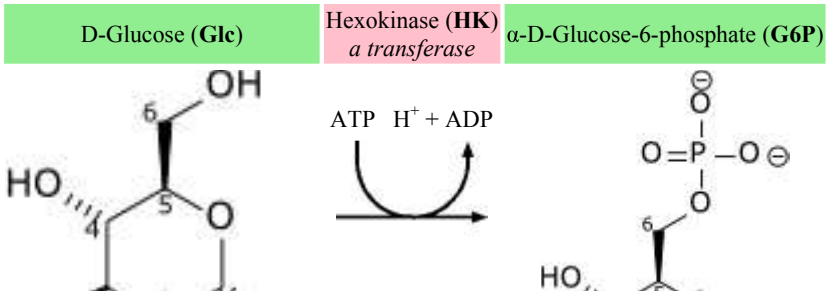
Glycolysis Metabolic Pathway



M: MET	mt, k, c/g/r/p/y/i, f/h/s/l/o/e, a/u, n, h	k, cgrp/y/i, f/h/s/l/o, au, n, h, epon	m(A16, C10),i(k, c/g/r/p/y/i, f/h/s/o/e, a/u, n, h)

Preparatory phase

The first five steps are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates (G3P).

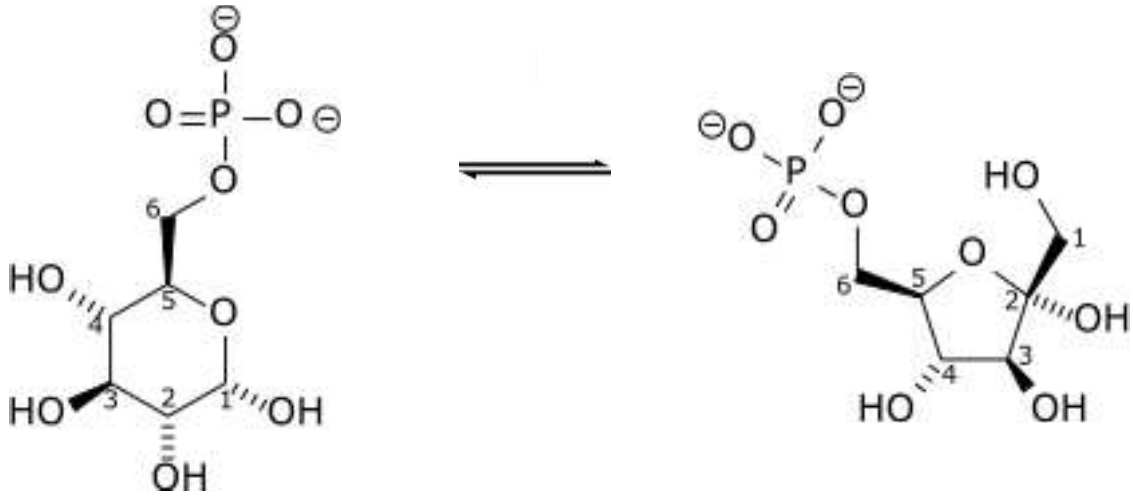


The first step in glycolysis is phosphorylation of glucose by a family of enzymes called hexokinases to form glucose 6-phosphate (G6P). This reaction consumes ATP, but it acts to keep the glucose concentration low, promoting continuous transport of glucose into the cell through the plasma membrane transporters. In addition, it blocks the glucose from leaking out - the cell lacks transporters for G6P. Glucose may alternatively be from the phosphorolysis or hydrolysis of intracellular starch or glycogen.

In animals, an isozyme of hexokinase called glucokinase is also used in the liver, which has a much lower affinity for glucose (K_m in the vicinity of normal glycemia), and differs in regulatory properties. The different substrate affinity and alternate regulation of this enzyme are a reflection of the role of the liver in maintaining blood sugar levels.

Cofactors: Mg^{2+}

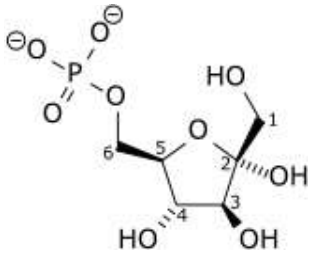
α-D-Glucose 6-phosphate (**G6P**)	Phosphoglucose isomerase *an isomerase*	β-D-Fructose 6-phosphate (**F6P**)



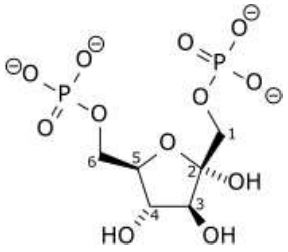
G6P is then rearranged into fructose 6-phosphate (F6P) by glucose phosphate isomerase. Fructose can also enter the glycolytic pathway by phosphorylation at this point.

The change in structure is an isomerization, in which the G6P has been converted to F6P. The reaction requires an enzyme, phosphohexose isomerase, to proceed. This reaction is freely reversible under normal cell conditions. However, it is often driven forward because of a low concentration of F6P, which is constantly consumed during the next step of glycolysis. Under conditions of high F6P concentration, this reaction readily runs in reverse. This phenomenon can be explained through Le Chatelier's Principle.

β-D-Fructose 6-phosphate (**F6P**)	phosphofructokinase (**PFK-1**) *a transferase*	β-D-Fructose 1,6-bisphosphate (**F1,6BP**)



ATP → H^+ + ADP

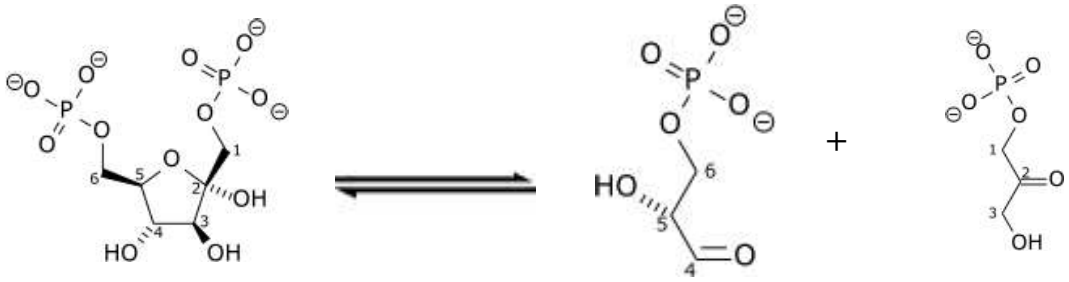


The energy expenditure of another ATP in this step is justified in 2 ways: The glycolytic process (up to this step) is now irreversible, and the energy supplied destabilizes the molecule. Because the reaction catalyzed by Phosphofructokinase 1 (PFK-1) is coupled to the hydrolysis of ATP, an energetically favorable step, it is, in essence, irreversible, and a different pathway must be used to do the reverse conversion during gluconeogenesis. This makes the reaction a key regulatory point. This is also the rate-limiting step.

The same reaction can also be catalysed by pyrophosphate-dependent phosphofructokinase (**PFP** or **PPi-PFK**), which is found in most plants, some bacteria, archea, and protists, but not in animals. This enzyme uses pyrophosphate (PPi) as a phosphate donor instead of ATP. It is a reversible reaction, increasing the flexibility of glycolytic metabolism. A rarer ADP-dependent PFK enzyme variant has been identified in archaean species.

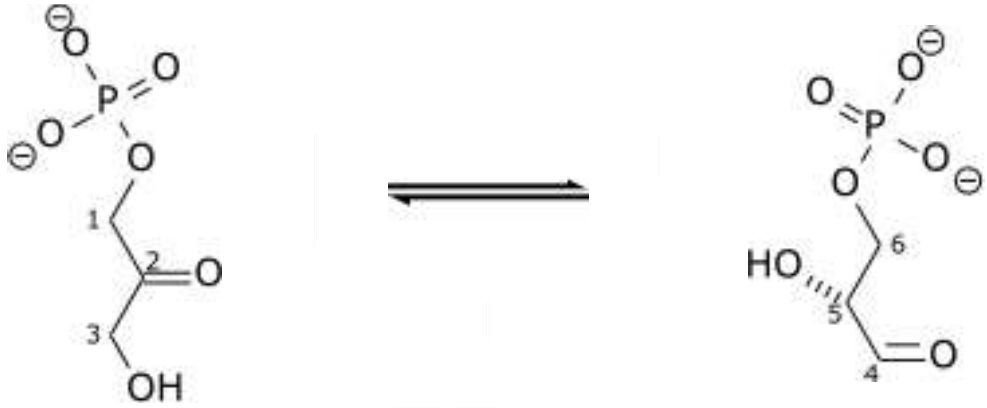
Cofactors: Mg^{2+}

β-D-Fructose 1,6-bisphosphate (**F1,6BP**)	fructose bisphosphate aldolase (**ALDO**) *a lyase*	D-glyceraldehyde 3-phosphate (**GADP**)		Dihydroxyacetone phosphate (**DHAP**)



Destabilizing the molecule in the previous reaction allows the hexose ring to be split by aldolase into two triose sugars, dihydroxyacetone phosphate, a ketone, and glyceraldehyde 3-phosphate, an aldehyde. There are two classes of aldolases: class I aldolases, present in animals and plants, and class II aldolases, present in fungi and bacteria; the two classes use different mechanisms in cleaving the ketose ring.

Dihydroxyacetone phosphate (**DHAP**)	triosephosphate isomerase (**TPI**) *an isomerase*	D-glyceraldehyde 3-phosphate (**GADP**)

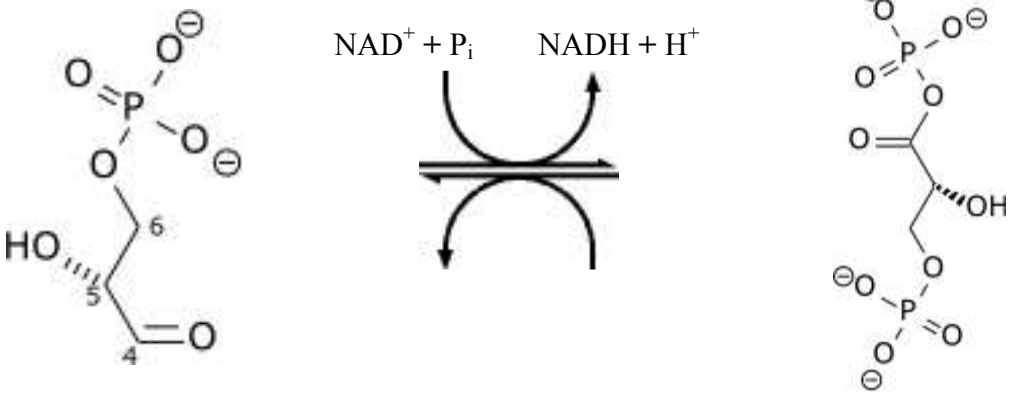


Triosephosphate isomerase rapidly interconverts dihydroxyacetone phosphate with glyceraldehyde 3-phosphate (**GADP**) that proceeds further into glycolysis. This is advantageous, as it directs dihydroxyacetone phosphate down the same pathway as glyceraldehyde 3-phosphate, simplifying regulation.

Pay-off phase

The second half of glycolysis is known as the pay-off phase, characterised by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose.

glyceraldehyde 3-phosphate (**GADP**)	glyceraldehyde phosphate dehydrogenase (**GAPDH**) *an oxidoreductase*	D-1,3-bisphosphoglycerate (**1,3BPG**)

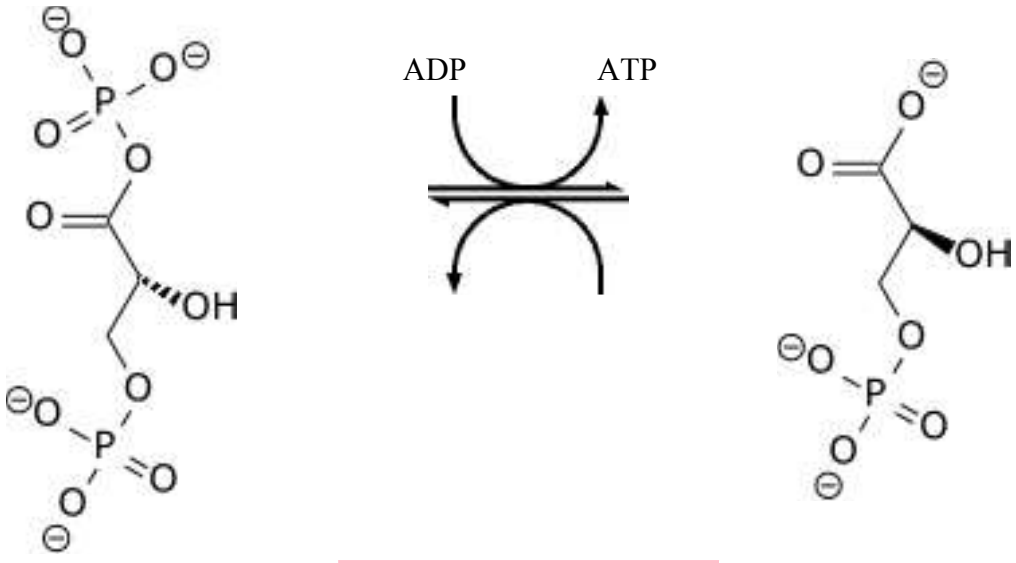


The triose sugars are dehydrogenated and inorganic phosphate is added to them, forming 1,3-bisphosphoglycerate.

The hydrogen is used to reduce two molecules of NAD^+, a hydrogen carrier, to give $NADH + H^+$ for each triose.

Hydrogen atom balance and charge balance are both maintained because the phosphate (P_i) group actually exists in the form of a hydrogen phosphate anion (HPO_4^{2-}), which dissociates to contribute the extra H^+ ion and gives a net charge of -3 on both sides.

1,3-bisphosphoglycerate (**1,3-BPG**)	phosphoglycerate kinase (**PGK**) *a transferase*	3-phosphoglycerate (**3-P-G**)



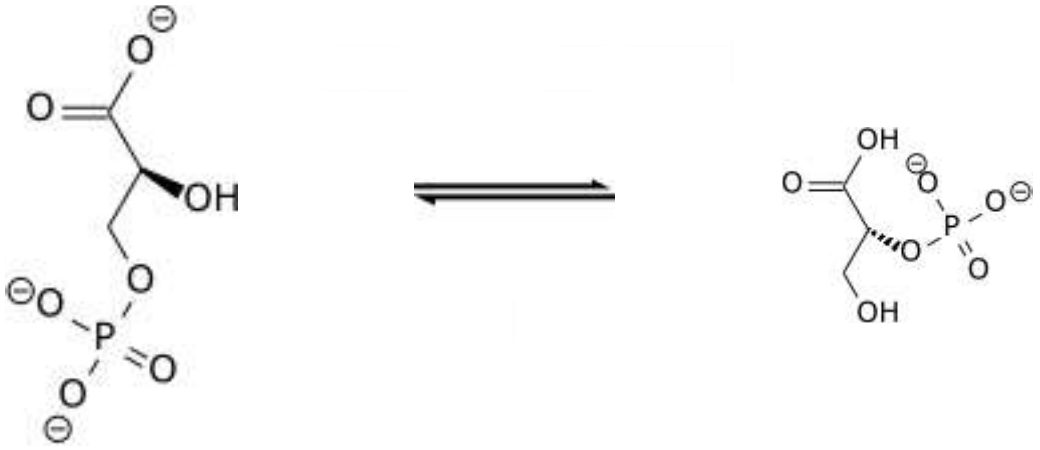
phosphoglycerate kinase (**PGK**)

This step is the enzymatic transfer of a phosphate group from 1,3-bisphosphoglycerate to ADP by phosphoglycerate kinase, forming ATP and 3-phosphoglycerate. At this step, glycolysis has reached the break-even point: 2 molecules of ATP were consumed, and 2 new molecules have now been synthesized. This step, one of the two substrate-level phosphorylation steps, requires ADP; thus, when the cell has plenty of ATP (and little ADP), this reaction does not occur. Because ATP decays relatively quickly when it is not metabolized, this is an important regulatory point in the glycolytic pathway.

ADP actually exists as $ADPMg^-$, and ATP as $ATPMg^{2-}$, balancing the charges at -5 both sides.

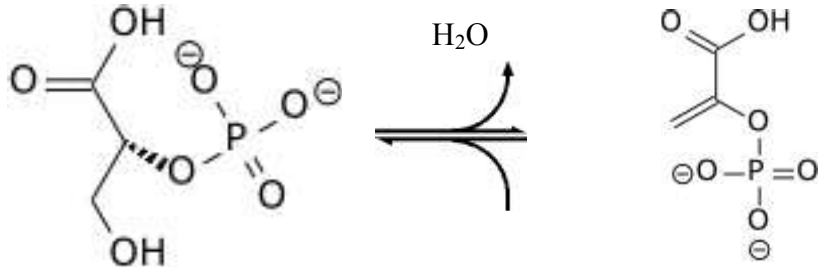
Cofactors: Mg^{2+}

3-phosphoglycerate (**3PG**)	phosphoglycerate mutase (**PGM**) *a mutase*	2-phosphoglycerate (**2PG**)



Phosphoglycerate mutase now forms 2-phosphoglycerate.

2-phosphoglycerate (**2PG**)	enolase (**ENO**) *a lyase*	phosphoenolpyruvate (**PEP**)

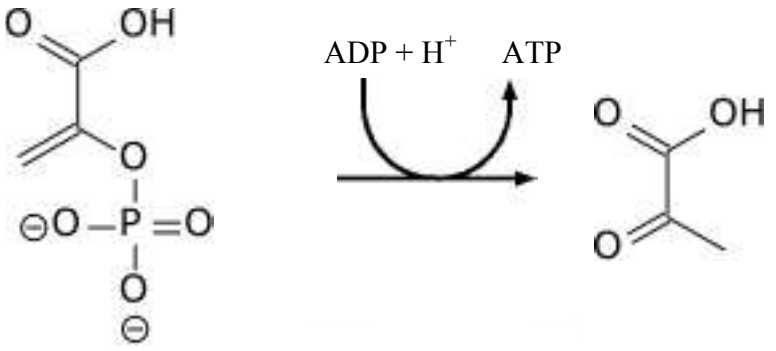


enolase (**ENO**)

Enolase next forms phosphoenolpyruvate from 2-phosphoglycerate.

Cofactors: 2 Mg^{2+}: one "conformational" ion to coordinate with the carboxylate group of the substrate, and one "catalytic" ion that participates in the dehydration.

phosphoenolpyruvate (**PEP**)	pyruvate kinase (**PK**) *a transferase*	pyruvate (**Pyr**)



A final substrate-level phosphorylation now forms a molecule of pyruvate and a molecule of ATP by means of the enzyme pyruvate kinase. This serves as an additional regulatory step, similar to the phosphoglycerate kinase step.

Cofactors: Mg^{2+}

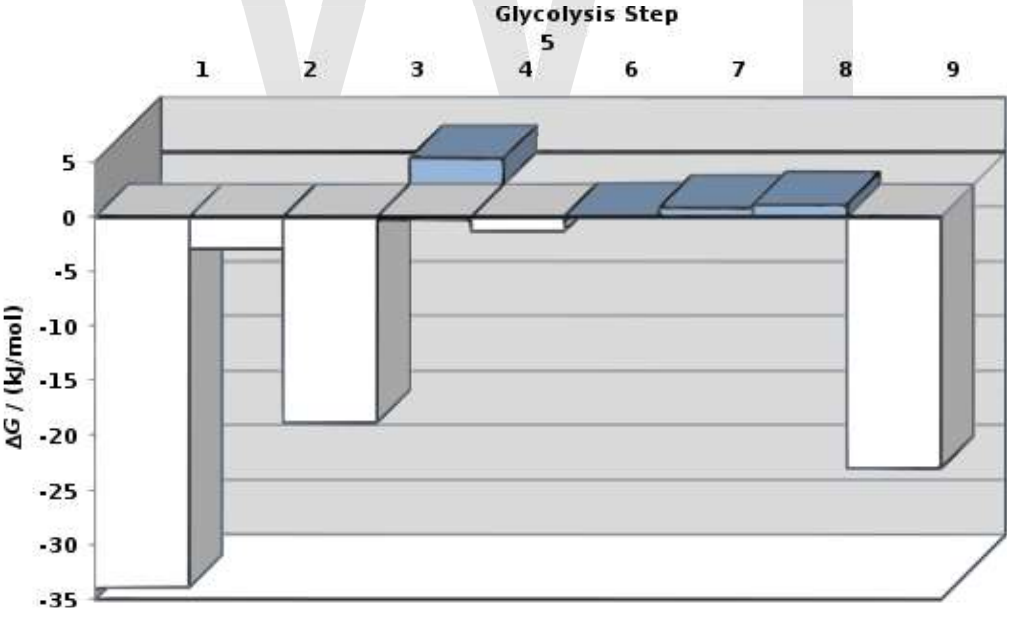
Regulation

Glycolysis is regulated by slowing down or speeding up certain steps in the glycolysis pathway. This is accomplished by inhibiting or activating the enzymes that are involved. The steps that are regulated may be determined by calculating the change in free energy, ΔG, for each step. If a step's products and reactants are in equilibrium, then the step is assumed to not be regulated. Since the change in free energy is zero for a system at equilibrium, *any step with a free energy change near zero is not being regulated*. If a step is being regulated, then that step's enzyme is not converting reactants into products as fast as it could, resulting in a build-up of reactants, which would be converted to products if the enzyme were operating faster. Since the reaction is thermodynamically favorable, the change in free energy for the step will be negative. *A step with a large negative change in free energy is assumed to be regulated.*

Free energy changes

Concentrations of metabolites in erythrocytes

Compound	Concentration / m*M*
glucose	5.0
glucose-6-phosphate	0.083
fructose-6-phosphate	0.014
fructose-1,6-bisphosphate	0.031
dihydroxyacetone phosphate	0.14
glyceraldehyde-3-phosphate	0.019
1,3-bisphosphoglycerate	0.001
2,3-bisphosphoglycerate	4.0
3-phosphoglycerate	0.12
2-phosphoglycerate	0.03
phosphoenolpyruvate	0.023
pyruvate	0.051
ATP	1.85
ADP	0.14
P_i	1.0



The change in free energy for each step of glycolysis estimated from the concentration of metabolites in a erythrocyte.

The change in free energy, ΔG, for each step in the glycolysis pathway can be calculated using $\Delta G = \Delta G^{\circ\prime} + RT\ln Q$, where Q is the reaction quotient. This requires knowing the concentrations of the metabolites. All of these values are available for erythrocytes, with the exception of the concentrations of NAD^+ and NADH. The ratio of NAD^+ to NADH is approximately 1, which results in these concentrations canceling out in the reaction quotient. (Since NAD^+ and NADH occur on opposite sides of the reactions, one will be in the numerator and the other in the denominator.)

Using the measured concentrations of each step, and the standard free energy changes, the actual free energy change can be calculated.

Change in free energy for each step of glycolysis

Step	Reaction	$\Delta G^{\circ\prime}$ / (kJ/mol)	ΔG / (kJ/mol)
1	glucose + ATP^{4-} → glucose-6-phosphate^{2-} + ADP^{3-} + H^+	-16.7	-34
2	glucose-6-phosphate^{2-} → fructose-6-phosphate^{2-}	1.67	-2.9
3	fructose-6-phosphate^{2-} + ATP^{4-} → fructose-1,6-bisphosphate^{4-} + ADP^{3-} + H^+	-14.2	-19
4	fructose-1,6-bisphosphate^{4-} → dihydroxyacetone phosphate^{2-} + glyceraldehyde-3-phosphate^{2-}	23.9	-0.23
5	dihydroxyacetone phosphate^{2-} → glyceraldehyde-3-phosphate^{2-}	7.56	2.4
6	glyceraldehyde-3-phosphate^{2-} + P_i^{2-} + NAD^+ → 1,3-bisphosphoglycerate^{4-} + NADH + H^+	6.30	-1.29
7	1,3-bisphosphoglycerate^{4-} + ADP^{3-} → 3-phosphoglycerate^{3-} + ATP^{4-}	-18.9	0.09
8	3-phosphoglycerate^{3-} → 2-phosphoglycerate^{3-}	4.4	0.83
9	2-phosphoglycerate^{3-} → phosphoenolpyruvate^{3-} + H_2O	1.8	1.1
10	phosphoenolpyruvate^{3-} + ADP^{3-} + H^+ → pyruvate$^-$ + ATP^{4-}	-31.7	-23.0

From measuring the physiological concentrations of metabolites in a erythrocyte it seems that about seven of the steps in glycolysis are in equilibrium for that cell type. Three of the steps — the ones with large negative free energy changes — are not in equilibrium and are referred to as *irreversible*; such steps are often subject to regulation.

Step 5 in the figure is shown behind the other steps, because that step is a side-reaction that can decrease or increase the concentration of the intermediate glyceraldehyde-3-phosphate. That compound is converted to dihydroxyacetone phosphate by the enzyme triose phosphate isomerase, which is a catalytically perfect enzyme; its rate is so fast that the reaction can be assumed to be in equilibrium. The fact that ΔG is not zero indicates that the actual concentrations in the erythrocyte are not accurately known.

Biochemical logic

The existence of more than one point of regulation indicates that intermediates between those points enter and leave the glycolysis pathway by other processes. For example, in the first regulated step, hexokinase converts glucose into glucose-6-phosphate. Instead of continuing through the glycolysis pathway, this intermediate can be converted into glucose storage molecules, such as glycogen or starch. The reverse reaction, breaking down, e.g., glycogen, produces mainly glucose-6-phosphate; very little free glucose is formed in the reaction. The glucose-6-phosphate so produced can enter glycolysis *after* the first control point.

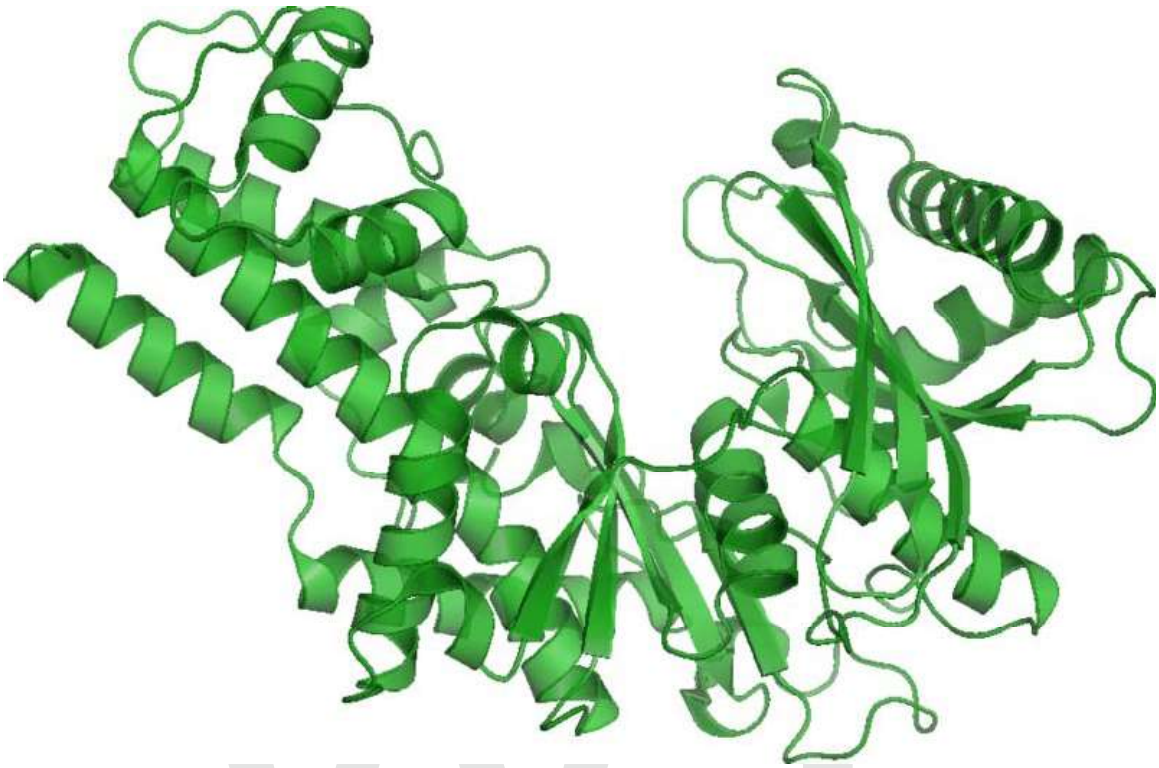
In the second regulated step (the third step of glycolysis), phosphofructokinase converts fructose-6-phosphate into fructose-1,6-bisphosphate, which then is converted into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The dihydroxyacetone phosphate can be removed from glycolysis by conversion into glycerol-3-phosphate, which can be used to form triglycerides. On the converse, triglycerides can be broken down into fatty acids and glycerol; the latter, in turn, can be converted into dihydroxyacetone phosphate, which can enter glycolysis *after* the second control point.

Regulation

The three regulated enzymes are hexokinase, phosphofructokinase, and pyruvate kinase.

The flux through the glycolytic pathway is adjusted in response to conditions both inside and outside the cell. The rate in liver is regulated to meet major cellular needs: (1) the production of ATP, (2) the provision of building blocks for biosynthetic reactions, and (3) to lower blood glucose, one of the major functions of the liver. When blood sugar falls, glycolysis is halted in the liver to allow the reverse process, gluconeogenesis. In glycolysis, the reactions catalyzed by hexokinase, phosphofructokinase, and pyruvate kinase are effectively irreversible in most organisms. In metabolic pathways, such enzymes are potential sites of control, and all three enzymes serve this purpose in glycolysis.

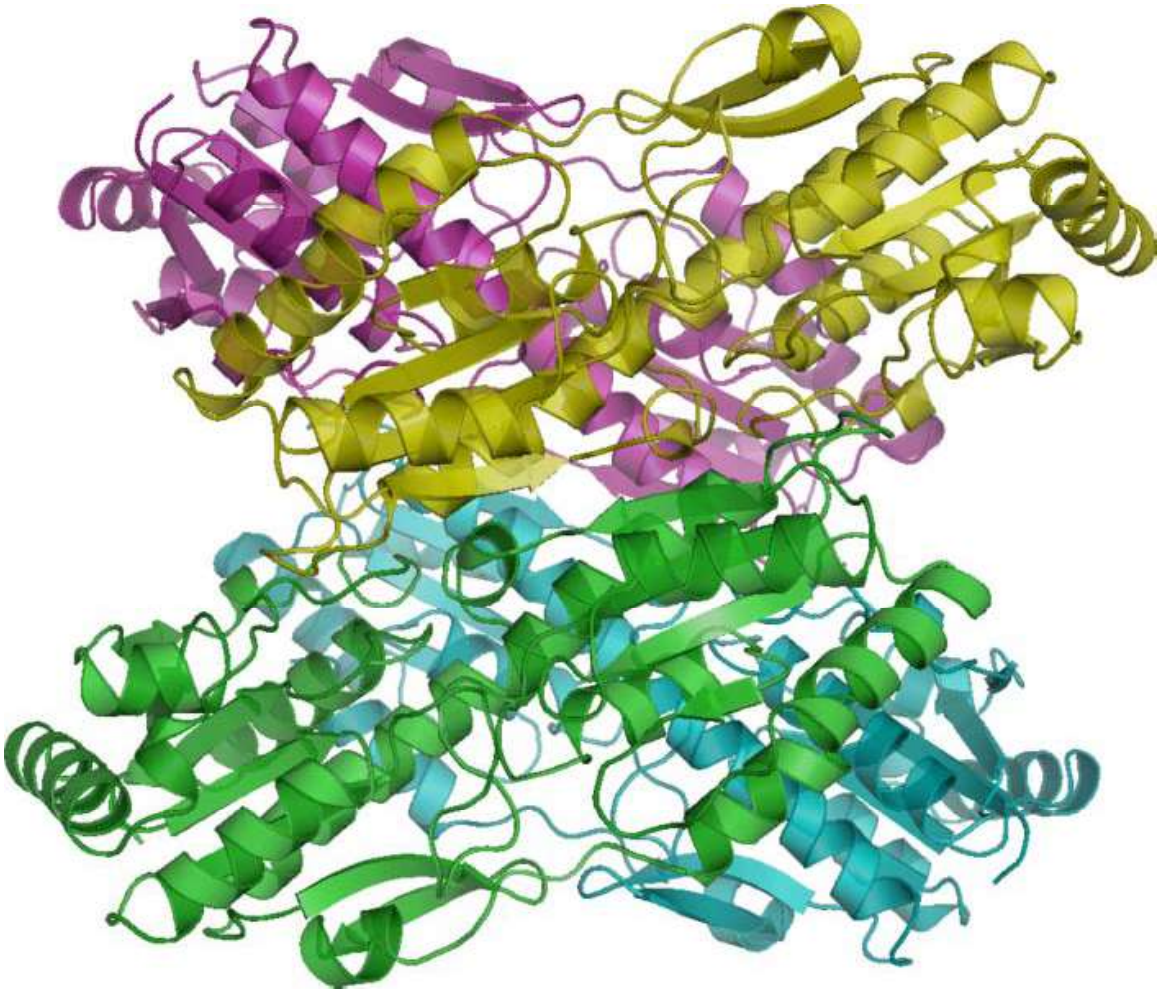
Hexokinase



Yeast hexokinase B. PDB 1IG8.

In animals, regulation of blood glucose levels by the liver is a vital part of homeostasis. In liver cells, extra G6P (glucose-6-phosphate) may be converted to G1P for conversion to glycogen, or it is alternatively converted by glycolysis to acetyl-CoA and then citrate. Excess citrate is exported to the cytosol, where ATP citrate lyase will regenerate acetyl-CoA and OAA. The acetyl-CoA is then used for fatty acid synthesis and cholesterol synthesis, two important ways of utilizing excess glucose when its concentration is high in blood. Liver contains both hexokinase and glucokinase; the latter catalyses the phosphorylation of glucose to G6P and is not inhibited by G6P. Thus, it allows glucose to be converted into glycogen, fatty acids, and cholesterol even when hexokinase activity is low. This is important when blood glucose levels are high. During hypoglycemia, the glycogen can be converted back to G6P and then converted to glucose by the liver-specific enzyme glucose 6-phosphatase. This reverse reaction is an important role of liver cells to maintain blood sugars levels during fasting. This is critical for brain function, since the brain utilizes glucose as an energy source under most conditions.

Phosphofructokinase



Bacillus stearothermophilus phosphofructokinase. PDB 6PFK.

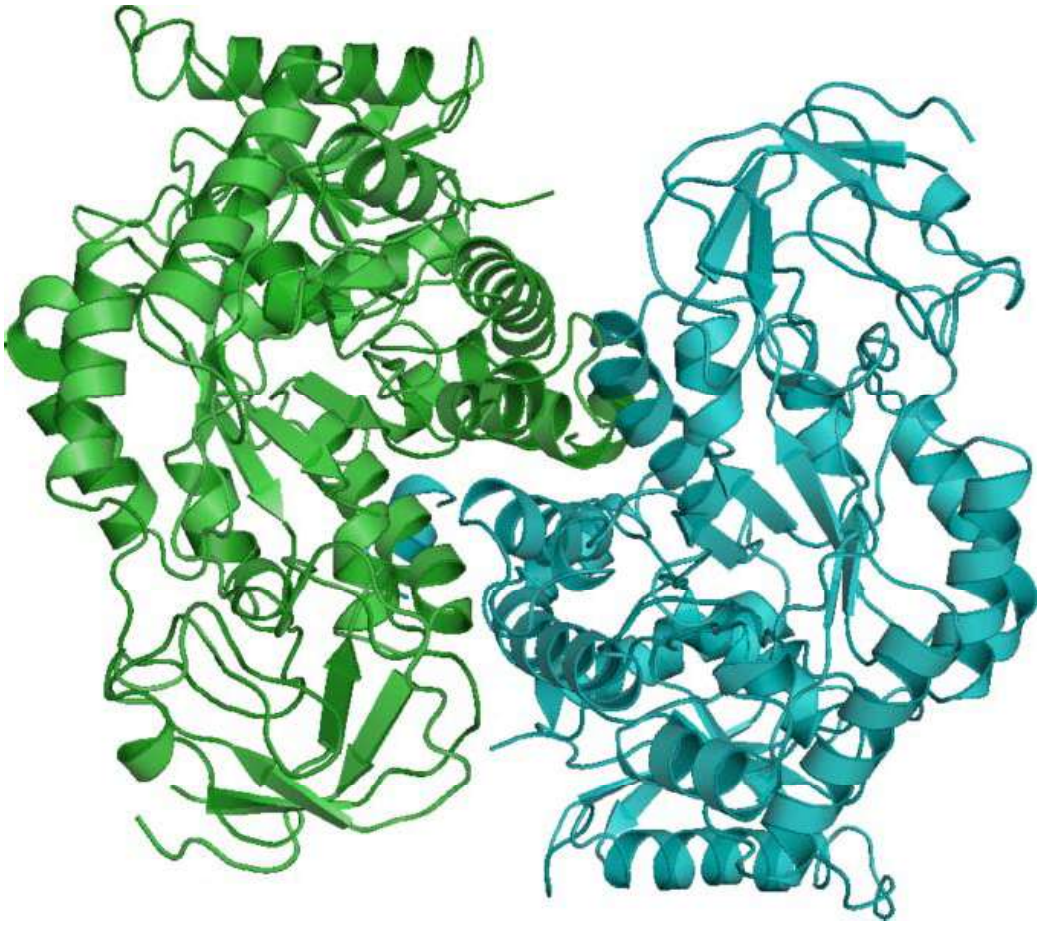
Phosphofructokinase is an important control point in the glycolytic pathway, since it is one of the irreversible steps and has key allosteric effectors, AMP and fructose 2,6-bisphosphate (F2,6BP).

Fructose 2,6-bisphosphate (F2,6BP) is a very potent activator of phosphofructokinase (PFK-1), which is synthesised when F6P is phosphorylated by a second phosphofructokinase (PFK2). In liver, when blood sugar is low and glucagon elevates cAMP, PFK2 is phosphorylated by protein kinase A. The phosphorylation inactivates PFK2, and another domain on this protein becomes active as fructose 2,6-bisphosphatase, which converts F2,6BP back to F6P. Both glucagon and epinephrine cause high levels of cAMP in the liver. The result of lower levels of liver fructose-2,6-bisphosphate is a decrease in activity of phosphofructokinase and an increase in activity of fructose 1,6-bisphosphatase, so that gluconeogenesis (in essence, "glycolysis in reverse") is favored. This is consistent with the role of the liver in such situations, since the response of the liver to these hormones is to release glucose to the blood.

ATP competes with AMP for the allosteric effector site on the PFK enzyme. ATP concentrations in cells are much higher than those of AMP, typically 100-fold higher, but the concentration of ATP does not change more than about 10% under physiological conditions, whereas a 10% drop in ATP results in a 6-fold increase in AMP. Thus, the relevance of ATP as an allosteric effector is questionable. An increase in AMP is a consequence of a decrease in energy charge in the cell.

Citrate inhibits phosphofructokinase when tested *in vitro* by enhancing the inhibitory effect of ATP. However, it is doubtful that this is a meaningful effect *in vivo*, because citrate in the cytosol is utilized mainly for conversion to acetyl-CoA for fatty acid and cholesterol synthesis.

Pyruvate kinase



Yeast pyruvate kinase. PDB 1A3W.

This enzyme catalyzes the last step of glycolysis, in which pyruvate and ATP are formed. Regulation of this enzyme is discussed in the main topic, pyruvate kinase.

Post-glycolysis processes

The overall process of glycolysis is:

$$\text{glucose} + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ \text{pyruvate} + 2\ NADH + 2\ H^+ + 2\ ATP + 2\ H_2O$$

If glycolysis were to continue indefinitely, all of the NAD^+ would be used up, and glycolysis would stop. To allow glycolysis to continue, organisms must be able to oxidize NADH back to NAD^+.

Fermentation

One method of doing this is to simply have the pyruvate do the oxidation; in this process, the pyruvate is converted to lactate (the conjugate base of lactic acid) in a process called lactic acid fermentation:

$$\text{pyruvate} + NADH + H^+ \rightarrow \text{lactate} + NAD^+$$

This process occurs in the bacteria involved in making yogurt (the lactic acid causes the milk to curdle). This process also occurs in animals under hypoxic (or partially-anaerobic) conditions, found, for example, in overworked muscles that are starved of oxygen, or in infarcted heart muscle cells. In many tissues, this is a cellular last resort for energy; most animal tissue cannot maintain anaerobic respiration for an extended length of time.

Some organisms, such as yeast, convert NADH back to NAD^+ in a process called ethanol fermentation. In this process, the pyruvate is converted first to acetaldehyde and carbon dioxide, then to ethanol.

Lactic acid fermentation and ethanol fermentation can occur in the absence of oxygen. This anaerobic fermentation allows many single-cell organisms to use glycolysis as their only energy source.

Anaerobic respiration

In the above two examples of fermentation, NADH is oxidized by transferring two electrons to pyruvate. However, anaerobic bacteria use a wide variety of compounds as the terminal electron acceptors in cellular respiration: nitrogenous compounds, such as nitrates and nitrites; sulfur compounds, such as sulfates, sulfites, sulfur dioxide, and elemental sulfur; carbon dioxide; iron compounds; manganese compounds; cobalt compounds; and uranium compounds.

Aerobic respiration

In aerobic organisms, a complex mechanism has been created to use the oxygen in air as the final electron acceptor of respiration.

- First, pyruvate is converted to acetyl-CoA and CO_2 within the mitochondria in a process called pyruvate decarboxylation.
- Second, the acetyl-CoA enters the citric acid cycle, also known as Krebs Cycle, where it is fully oxidized to carbon dioxide and water, producing yet more NADH.
- Third, the NADH is oxidized to NAD^+ by the electron transport chain, using oxygen as the final electron acceptor. This process creates a "hydrogen ion gradient" across the inner membrane of the mitochondria.
- Fourth, the proton gradient is used to produce a large amount of ATP in a process called oxidative phosphorylation.

Intermediates for other pathways

Here we, concentrates on the catabolic role of glycolysis with regard to converting potential chemical energy to usable chemical energy during the oxidation of glucose to pyruvate. However, many of the metabolites in the glycolytic pathway are also used by anabolic pathways, and, as a consequence, flux through the pathway is critical to maintain a supply of carbon skeletons for biosynthesis.

In addition, not all carbon entering the pathway leaves as pyruvate and may be extracted at earlier stages to provide carbon compounds for other pathways.

These metabolic pathways are all strongly reliant on glycolysis as a source of metabolites:

- Gluconeogenesis
- Lipid metabolism
- Pentose phosphate pathway
- Citric acid cycle, which in turn leads to:
    - Amino acid synthesis
    - Nucleotide synthesis
    - Tetrapyrrole synthesis

From an anabolic metabolism perspective, the NADH has a role to drive synthetic reactions, doing so by directly or indirectly reducing the pool of NADP+ in the cell to NADPH, which is another important reducing agent for biosynthetic pathways in a cell.

Glycolysis in disease

Genetic diseases

Glycolytic mutations are generally rare due to importance of the metabolic pathway, this means that the majority of occurring mutations result in an inability for the cell to respire, and therefore cause the death of the cell at an early stage. However, some mutations are

seen with one notable example being Pyruvate kinase deficiency, leading to chronic hemolytic anemia.

Cancer

Malignant rapidly-growing tumor cells typically have glycolytic rates that are up to 200 times higher than those of their normal tissues of origin. This phenomenon was first described in 1930 by Otto Warburg and is referred to as the Warburg effect. The Warburg hypothesis claims that cancer is primarily caused by dysfunctionality in mitochondrial metabolism, rather than because of uncontrolled growth of cells. A number of theories have been advanced to explain the Warburg effect.

This high glycolysis rate has important medical applications, as high aerobic glycolysis by malignant tumors is utilized clinically to diagnose and monitor treatment responses of cancers by imaging uptake of 2-^{18}F-2-deoxyglucose (FDG) (a radioactive modified hexokinase substrate) with positron emission tomography (PET).

There is ongoing research to affect mitochondrial metabolism and treat cancer by reducing glycolysis and thus starving cancerous cells in various new ways, including a ketogenic diet.

Alzheimer's disease

Disfunctioning glycolysis or glucose metabolism in fronto-temporo-parietal and cingulate cortices has been associated with Alzheimer's disease, probably due to the decreased amyloid β (1-42) (Aβ42) and increased tau, phosphorylated tau in cerebrospinal fluid (CSF)

Alternative nomenclature

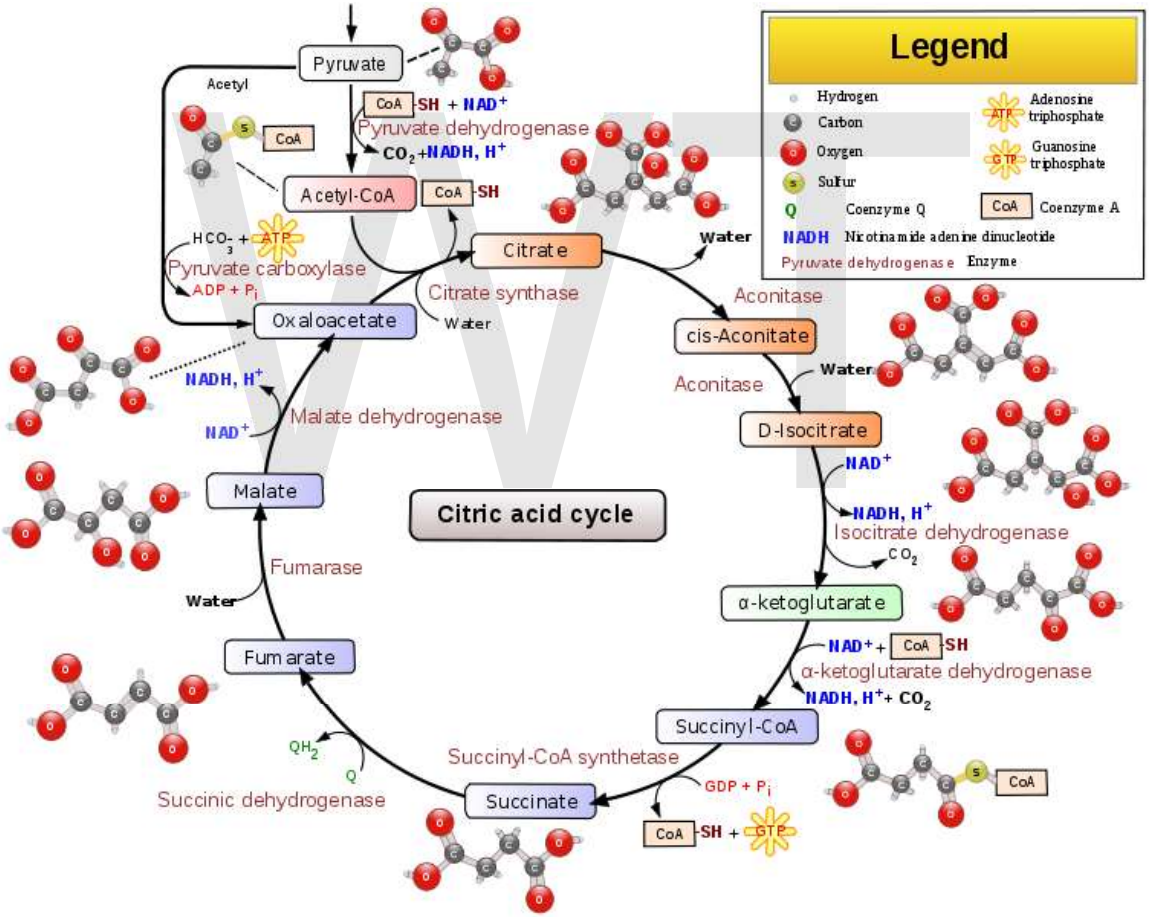
Some of the metabolites in glycolysis have alternative names and nomenclature. In part, this is because some of them are common to other pathways, such as the Calvin cycle.

	Given Names		Alternative names	Alternative nomenclature
1	glucose	**Glc**	dextrose	
3	fructose 6-phosphate	**F6P**		
4	fructose 1,6-bisphosphate	**F1,6BP**	fructose 1,6-diphosphate	**FBP**, **FDP**, **F1,6DP**
5	dihydroxyacetone phosphate	**DHAP**	glycerone phosphate	
6	glyceraldehyde 3-phosphate	**GADP**	3-phosphoglyceraldehyde	**PGAL**, **G3P**, **GALP**,**GAP**,**TP**
7	1,3-bisphosphoglycerate	**1,3BPG**	glycerate 1,3-bisphosphate,	**PGAP**, **BPG**, **DPG**

			glycerate 1,3-diphosphate, 1,3-diphosphoglycerate	
8	3-phosphoglycerate	**3PG**	glycerate 3-phosphate	**PGA**, **GP**
9	2-phosphoglycerate	**2PG**	glycerate 2-phosphate	
10	phosphoenolpyruvate	**PEP**		
11	pyruvate	**Pyr**	pyruvic acid	

Chapter- 10

Citric Acid Cycle



Overview of the citric acid cycle

The **citric acid cycle** — also known as the **tricarboxylic acid cycle** (**TCA cycle**), the **Krebs cycle**, or the **Szent-Györgyi-Krebs cycle** — is a series of enzyme-catalysed chemical reactions, which is of central importance in all living cells that use oxygen as part of cellular respiration. In eukaryotic cells, the citric acid cycle occurs in the matrix of

the mitochondrion. The components and reactions of the citric acid cycle were established by seminal work from Albert Szent-Györgyi and Hans Krebs.

In aerobic organisms, the citric acid cycle is part of a metabolic pathway involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy. Other relevant reactions in the pathway include those in glycolysis and pyruvate oxidation before the citric acid cycle, and oxidative phosphorylation after it. In addition, it provides precursors for many compounds including some amino acids and is therefore functional even in cells performing fermentation.

A simplified view of the process

- The citric acid cycle begins with the transfer of a two-carbon acetyl group from acetyl-CoA to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).
- The citrate then goes through a series of chemical transformations, losing two carboxyl groups as CO_2. The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA. The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle. Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric acid cycle in anabolism, they may not be lost, since many TCA cycle intermediates are also used as precursors for the biosynthesis of other molecules.
- Most of the energy made available by the oxidative steps of the cycle is transferred as energy-rich electrons to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced.
- Electrons are also transferred to the electron acceptor Q, forming QH_2.
- At the end of each cycle, the four-carbon oxaloacetate has been regenerated, and the cycle continues.

Steps

Two carbon atoms are oxidized to CO_2, the energy from these reactions being transferred to other metabolic processes by GTP (or ATP), and as electrons in NADH and QH_2. The NADH generated in the TCA cycle may later donate its electrons in oxidative phosphorylation to drive ATP synthesis; $FADH_2$ is covalently attached to succinate dehydrogenase, an enzyme functioning both in the TCA cycle and the mitochondrial electron transport chain in oxidative phosphorylation. $FADH_2$, therefore, facilitates transfer of electrons to coenzyme Q, which is the final electron acceptor of the reaction catalyzed by the Succinate:ubiquinone oxidoreductase complex, also acting as an intermediate in the electron transport chain.

The citric acid cycle is continuously supplied with new carbon in the form of acetyl-CoA, entering at step 1 below.

	Substrates	Products	Enzyme	Reaction type	Comment
1	Oxaloacetate + Acetyl CoA + H_2O	Citrate + CoA-SH	Citrate synthase	Aldol condensation	rate-limiting stage (irreversible), extends the 4C oxaloacetate to a 6C molecule
2	Citrate	*cis*-Aconitate + H_2O	Aconitase	Dehydration	reversible isomerisation
3	*cis*-Aconitate + H_2O	Isocitrate		Hydration	
4	Isocitrate + NAD^+	Oxalosuccinate + NADH + H^+	Isocitrate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)
5	Oxalosuccinate	α-Ketoglutarate + CO_2		Decarboxylation	irreversible stage, generates a 5C molecule
6	α-Ketoglutarate + NAD^+ + CoA-SH	Succinyl-CoA + NADH + H^+ + CO_2	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	irreversible stage, generates NADH (equivalent of 2.5 ATP), regenerates the 4C chain (CoA excluded)
7	Succinyl-CoA + GDP + P_i	Succinate + CoA-SH + GTP	Succinyl-CoA synthetase	substrate-level phosphorylation	or ADP→ATP instead of GDP→GTP, generates 1 ATP or equivalent
8	Succinate + ubiquinone (Q)	Fumarate + ubiquinol (QH_2)	Succinate dehydrogenase	Oxidation	uses FAD as a prosthetic group (FAD→$FADH_2$ in the first step of the reaction) in the enzyme, generates the equivalent of 1.5 ATP
9	Fumarate + H_2O	*L*-Malate	Fumarase	H_2O addition (*hydration*)	
10	*L*-Malate + NAD^+	Oxaloacetate + NADH + H^+	Malate dehydrogenase	Oxidation	generates NADH (equivalent of 2.5 ATP)

Mitochondria in animals, including humans, possess two succinyl-CoA synthetases: one that produces GTP from GDP, and another that produces ATP from ADP. Plants have the type that produces ATP (ADP-forming succinyl-CoA synthetase). Several of the enzymes in the cycle may be loosely-associated in a multienzyme protein complex within the mitochondrial matrix.

The GTP that is formed by GDP-forming succinyl-CoA synthetase may be utilized by nucleoside-diphosphate kinase to form ATP (the catalyzed reaction is GTP + ADP → GDP + ATP).

Products

Products of the first turn of the cycle are: *one GTP (or ATP), three NADH, one QH_2, two CO_2*.

Because two acetyl-CoA molecules are produced from each glucose molecule, two cycles are required per glucose molecule. Therefore, at the end of two cycles, the products are: two GTP, six NADH, two QH_2, and four CO_2

Description	Reactants	Products
The sum of all reactions in the citric acid cycle is:	Acetyl-CoA + 3 NAD^+ + Q + GDP + P_i + 2 H_2O	→ CoA-SH + 3 NADH + 3 H^+ + QH_2 + GTP + 2 CO_2
Combining the reactions occurring during the pyruvate oxidation with those occurring during the citric acid cycle, the following overall pyruvate oxidation reaction is obtained:	Pyruvate ion + 4 NAD^+ + Q + GDP + P_i + 2 H_2O	→ 4 NADH + 3 H^+ + QH_2 + GTP + 3 CO_2
Combining the above reaction with the ones occurring in the course of glycolysis, the following overall glucose oxidation reaction (excluding reactions in the respiratory chain) is obtained:	Glucose + 10 NAD^+ + 2 Q + 2 ADP + 2 GDP + 4 P_i + 2 H_2O	→ 10 NADH + 10 H^+ + 2 QH_2 + 2 ATP + 2 GTP + 6 CO_2

The above reactions are balanced if P_i represents the $H_2PO_4^-$ ion, ADP and GDP the ADP^{2-} and GDP^{2-} ions, respectively, and ATP and GTP the ATP^{3-} and GTP^{3-} ions, respectively.

The total number of ATP obtained after complete oxidation of one glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is estimated to be between 30 and 38. A recent assessment of the total ATP yield with the updated proton-to-ATP ratios provides an estimate of 29.85 ATP per glucose molecule.

Regulation

Although pyruvate dehydrogenase is not technically a part of the citric acid cycle, its regulation is included here.

The regulation of the TCA cycle is largely determined by substrate availability and product inhibition. NADH, a product of all dehydrogenases in the TCA cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase. Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits succinyl-CoA synthetase and citrate synthase. When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

Calcium is used as a regulator. It activates pyruvate dehydrogenase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.

Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate,a precursor of pyruvate. This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.

Recent work has demonstrated an important link between intermediates of the citric acid cycle and the regulation of hypoxia-inducible factors (HIF). HIF plays a role in the regulation of oxygen homeostasis, and is a transcription factor that targets angiogenesis, vascular remodeling, glucose utilization, iron transport and apoptosis. HIF is synthesized consititutively, and hydroxylation of at least one of two critical proline residues mediates their interaction with the von Hippel Lindau E3 ubiquitin ligase complex, which targets them for rapid degradation. This reaction is calalysed by prolyl 4-hydroxylases. Fumarate and succinate have been identified as potent inhibitors of prolyl hydroxylases, thus leading to the stabilisation of HIF.

Major metabolic pathways converging on the TCA cycle

Several catabolic pathways converge on the TCA cycle. Reactions that form intermediates of the TCA cycle in order to replenish them (especially during the scarcity of the intermediates) are called anaplerotic reactions.

The citric acid cycle is the third step in carbohydrate catabolism (the breakdown of sugars). Glycolysis breaks glucose (a six-carbon-molecule) down into pyruvate (a three-

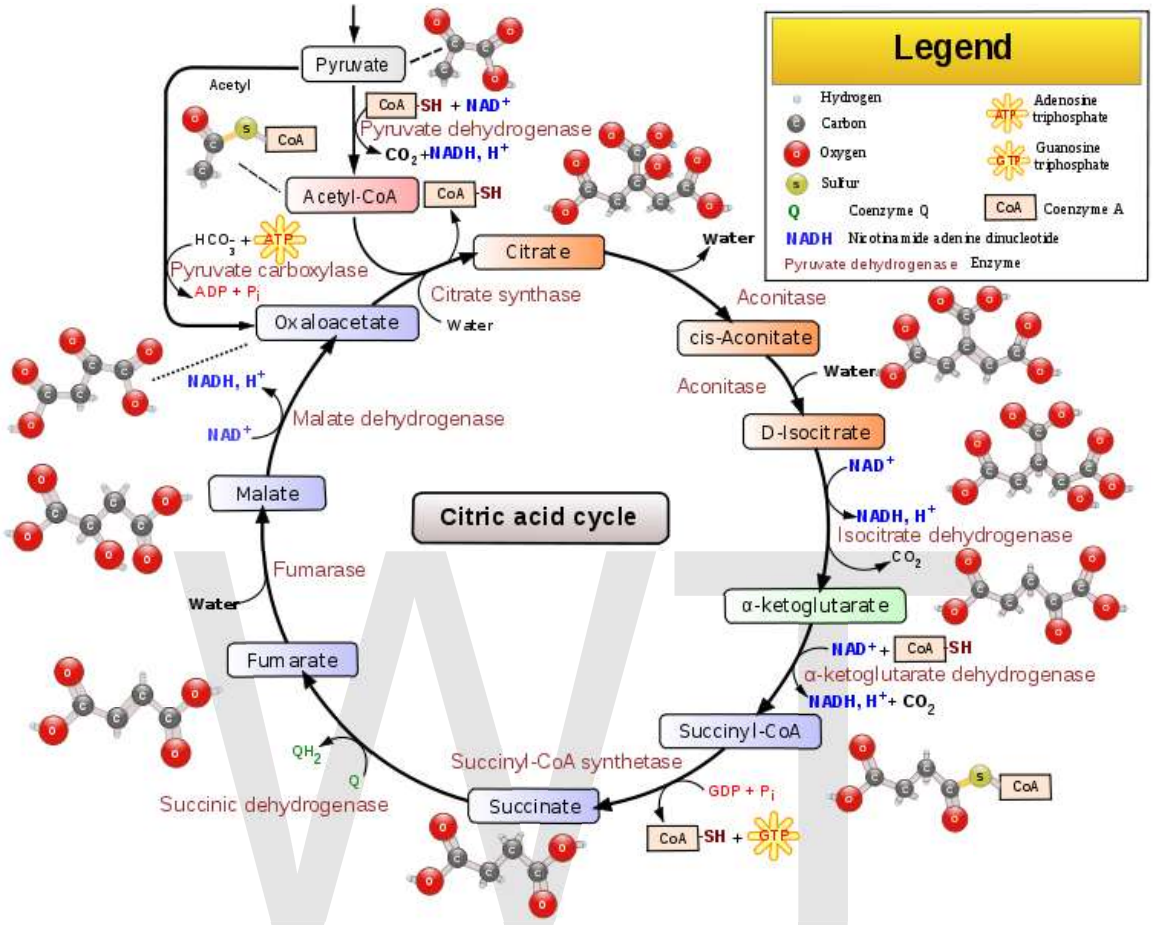
carbon molecule). In eukaryotes, pyruvate moves into the mitochondria. It is converted into acetyl-CoA by decarboxylation and enters the citric acid cycle.

In protein catabolism, proteins are broken down by proteases into their constituent amino acids. The carbon backbone of these amino acids can become a source of energy by being converted to acetyl-CoA and entering into the citric acid cycle.

In fat catabolism, triglycerides are hydrolyzed to break them into fatty acids and glycerol. In the liver the glycerol can be converted into glucose via dihydroxyacetone phosphate and glyceraldehyde-3-phosphate by way of gluconeogenesis. In many tissues, especially heart tissue, fatty acids are broken down through a process known as beta oxidation, which results in acetyl-CoA, which can be used in the citric acid cycle. Beta oxidation of fatty acids with an odd number of methylene groups produces propionyl CoA, which is then converted into succinyl-CoA and fed into the citric acid cycle.

The total energy gained from the complete breakdown of one molecule of glucose by glycolysis, the citric acid cycle, and oxidative phosphorylation equals about 30 ATP molecules, in eukaryotes. The citric acid cycle is called an amphibolic pathway because it participates in both catabolism and anabolism.

Interactive pathway map



Citric acid cycle

Chapter- 11

Ethanol Fermentation

A laboratory vessel being used for the fermentation of straw.

Ethanol fermentation, also referred to as **alcoholic fermentation**, is a biological process in which sugars such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this process in the absence of oxygen, ethanol fermentation is classified as anaerobic. Ethanol fermentation occurs in the production of alcoholic beverages and ethanol fuel, and in the rising of bread dough.

The chemical process of fermentation of glucose

The chemical equation below summarizes the fermentation of glucose, whose chemical formula is $C_6H_{12}O_6$. One mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide:

$$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow C_6H_{12}O_6$$
$$C_6H_{12}O_6 + \text{Zymase} \rightarrow 2C_2H_5OH + 2CO_2$$

C_2H_5OH is the chemical formula for ethanol.

Before fermentation takes place, one glucose molecule is broken down into two pyruvic molecules. This is known as glycolysis. Glycolysis is summarized by the chemical equation:

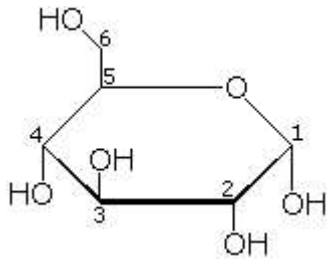
$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H^+$$

The chemical formula of pyruvate is CH_3COCOO^-. P_i stands for the inorganic phosphate. As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation.

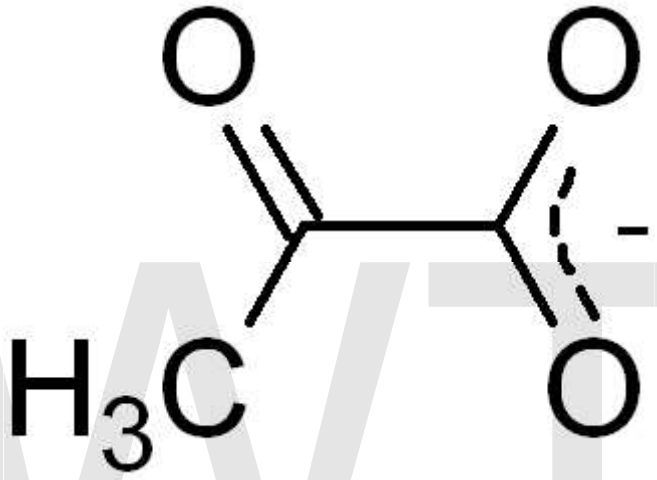
Effect of oxygen

The fermentation process does not require oxygen. If oxygen is present, some species of yeast (*Kluyveromyces lactis*, *Kluyveromyces lipolytica*) oxidize pyruvate completely to carbon dioxide and water. This process is called respiration. Thus these yeasts produce ethanol only in an anaerobic environment.

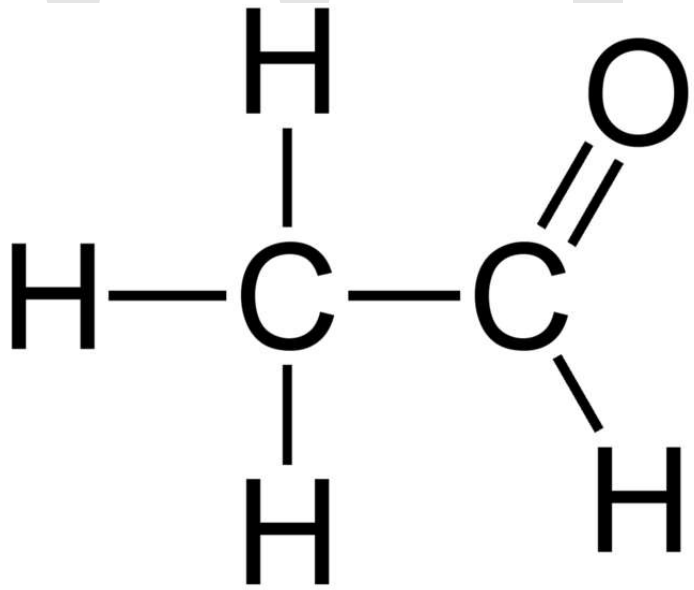
However, many yeasts such as the commonly used baker's yeast *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*, prefer fermentation to respiration. These yeasts will produce ethanol even under aerobic conditions given the right sources of nutrition.



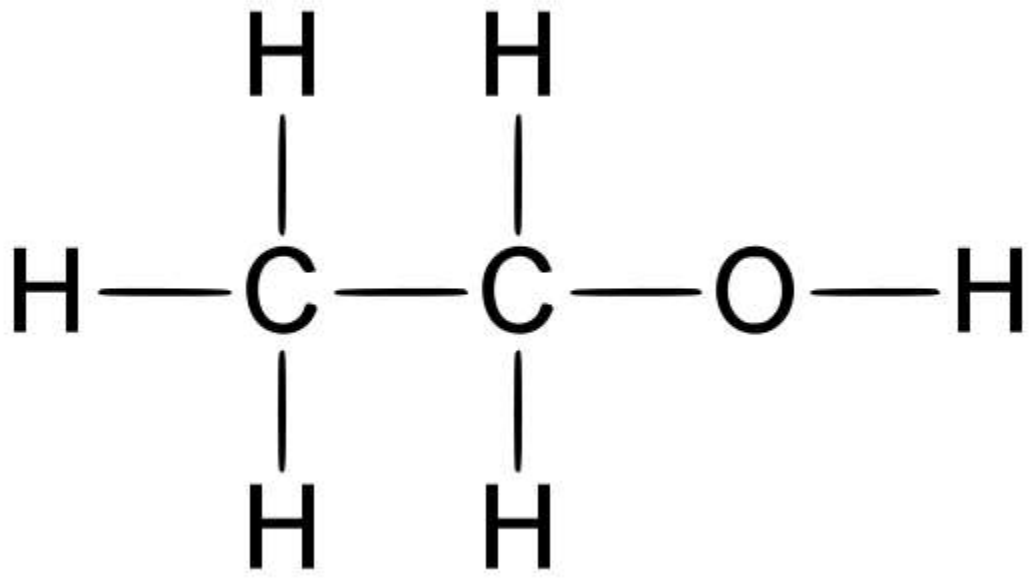
Glucose depicted in Haworth projection



Pyruvate



Acetaldehyde



Ethanol

Uses

The formation of carbon dioxide — a byproduct of yeast's respiration — causes bread to rise.

Ethanol fermentation is responsible for the rising of bread dough. Yeast organisms consume sugars in the dough and produce ethanol and carbon dioxide as waste products. The carbon dioxide forms bubbles in the dough, expanding it into something of a foam. Nearly all the ethanol evaporates from the dough when the bread is baked.

All alcoholic beverages, except those produced by carbonic maceration, are produced by ethanol fermentation by yeast. Wine and brandy are produced by fermentation of the natural sugars present in fruits, especially grapes. Beer and whiskey are produced by fermentation of grain starches that have been converted to sugar by the enzyme amylase, which is present in grain kernels that have been germinated. Amylase-treated grain or amylase-treated potatoes are fermented for the production of vodka. Rum is produced by fermentation of cane sugar. In all cases, the fermentation must take place in a vessel that allows carbon dioxide to escape, but prevents outside air from coming in, as exposure to oxygen would prevent the formation of ethanol.

Similar yeast fermentation of various carbohydrate products is used to produce much of the ethanol used for fuel.

Feedstocks for fuel production

The dominant ethanol feedstock in warmer regions is sugarcane. In temperate regions, sugar beet is sometimes used instead.

In the United States, the main feedstock for the production of ethanol is currently corn. Approximately 2.8 gallons of ethanol are produced from one bushel of corn (0.42 liter per kilogram). While much of the corn turns into ethanol, some of the corn also yields by-products such as DDGS (distillers dried grains with solubles) that can be used as feed for livestock. A bushel of corn produces about 18 pounds of DDGS (320 kilograms of DDGS per metric ton of maize). Although most of the fermentation plants have been built in corn-producing regions, sorghum is also an important feedstock for ethanol production in the Plains states. Pearl millet is showing promise as an ethanol feedstock for the southeastern U.S. and the potential of duckweed is being studied.

In some parts of Europe, particularly France and Italy, grapes have become a *de facto* feedstock for fuel ethanol by the distillation of surplus wine. In Japan, it has been proposed to use rice normally made into sake as an ethanol source.

Cassava as ethanol feedstock

Ethanol can be made from mineral oil or from sugars or starches. Starches are cheapest. The starchy crop with highest energy content per acre is cassava, which grows in tropical countries.

Thailand already had a large cassava industry in the 1990s, for use as cattle feed and as a cheap admixture to wheat flour. Nigeria and Ghana are already establishing cassava-to-ethanol plants. Brazil is doing that too (sugarcane and cassava grow on very different

types of soil). So are many other countries. Production of ethanol from cassava is currently economically feasible when crude oil prices are above US$120 per barrel.

New varieties of cassava are being developed, so the future situation remains uncertain. Currently, cassava can yield between 25-40 tonnes per hectare (with irrigation and fertilizer), and from a tonne of cassava roots, circa 200 liters of ethanol can be produced (assuming cassava with 22% starch content). A liter of ethanol contains circa 21.46 MJ of energy. The overall energy efficiency of cassava-root to ethanol conversion is circa 32%.

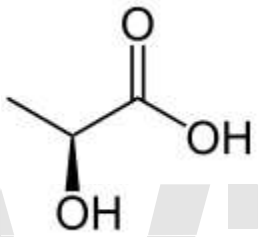
The yeast used for processing cassava is *Endomycopsis fibuligera*, sometimes used together with bacterium *Zymomonas mobilis*.

Microbes used in ethanol fermentation

- Yeast
- Zymomonas mobilis

Chapter- 12

Lactic Acid Fermentation



One isomer of lactic acid

Lactic acid fermentation is a biological process by which sugars such as glucose, fructose, and sucrose, are converted into cellular energy and the metabolic byproduct lactate. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells, in the absence of oxygen. If oxygen is present in the cell, many organisms will bypass fermentation and undergo cellular respiration; however, facultative anaerobic organisms will both ferment and undergo respiration in the presence of oxygen.

In *homofermentative fermentation*, one molecule of glucose is ultimately converted to two molecules of lactic acid. *Heterofermentative fermentation*, in contrast, yields carbon dioxide and ethanol in addition to lactic acid, in a process called the phosphoketolase pathway.

Chemical process

The process of lactic acid fermentation using glucose is summarized below. In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$$

In heterolactic fermentation, the reaction proceeds as follows, with one molecule of glucose converted to one molecule of lactic acid, one molecule of ethanol, and one molecule of carbon dioxide:

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Before lactic acid fermentation can occur, the molecule of glucose must be split into two molecules of pyruvate. This process is called glycolysis.

Glycolysis

To extract chemical energy from glucose, the glucose molecule must be split up into two molecules of pyruvate. This process also generates two molecules of adenosine triphosphate as an immediate energy yield and two molecules of NADH.

$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H^+$$

In aerobic respiration, the pyruvate is further oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation. However, this can occur only in the presence of oxygen. Oxygen is toxic to organisms that are obligate anaerobes, and is not required by facultative anaerobic organisms. In the absence of oxygen, one of the fermentation pathways occurs in order to regenerate NAD^+; lactic acid fermentation is one of these pathways.

Fermentation

Lactic acid fermentation is the simplest type of fermentation. In essence, it is a redox reaction. In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces – that is, transfers electrons to – NAD^+, forming NADH. However, there is only a limited supply of NAD^+ available in a cell. For glycolysis to continue, NADH must be oxidized – that is, have electrons taken away – to regenerate the NAD^+. This is usually done through an electron transport chain in a process called oxidative phosphorylation; however, this mechanism is not available without oxygen.

Instead, the NADH donates its extra electrons to the pyruvate molecules formed during glycolysis. Since the NADH has lost electrons, NAD^+ regenerates and is again available for glycolysis. Lactic acid, for which this process is named, is formed by the reduction of pyruvate.

In homolactic acid fermentation, both molecules of pyruvate are converted to lactate. In heterolactic acid fermentation, one molecule of pyruvate is converted to lactate; the other is converted to ethanol and carbon dioxide. Homolactic acid fermentation is unique in that it is one of the only respiration processes that do not produce a gas as a byproduct.

Purpose

Some bacteria and yeasts organisms are unable to cope with the presence of oxygen. These organisms use fermentation as a method of obtaining energy in the form of ATP.

Because the production of lactic acid frees up NAD^+, the process of glycolysis can continue.

Lactic acid fermentation also occurs in animal muscle cells under conditions when oxygen is low. Extreme exercise would be an example of this. In this situation, the lactate is carried away by the circulatory system to the liver, where it is converted back to pyruvate through the Cori cycle.

Fermentation, however, is far less effective than cellular respiration, producing only two ATP molecules per glucose molecule consumed. The typical yield from cellular respiration is anywhere from 34-38 molecules of ATP. Thus, it is typically seen only in small organisms, such as bacteria and yeast, that can survive on this low energy yield.

Applications

Lactic acid fermentation is used in many areas of the world to produce foods that cannot be produced through other methods. The most commercially important genus of lactic acid-fermenting bacteria is *Lactobacillus*, though other bacteria and even yeast are sometimes used. Two of the most common applications of lactic acid fermentation are in the production of yogurt and sauerkraut.

Yogurt production

The main method of producing yogurt is through the lactic acid fermentation of milk with harmless bacteria. The primary bacteria used are typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and US law requires all yogurts to contain these two cultures (though others may be added as probiotic cultures). These bacteria produce lactic acid in the milk culture, decreasing its pH and causing it to congeal. The bacteria also produce compounds that give yogurt its distinctive flavor. An additional effect of the lowered pH is the incompatibility of the acidic environment with many other types of harmful bacteria.

For a probiotic yogurt, additional types of bacteria such as *Lactobacillus acidophilus* are also added to the culture.

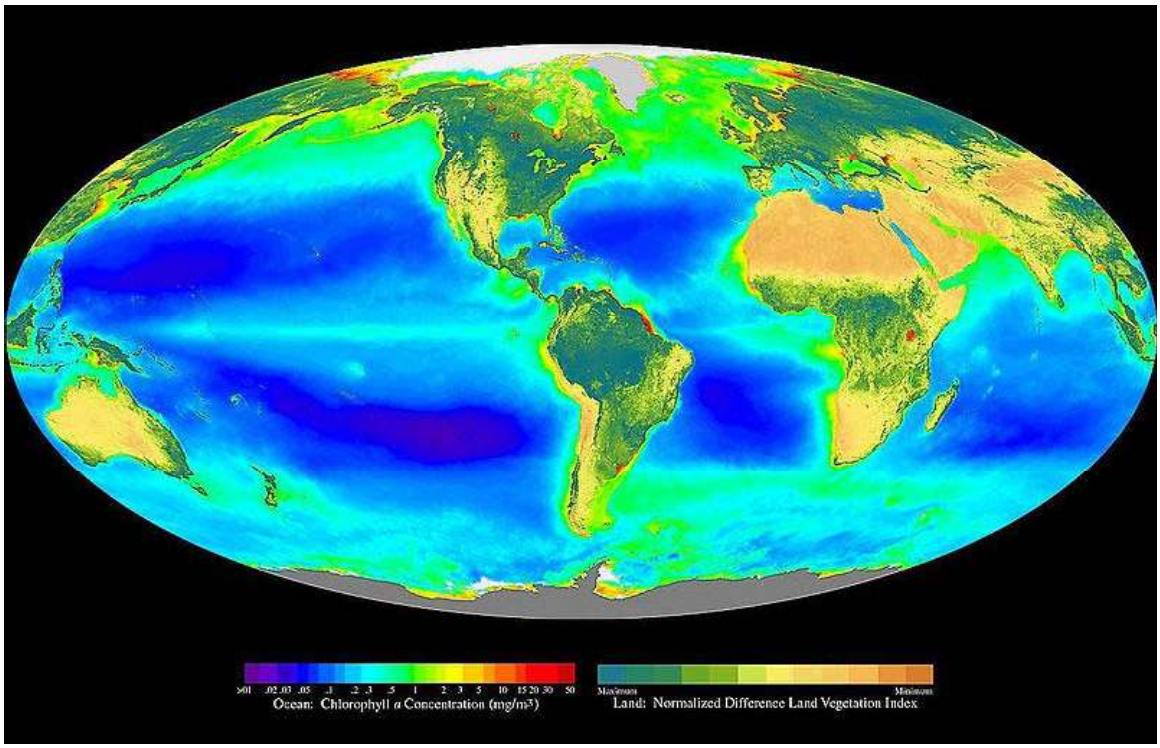
Sauerkraut

Lactic acid fermentation is also used in the production of sauerkraut. The main type of bacteria used in the production of sauerkraut is of the genus *Leuconostoc*.

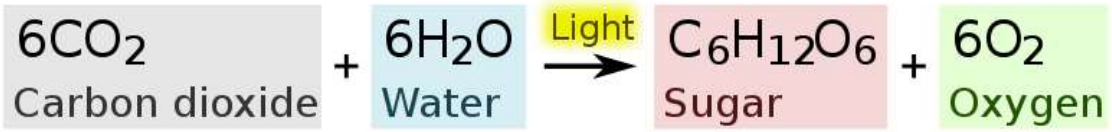
As in yogurt, when the acidity rises due to lactic acid-fermenting organisms, many other pathogenic microorganisms are killed. The bacteria produce lactic acid, as well as simple alcohols and other hydrocarbons. These may then combine to form esters, contributing to the unique flavor of sauerkraut.

Chapter- 13

Photosynthesis



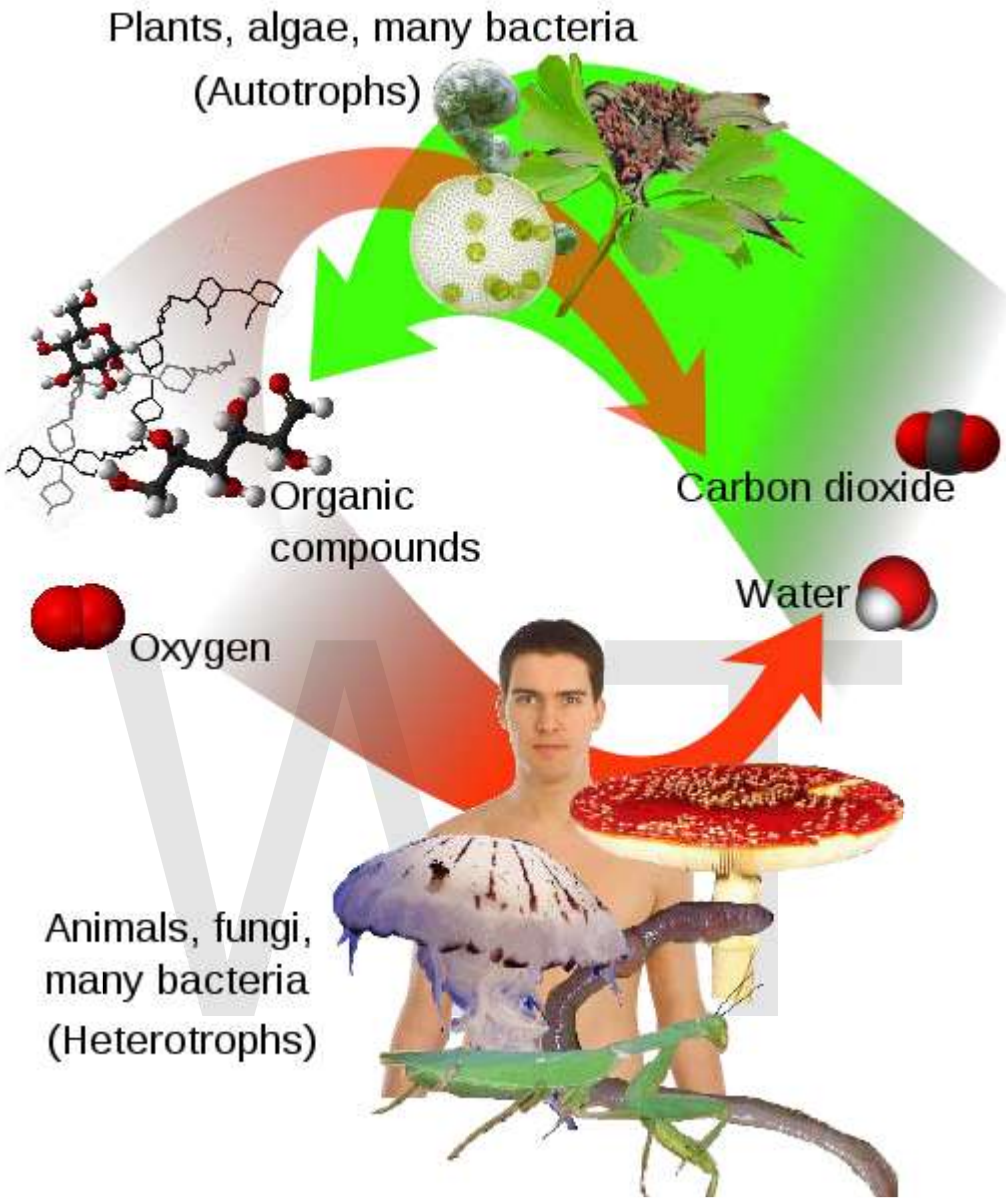
Composite image showing the global distribution of photosynthesis, including both oceanic phytoplankton and vegetation



Overall equation for the type of photosynthesis that occurs in plants

Photosynthesis is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. Photosynthesis occurs in plants, algae, and many species of bacteria, but not in archaea. Photosynthetic organisms are called *photoautotrophs*, since they can create their own food. In plants, algae, and cyanobacteria, photosynthesis uses carbon dioxide and water, releasing oxygen as a waste product. Photosynthesis is vital for all aerobic life on Earth. As well as maintaining the normal level of oxygen in the atmosphere, nearly all life either depends on it directly as a source of energy, or indirectly as the ultimate source of the energy in their food (the exceptions are chemoautotrophs that live in rocks or around deep sea hydrothermal vents). The rate of energy capture by photosynthesis is immense, approximately 100 terawatts, which is about six times larger than the power consumption of human civilization. As well as energy, photosynthesis is also the source of the carbon in all the organic compounds within organisms' bodies. In all, photosynthetic organisms convert around 100–115 teragrams of carbon into biomass per year.

Although photosynthesis can happen in different ways in different species, some features are always the same. For example, the process always begins when energy from light is absorbed by proteins called photosynthetic reaction centers that contain chlorophylls. In plants, these proteins are held inside organelles called chloroplasts, while in bacteria they are embedded in the plasma membrane. Some of the light energy gathered by chlorophylls is stored in the form of adenosine triphosphate (ATP). The rest of the energy is used to remove electrons from a substance such as water. These electrons are then used in the reactions that turn carbon dioxide into organic compounds. In plants, algae and cyanobacteria, this is done by a sequence of reactions called the Calvin cycle, but different sets of reactions are found in some bacteria, such as the reverse Krebs cycle in *Chlorobium*. Many photosynthetic organisms have adaptations that concentrate or store carbon dioxide. This helps reduce a wasteful process called photorespiration that can consume part of the sugar produced during photosynthesis.

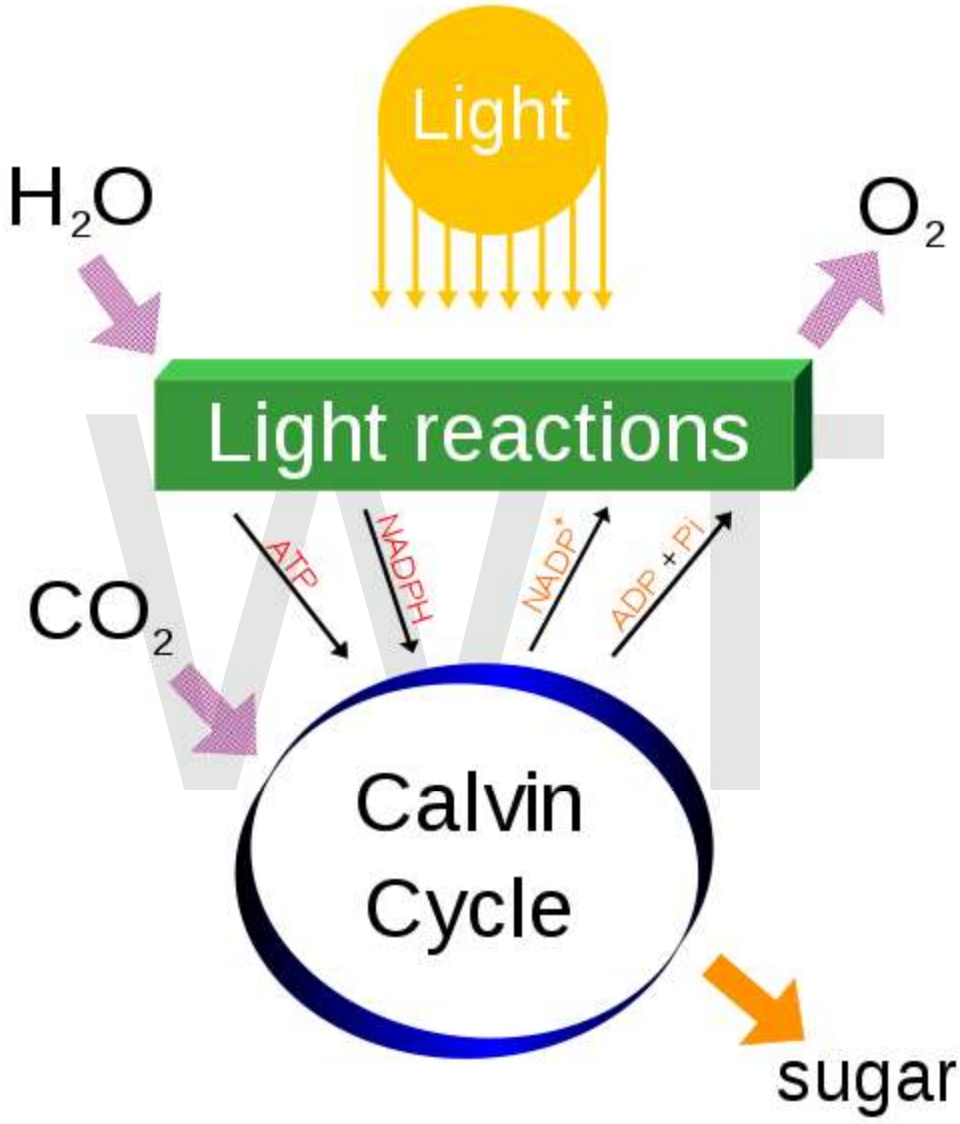


Overview of cycle between autotrophs and heterotrophs. Photosynthesis is the main means by which plants, algae and many bacteria produce organic compounds and oxygen from carbon dioxide and water (green arrow).

The first photosynthetic organisms probably evolved about 3,500 million years ago, early in the evolutionary history of life, when all forms of life on Earth were microorganisms and the atmosphere had much more carbon dioxide. They most likely used hydrogen or hydrogen sulfide as sources of electrons, rather than water. Cyanobacteria appeared later, around 3,000 million years ago, and drastically changed the Earth when they began to oxygenate the atmosphere, beginning about 2,400 million years ago. This new atmosphere allowed the evolution of complex life such as protists. Eventually, no later than a billion years ago, one of these protists formed a symbiotic relationship with a

cyanobacterium, producing the ancestor of many plants and algae. The chloroplasts in modern plants are the descendants of these ancient symbiotic cyanobacteria.

Overview



Photosynthesis changes the energy from the sun into chemical energy, splits water to liberate O_2, and fixes CO_2 into sugar.

Photosynthetic organisms are photoautotrophs, which means that they are able to synthesize food directly from carbon dioxide using energy from light. However, not all organisms that use light as a source of energy carry out photosynthesis, since *photoheterotrophs* use organic compounds, rather than carbon dioxide, as a source of

carbon. In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called *oxygenic photosynthesis*. Although there are some differences between oxygenic photosynthesis in plants, algae and cyanobacteria, the overall process is quite similar in these organisms. However, there are some types of bacteria that carry out anoxygenic photosynthesis, which consumes carbon dioxide but does not release oxygen.

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs to supply both a source of energy to drive this process, and the electrons needed to convert carbon dioxide into carbohydrate, which is a reduction reaction. In general outline, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy. However, the two processes take place through a different sequence of chemical reactions and in different cellular compartments.

The general equation for photosynthesis is therefore:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + n\ O_2 + 2n\ A$$

Carbon dioxide + electron donor + light energy → carbohydrate + oxygen + oxidized electron donor

Since water is used as the electron donor in oxygenic photosynthesis, the equation for this process is:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2$$

carbon dioxide + water + light energy → carbohydrate + oxygen

Other processes substitute other compounds (such as arsenite) for water in the electron-supply role; the microbes use sunlight to oxidize arsenite to arsenate: The equation for this reaction is:

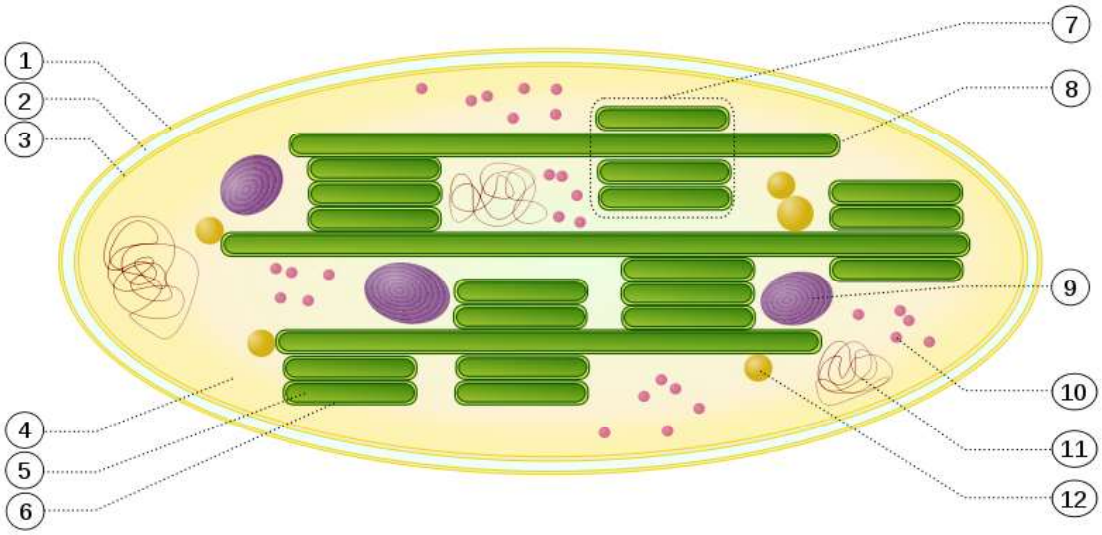
$$(AsO_3^{3-}) + CO_2 + \text{photons} \rightarrow CO + (AsO_4^{3-})$$

carbon dioxide + arsenite + light energy → arsenate + carbon monoxide (used to build other compounds in subsequent reactions)

Photosynthesis occurs in two stages. In the first stage, *light-dependent reactions* or *light reactions* capture the energy of light and use it to make the energy-storage molecules ATP and NADPH. During the second stage, the *light-independent reactions* use these products to capture and reduce carbon dioxide.

Most organisms that utilize photosynthesis to produce oxygen use visible light to do so, although at least three use infrared radiation.

Photosynthetic membranes and organelles



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 proteins that gather light for photosynthesis are embedded within cell membranes. The simplest way these are arranged is in photosynthetic bacteria, where these proteins are held within the plasma membrane. However, this membrane may be tightly folded into cylindrical sheets called thylakoids, or bunched up into round vesicles called *intracytoplasmic membranes*. These structures can fill most of the interior of a cell, giving the membrane a very large surface area and therefore increasing the amount of light that the bacteria can absorb.

In plants and algae, photosynthesis takes place in organelles called chloroplasts. A typical plant cell contains about 10 to 100 chloroplasts. The chloroplast is enclosed by a membrane. This membrane is composed of a phospholipid inner membrane, a phospholipid outer membrane, and an intermembrane space between them. Within the membrane is an aqueous fluid called the stroma. The stroma contains stacks (grana) of thylakoids, which are the site of photosynthesis. The thylakoids are flattened disks,

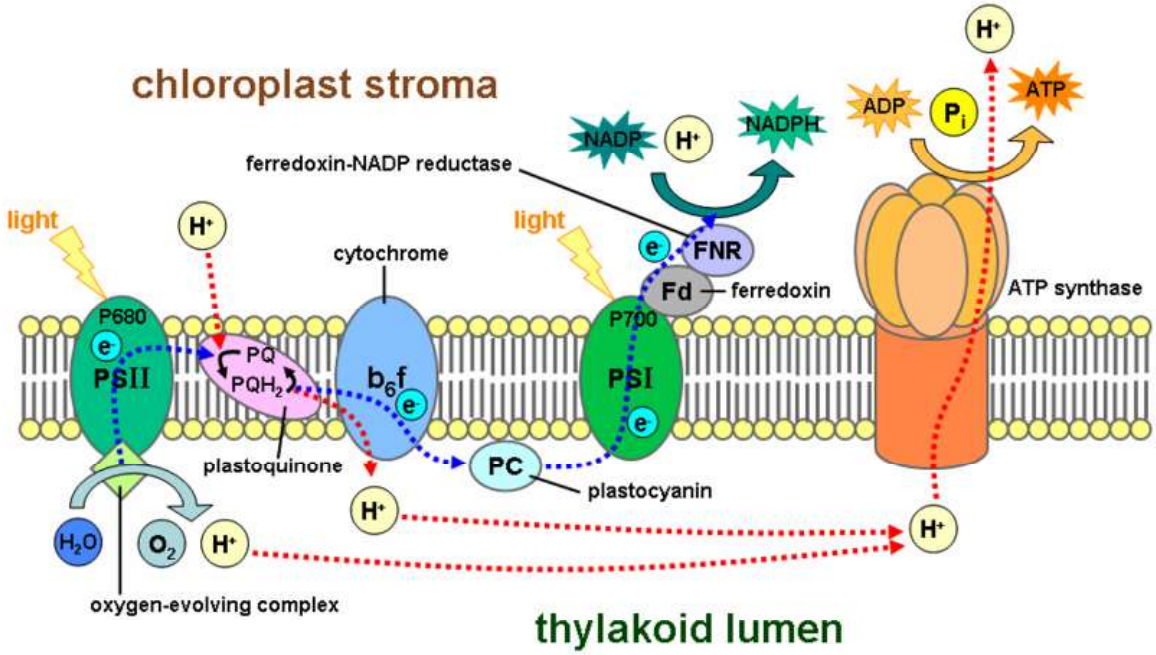
bounded by a membrane with a lumen or thylakoid space within it. The site of photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems.

Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green color. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Algae also use chlorophyll, but various other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthin in brown algae and diatoms resulting in a wide variety of colors.

These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a light-harvesting complex.

Although all cells in the green parts of a plant have chloroplasts, most of the energy is captured in the leaves. The cells in the interior tissues of a leaf, called the mesophyll, can contain between 450,000 and 800,000 chloroplasts for every square millimeter of leaf. The surface of the leaf is uniformly coated with a water-resistant waxy cuticle that protects the leaf from excessive evaporation of water and decreases the absorption of ultraviolet or blue light to reduce heating. The transparent epidermis layer allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

Light reactions



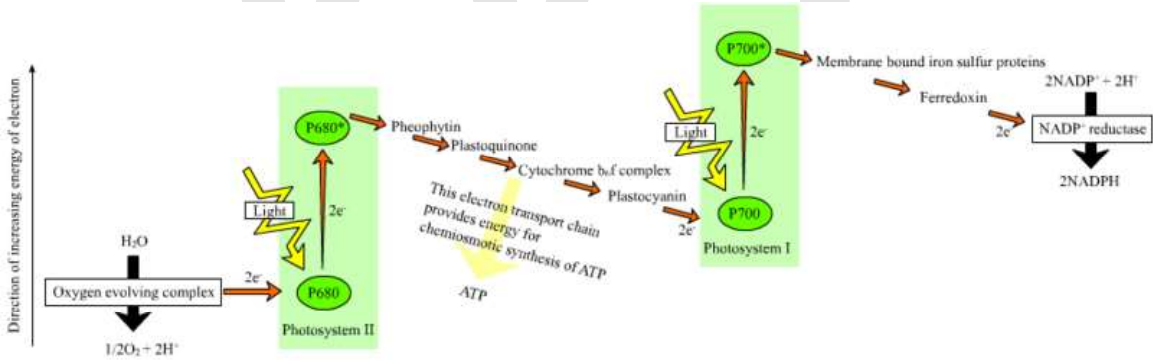
Light-dependent reactions of photosynthesis at the thylakoid membrane

In the light reactions, one molecule of the pigment chlorophyll absorbs one photon and loses one electron. This electron is passed to a modified form of chlorophyll called pheophytin, which passes the electron to a quinone molecule, allowing the start of a flow of electrons down an electron transport chain that leads to the ultimate reduction of NADP to NADPH. In addition, this creates a proton gradient across the chloroplast membrane; its dissipation is used by ATP synthase for the concomitant synthesis of ATP. The chlorophyll molecule regains the lost electron from a water molecule through a process called photolysis, which releases a dioxygen (O_2) molecule. The overall equation for the light-dependent reactions under the conditions of non-cyclic electron flow in green plants is:

$$2\ H_2O + 2\ NADP^+ + 3\ ADP + 3\ P_i + light \rightarrow 2\ NADPH + 2\ H^+ + 3\ ATP + O_2$$

Not all wavelengths of light can support photosynthesis. The photosynthetic action spectrum depends on the type of accessory pigments present. For example, in green plants, the action spectrum resembles the absorption spectrum for chlorophylls and carotenoids with peaks for violet-blue and red light. In red algae, the action spectrum overlaps with the absorption spectrum of phycobilins for blue-green light, which allows these algae to grow in deeper waters that filter out the longer wavelengths used by green plants. The non-absorbed part of the light spectrum is what gives photosynthetic organisms their color (e.g., green plants, red algae, purple bacteria) and is the least effective for photosynthesis in the respective organisms.

Z scheme



The "Z scheme"

In plants, light-dependent reactions occur in the thylakoid membranes of the chloroplasts and use light energy to synthesize ATP and NADPH. The light-dependent reaction has two forms: cyclic and non-cyclic. In the non-cyclic reaction, the photons are captured in the light-harvesting antenna complexes of photosystem II by chlorophyll and other accessory pigments. When a chlorophyll molecule at the core of the photosystem II reaction center obtains sufficient excitation energy from the adjacent antenna pigments, an electron is transferred to the primary electron-acceptor molecule, pheophytin, through a process called photoinduced charge separation. These electrons are shuttled through an electron transport chain, the so called ***Z-scheme*** shown in the diagram, that initially

functions to generate a chemiosmotic potential across the membrane. An ATP synthase enzyme uses the chemiosmotic potential to make ATP during photophosphorylation, whereas NADPH is a product of the terminal redox reaction in the *Z-scheme*. The electron enters a chlorophyll molecule in Photosystem I. The electron is excited due to the light absorbed by the photosystem. A second electron carrier accepts the electron, which again is passed down lowering energies of electron acceptors. The energy created by the electron acceptors is used to move hydrogen ions across the thylakoid membrane into the lumen. The electron is used to reduce the co-enzyme NADP, which has functions in the light-independent reaction. The cyclic reaction is similar to that of the non-cyclic, but differs in the form that it generates only ATP, and no reduced NADP (NADPH) is created. The cyclic reaction takes place only at photosystem I. Once the electron is displaced from the photosystem, the electron is passed down the electron acceptor molecules and returns back to photosystem I, from where it was emitted, hence the name *cyclic reaction*.

Water photolysis

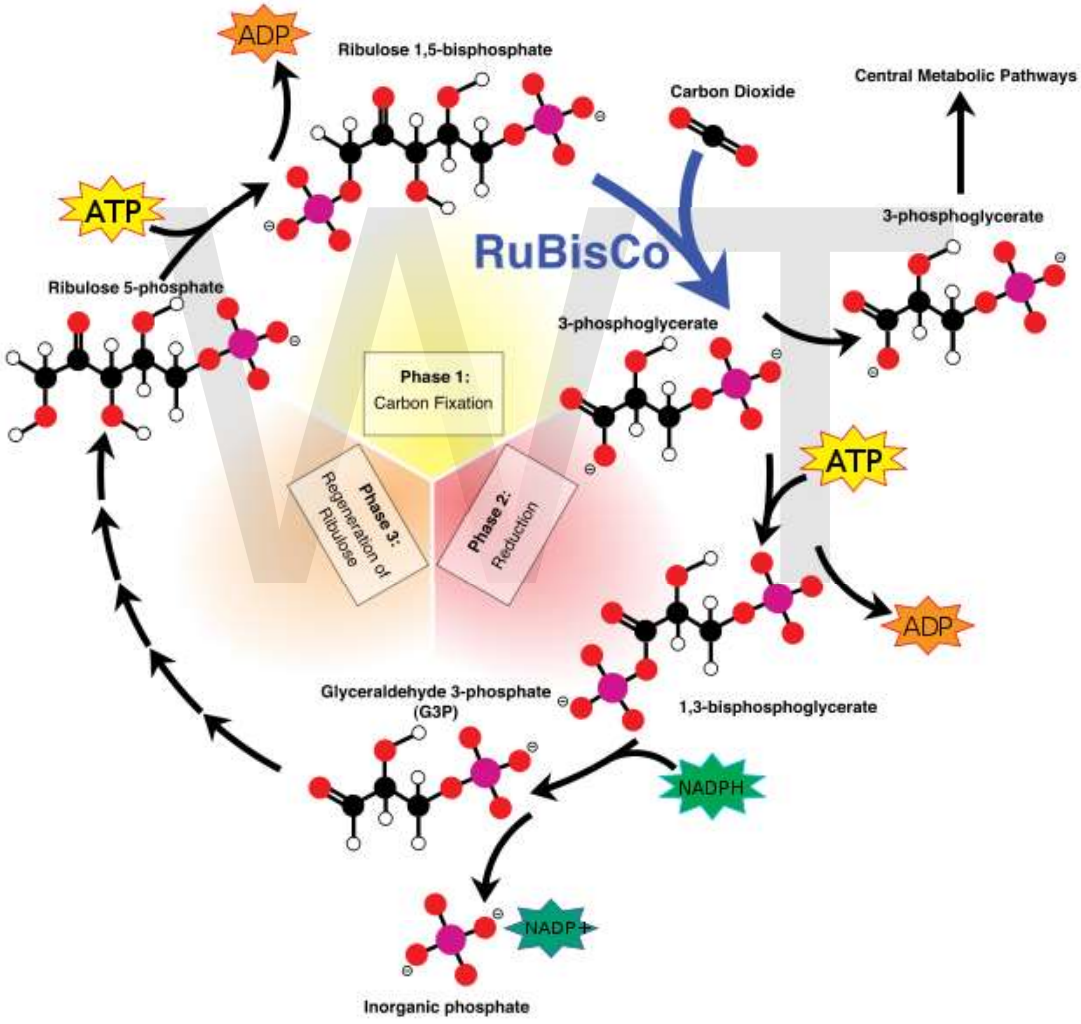
The NADPH is the main reducing agent in chloroplasts, providing a source of energetic electrons to other reactions. Its production leaves chlorophyll with a deficit of electrons (oxidized), which must be obtained from some other reducing agent. The excited electrons lost from chlorophyll in photosystem I are replaced from the electron transport chain by plastocyanin. However, since photosystem II includes the first steps of the *Z-scheme*, an external source of electrons is required to reduce its oxidized **chlorophyll *a*** molecules. The source of electrons in green-plant and cyanobacterial photosynthesis is water. Two water molecules are oxidized by four successive charge-separation reactions by photosystem II to yield a molecule of diatomic oxygen and four hydrogen ions; the electron yielded in each step is transferred to a redox-active tyrosine residue that then reduces the photoxidized paired-chlorophyll *a* species called P680 that serves as the primary (light-driven) electron donor in the photosystem II reaction center. The oxidation of water is catalyzed in photosystem II by a redox-active structure that contains four manganese ions and a calcium ion; this oxygen-evolving complex binds two water molecules and stores the four oxidizing equivalents that are required to drive the water-oxidizing reaction. Photosystem II is the only known biological enzyme that carries out this oxidation of water. The hydrogen ions contribute to the transmembrane chemiosmotic potential that leads to ATP synthesis. Oxygen is a waste product of light-dependent reactions, but the majority of organisms on Earth use oxygen for cellular respiration, including photosynthetic organisms.

Light-independent reactions

The Calvin Cycle

In the Light-independent or dark reactions the enzyme RuBisCO captures CO_2 from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson Cycle, releases three-carbon sugars, which are later combined to form sucrose and starch. The overall equation for the light-independent reactions in green plants is:

$3\ CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ P_i + 6\ NADP^+ + 3\ H_2O$



Overview of the Calvin cycle and carbon fixation

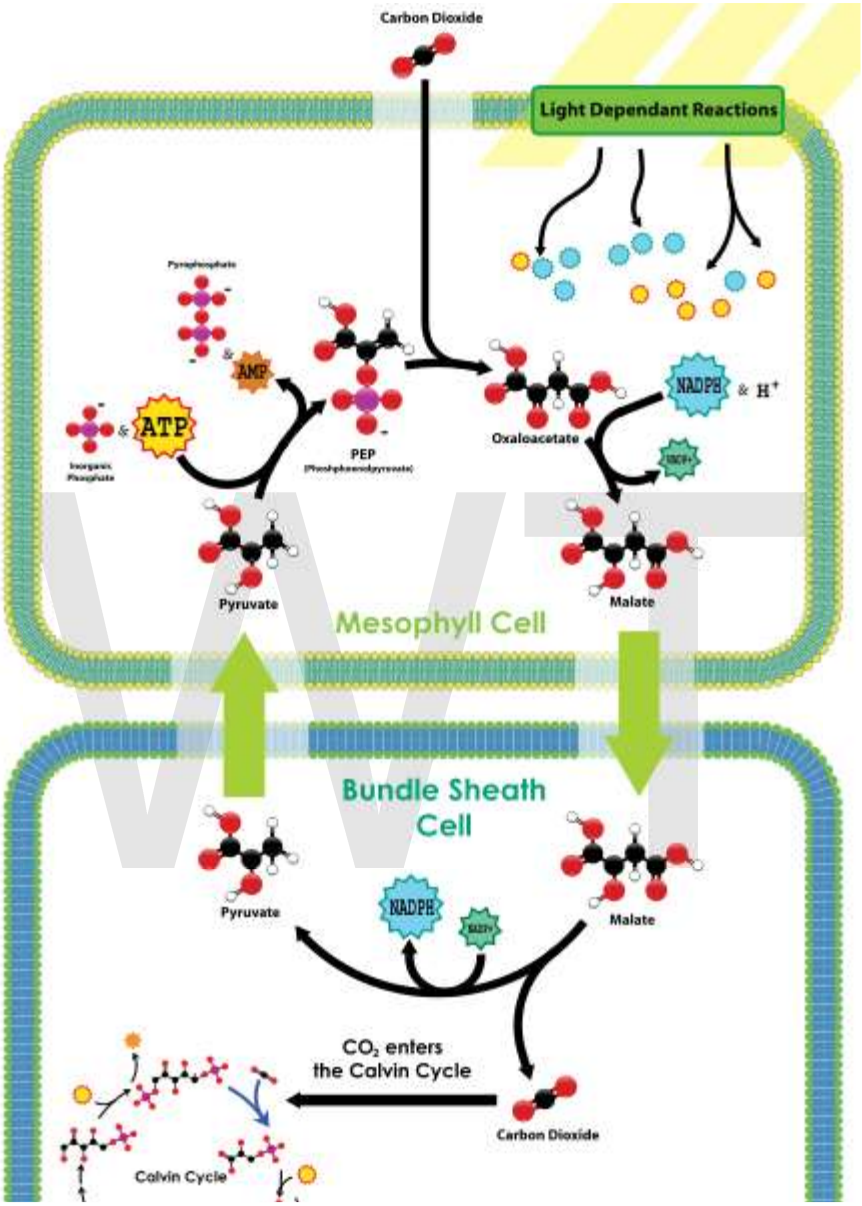
To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then variously used to form other organic compounds, such as the

building material cellulose, as precursors for lipid and amino acid biosynthesis, or as a fuel in cellular respiration. The latter occurs not only in plants but also in animals when the energy from plants gets passed through a food chain.

The fixation or reduction of carbon dioxide is a process in which carbon dioxide combines with a five-carbon sugar, ribulose 1,5-bisphosphate (RuBP), to yield two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). GP, in the presence of ATP and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Triose is a 3-carbon sugar. Most (5 out of 6 molecules) of the G3P produced is used to regenerate RuBP so the process can continue. The 1 out of 6 molecules of the triose phosphates not "recycled" often condense to form hexose phosphates, which ultimately yield sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids.

Carbon concentrating mechanisms

On land



Overview of C4 carbon fixation

In hot and dry conditions, plants close their stomata to prevent the loss of water. Under these conditions, CO_2 will decrease, and oxygen gas, produced by the light reactions of photosynthesis, will decrease in the stem, not leaves, causing an increase of photorespiration by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase and decrease in carbon fixation. Some plants have evolved mechanisms to increase the CO_2 concentration in the leaves under these conditions.

C_4 plants chemically fix carbon dioxide in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEP carboxylase and which creates the four-carbon organic acid, oxaloacetic acid. Oxaloacetic acid or malate synthesized by this process is then translocated to specialized bundle sheath cells where the enzyme, rubisco, and other Calvin cycle enzymes are located, and where CO_2 released by decarboxylation of the four-carbon acids is then fixed by rubisco activity to the three-carbon sugar 3-phosphoglyceric acids. The physical separation of rubisco from the oxygen-generating light reactions reduces photorespiration and increases CO_2 fixation and thus photosynthetic capacity of the leaf. C_4 plants can produce more sugar than C_3 plants in conditions of high light and temperature. Many important crop plants are C_4 plants, including maize, sorghum, sugarcane, and millet. Plants that do not use PEP-carboxylase in carbon fixation are called C_3 plants because the primary carboxylation reaction, catalyzed by rubisco, produces the three-carbon sugar 3-phosphoglyceric acids directly in the Calvin-Benson cycle. Over 90% of plants use C_3 carbon fixation, compared to 3% that use C_4 carbon fixation.

Xerophytes, such as cacti and most succulents, also use PEP carboxylase to capture carbon dioxide in a process called Crassulacean acid metabolism (CAM). In contrast to C_4 metabolism, which *physically* separates the CO_2 fixation to PEP from the Calvin cycle, CAM *temporally* separates these two processes. CAM plants have a different leaf anatomy from C_3 plants, and fix the CO_2 at night, when their stomata are open. CAM plants store the CO_2 mostly in the form of malic acid via carboxylation of phosphoenolpyruvate to oxaloacetate, which is then reduced to malate. Decarboxylation of malate during the day releases CO_2 inside the leaves, thus allowing carbon fixation to 3-phosphoglycerate by rubisco. 16,000 species of plants use CAM.

In water

Cyanobacteria possess carboxysomes which increase the concentration of CO_2 around rubisco to increase the rate of photosynthesis. This operates by carbonic anhydrase producing hydrocarbonate ions (HCO_3^-) which are then pumped into the carboxysome, before being processed by a different carbonic anhydrase to produce CO_2. Pyrenoids in algae and hornworts also act to concentrate CO_2 around rubisco.

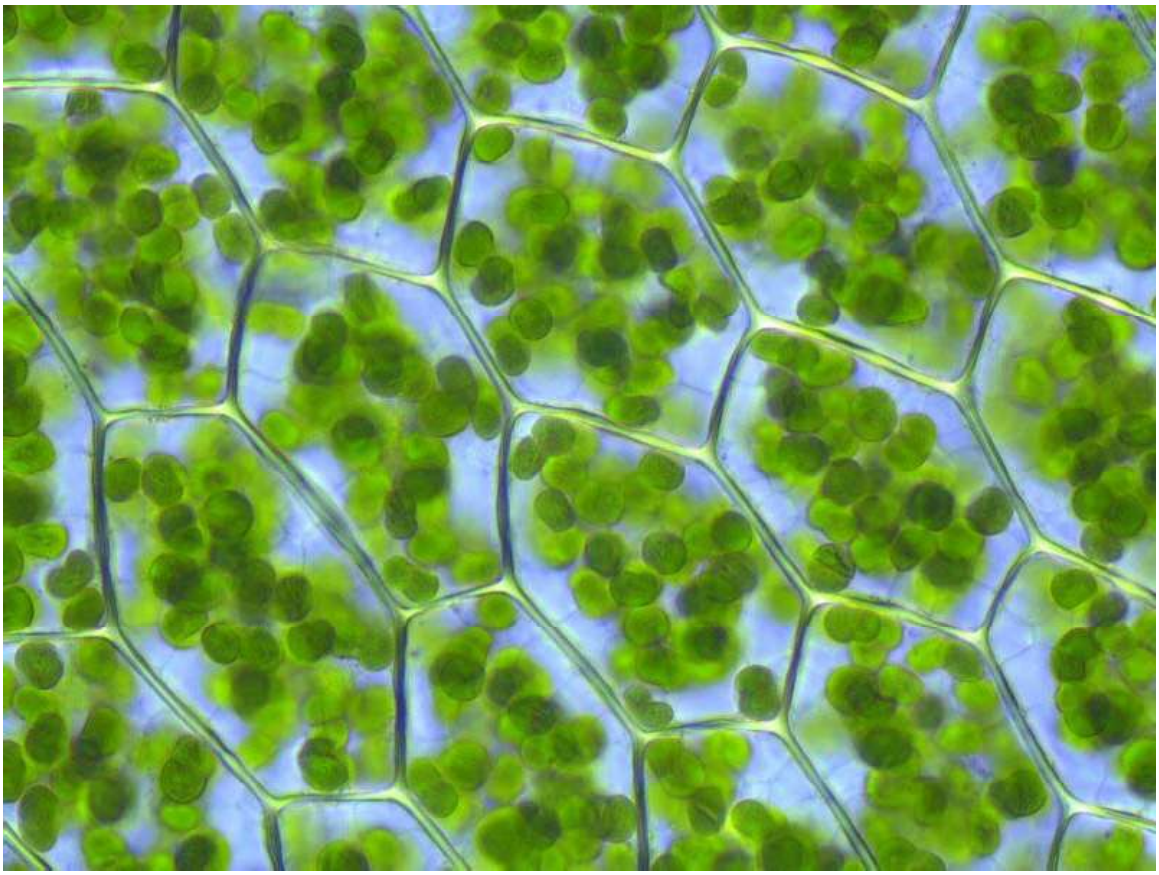
Order and kinetics

The overall process of photosynthesis takes place in four stages. The first, energy transfer in antenna chlorophyll takes place in the femtosecond (1 femtosecond (fs) = 10^{-15} s) to picosecond (1 picosecond (ps) = 10^{-12} s) time scale. The next phase, the transfer of electrons in photochemical reactions, takes place in the picosecond to nanosecond time scale (1 nanosecond (ns) = 10^{-9} s). The third phase, the electron transport chain and ATP synthesis, takes place on the microsecond (1 microsecond (μs) = 10^{-6} s) to millisecond (1 millisecond (ms) = 10^{-3} s) time scale. The final phase is carbon fixation and export of stable products and takes place in the millisecond to second time scale. The first three stages occur in the thylakoid membranes.

Efficiency

Plants usually convert light into chemical energy with a photosynthetic efficiency of 3–6%. Actual plants' photosynthetic efficiency varies with the frequency of the light being converted, light intensity, temperature and proportion of carbon dioxide in the atmosphere, and can vary from 0.1% to 8%. By comparison, solar panels convert light into electric energy at an efficiency of approximately 6–20% for mass-produced panels, and up to 41% in a research laboratory.

Evolution



Plant cells with visible chloroplasts (from a moss, *Plagiomnium affine*)

Early photosynthetic systems, such as those from green and purple sulfur and green and purple nonsulfur bacteria, are thought to have been anoxygenic, using various molecules as electron donors. Green and purple sulfur bacteria are thought to have used hydrogen and sulfur as an electron donor. Green nonsulfur bacteria used various amino and other organic acids. Purple nonsulfur bacteria used a variety of nonspecific organic molecules. The use of these molecules is consistent with the geological evidence that the atmosphere was highly reduced at that time.

Fossils of what are thought to be filamentous photosynthetic organisms have been dated at 3.4 billion years old.

The main source of oxygen in the atmosphere is oxygenic photosynthesis, and its first appearance is sometimes referred to as the oxygen catastrophe. Geological evidence suggests that oxygenic photosynthesis, such as that in cyanobacteria, became important during the Paleoproterozoic era around 2 billion years ago. Modern photosynthesis in plants and most photosynthetic prokaryotes is oxygenic. Oxygenic photosynthesis uses water as an electron donor, which is oxidized to molecular oxygen (O_2) in the photosynthetic reaction center.

Symbiosis and the origin of chloroplasts

Several groups of animals have formed symbiotic relationships with photosynthetic algae. These are most common in corals, sponges and sea anemones, possibly due to these animals having particularly simple body plans and large surface areas compared to their volumes. In addition, a few marine mollusks *Elysia viridis* and *Elysia chlorotica* also maintain a symbiotic relationship with chloroplasts they capture from the algae in their diet and then store in their bodies. This allows the molluscs to survive solely by photosynthesis for several months at a time. Some of the genes from the plant cell nucleus have even been transferred to the slugs, so that the chloroplasts can be supplied with proteins that they need to survive.

An even closer form of symbiosis may explain the origin of chloroplasts. Chloroplasts have many similarities with photosynthetic bacteria, including a circular chromosome, prokaryotic-type ribosomes, and similar proteins in the photosynthetic reaction center. The endosymbiotic theory suggests that photosynthetic bacteria were acquired (by endocytosis) by early eukaryotic cells to form the first plant cells. Therefore, chloroplasts may be photosynthetic bacteria that adapted to life inside plant cells. Like mitochondria, chloroplasts still possess their own DNA, separate from the nuclear DNA of their plant host cells and the genes in this chloroplast DNA resemble those in cyanobacteria. DNA in chloroplasts codes for redox proteins such as photosynthetic reaction centers. The CoRR Hypothesis proposes that this **Co**-location is required for **R**edox **R**egulation.

Cyanobacteria and the evolution of photosynthesis

The biochemical capacity to use water as the source for electrons in photosynthesis evolved once, in a common ancestor of extant cyanobacteria. The geological record indicates that this transforming event took place early in Earth's history, at least 2450–2320 million years ago (Ma), and possibly much earlier. Available evidence from geobiological studies of Archean (>2500 Ma) sedimentary rocks indicates that life existed 3500 Ma, but the question of when oxygenic photosynthesis evolved is still unanswered. A clear paleontological window on cyanobacterial evolution opened about 2000 Ma, revealing an already-diverse biota of blue-greens. Cyanobacteria remained principal primary producers throughout the Proterozoic Eon (2500–543 Ma), in part because the redox structure of the oceans favored photoautotrophs capable of nitrogen

fixation. Green algae joined blue-greens as major primary producers on continental shelves near the end of the Proterozoic, but only with the Mesozoic (251–65 Ma) radiations of dinoflagellates, coccolithophorids, and diatoms did primary production in marine shelf waters take modern form. Cyanobacteria remain critical to marine ecosystems as primary producers in oceanic gyres, as agents of biological nitrogen fixation, and, in modified form, as the plastids of marine algae.

A 2010 study by researchers at Tel Aviv University discovered that the Oriental hornet (*Vespa orientalis*) converts sunlight into electric power using a pigment called xanthopterin. This is the first scientific evidence of a member of the animal kingdom engaging in photosynthesis.

Discovery

Although some of the steps in photosynthesis are still not completely understood, the overall photosynthetic equation has been known since the 19th century.

Jan van Helmont began the research of the process in the mid-17th century when he carefully measured the mass of the soil used by a plant and the mass of the plant as it grew. After noticing that the soil mass changed very little, he hypothesized that the mass of the growing plant must come from the water, the only substance he added to the potted plant. His hypothesis was partially accurate—much of the gained mass also comes from carbon dioxide as well as water. However, this was a signaling point to the idea that the bulk of a plant's biomass comes from the inputs of photosynthesis, not the soil itself.

Joseph Priestley, a chemist and minister, discovered that when he isolated a volume of air under an inverted jar, and burned a candle in it, the candle would burn out very quickly, much before it ran out of wax. He further discovered that a mouse could similarly "injure" air. He then showed that the air that had been "injured" by the candle and the mouse could be restored by a plant.

In 1778, Jan Ingenhousz, court physician to the Austrian Empress, repeated Priestley's experiments. He discovered that it was the influence of sunlight on the plant that could cause it to revive a mouse in a matter of hours.

In 1796, Jean Senebier, a Swiss pastor, botanist, and naturalist, demonstrated that green plants consume carbon dioxide and release oxygen under the influence of light. Soon afterwards, Nicolas-Théodore de Saussure showed that the increase in mass of the plant as it grows could not be due only to uptake of CO_2, but also to the incorporation of water. Thus the basic reaction by which photosynthesis is used to produce food (such as glucose) was outlined.

Cornelis Van Niel made key discoveries explaining the chemistry of photosynthesis. By studying purple sulfur bacteria and green bacteria he was the first scientist to demonstrate that photosynthesis is a light-dependent redox reaction, in which hydrogen reduces carbon dioxide.

Robert Emerson discovered two light reactions by testing plant productivity using different wavelengths of light. With the red alone, the light reactions were suppressed. When blue and red were combined, the output was much more substantial. Thus, there were two photosystems, one aborbing up to 600 nm wavelengths, the other up to 700. The former is known as PSII, the latter is PSI. PSI contains only chlorophyll a, PSII contains primarily chlorophyll a with most of the available chlorophyll b, among other pigments.

Further experiments to prove that the oxygen developed during the photosynthesis of green plants came from water, were performed by Robert Hill in 1937 and 1939. He showed that isolated chloroplasts give off oxygen in the presence of unnatural reducing agents like iron oxalate, ferricyanide or benzoquinone after exposure to light. The Hill reaction is as follows:

$$2\ H_2O + 2\ A + (\text{light, chloroplasts}) \rightarrow 2\ AH_2 + O_2$$

where A is the electron acceptor. Therefore, in light the electron acceptor is reduced and oxygen is evolved. Cyt b_6, now known as a plastoquinone, is one electron acceptor.

Samuel Ruben and Martin Kamen used radioactive isotopes to determine that the oxygen liberated in photosynthesis came from the water.

Melvin Calvin and Andrew Benson, along with James Bassham, elucidated the path of carbon assimilation (the photosynthetic carbon reduction cycle) in plants. The carbon reduction cycle is known as the Calvin cycle, which inappropriately ignores the contribution of Bassham and Benson. Many scientists refer to the cycle as the Calvin-Benson Cycle, Benson-Calvin, and some even call it the Calvin-Benson-Bassham (or CBB) Cycle.

A Nobel Prize winning scientist, Rudolph A. Marcus, was able to discover the function and significance of the electron transport chain.

Otto Heinrich Warburg and Dean Burk discovered the I-quantum photosynthesis reaction that splits the CO2, activated by the respiration.

Factors

The leaf is the primary site of photosynthesis in plants.

There are three main factors affecting photosynthesis and several corollary factors. The three main are:

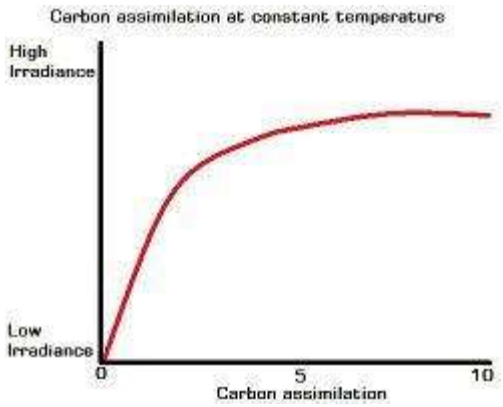
- Light irradiance and wavelength
- Carbon dioxide concentration
- Temperature.

Light intensity (irradiance), wavelength and temperature

In the early 20th century Frederick Frost Blackman along with Albert Einstein investigated the effects of light intensity (irradiance) and temperature on the rate of carbon assimilation.

- At constant temperature, the rate of carbon assimilation varies with irradiance, initially increasing as the irradiance increases. However at higher irradiance this relationship no longer holds and the rate of carbon assimilation reaches a plateau.

- At constant irradiance, the rate of carbon assimilation increases as the temperature is increased over a limited range. This effect is only seen at high irradiance levels. At low irradiance, increasing the temperature has little influence on the rate of carbon assimilation.



Carbon assimilation at a constant temperature.

These two experiments illustrate vital points: firstly, from research it is known that photochemical reactions are not generally affected by temperature. However, these experiments clearly show that temperature affects the rate of carbon assimilation, so there must be two sets of reactions in the full process of carbon assimilation. These are of course the light-dependent 'photochemical' stage and the light-independent, temperature-dependent stage. Second, Blackman's experiments illustrate the concept of limiting factors. Another limiting factor is the wavelength of light. Cyanobacteria, which reside several meters underwater, cannot receive the correct wavelengths required to cause photoinduced charge separation in conventional photosynthetic pigments. To combat this problem, a series of proteins with different pigments surround the reaction center.This unit is called a phycobilisome.

Carbon dioxide levels and photorespiration

As carbon dioxide concentrations rise, the rate at which sugars are made by the light-independent reactions increases until limited by other factors. RuBisCO, the enzyme that captures carbon dioxide in the light-independent reactions, has a binding affinity for both carbon dioxide and oxygen. When the concentration of carbon dioxide is high, RuBisCO will fix carbon dioxide. However, if the carbon dioxide concentration is low, RuBisCO will bind oxygen instead of carbon dioxide. This process, called photorespiration, uses energy, but does not produce sugars.

RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

1. One product of oxygenase activity is phosphoglycolate (2 carbon) instead of 3-phosphoglycerate (3 carbon). Phosphoglycolate cannot be metabolized by the Calvin-Benson cycle and represents carbon lost from the cycle. A high oxygenase

activity, therefore, drains the sugars that are required to recycle ribulose 5-bisphosphate and for the continuation of the Calvin-Benson cycle.
2. Phosphoglycolate is quickly metabolized to glycolate that is toxic to a plant at a high concentration; it inhibits photosynthesis.
3. Salvaging glycolate is an energetically expensive process that uses the glycolate pathway and only 75% of the carbon is returned to the Calvin-Benson cycle as 3-phosphoglycerate. The reactions also produce ammonia (NH_3) which is able to diffuse out of the plant leading to a loss of nitrogen.

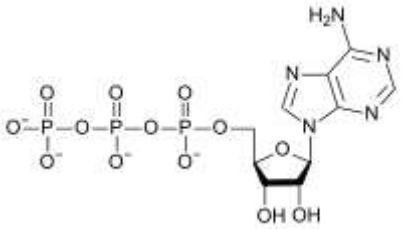
   A highly simplified summary is:
   2 glycolate + ATP → 3-phosphoglycerate + carbon dioxide + ADP +NH_3

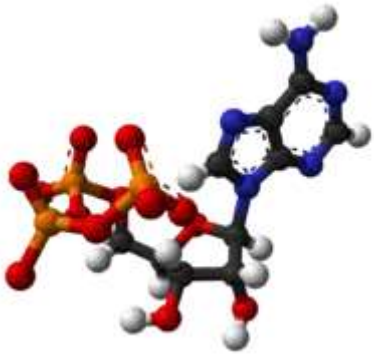
The salvaging pathway for the products of RuBisCO oxygenase activity is more commonly known as photorespiration, since it is characterized by light-dependent oxygen consumption and the release of carbon dioxide.

Chapter- 14

Adenosine Triphosphate

Adenosine triphosphate





IUPAC name
[(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl(hydroxyphosphonooxyphosphoryl)hydrogen phosphate

Other names
adenosine 5'-(tetrahydrogen triphosphate)

Identifiers	
CAS number	56-65-5
PubChem	5957
ChemSpider	5742
UNII	8L70Q75FXE
ChEMBL	CHEMBL14249
IUPHAR ligand	1713
Properties	
Molecular formula	$C_{10}H_{16}N_5O_{13}P_3$
Molar mass	507.18 g mol^{-1}
Density	1.04 g/cm^3 (disodium salt)
Melting point	187 °C (disodium salt) *decomposes*
Acidity (pK_a)	6.5

Adenosine-5'-triphosphate (**ATP**) is a multifunctional nucleotide used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer. ATP transports chemical energy within cells for metabolism. It is produced by photophosphorylation and cellular respiration and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains three phosphate groups, and it is produced by ATP synthase from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP).

Metabolic processes that use ATP as an energy source convert it back into its precursors. ATP is therefore continuously recycled in organisms: the human body, which on average contains 250 grams (8.8 oz) of ATP turns over its own weight in ATP each day.

ATP is used as a substrate in signal transduction pathways by kinases that phosphorylate proteins and lipids, as well as by adenylate cyclase, which uses ATP to produce the second messenger molecule cyclic AMP. The ratio between ATP and AMP is used as a way for a cell to sense how much energy is available and control the metabolic pathways that produce and consume ATP. Apart from its roles in energy metabolism and signaling, ATP is also incorporated into nucleic acids by polymerases in the processes of DNA replication and transcription.

The structure of this molecule consists of a purine base (adenine) attached to the 1' carbon atom of a pentose sugar (ribose). Three phosphate groups are attached at the 5' carbon atom of the pentose sugar. It is the addition and removal of these phosphate groups that inter-convert ATP, ADP and AMP. When ATP is used in DNA synthesis, the ribose sugar is first converted to deoxyribose by ribonucleotide reductase.

ATP was discovered in 1929 by Karl Lohmann, but its correct structure was not determined until some years later. It was proposed to be the main energy-transfer

molecule in the cell by Fritz Albert Lipmann in 1941. It was first artificially synthesized by Alexander Todd in 1948.

Physical and chemical properties

ATP consists of adenosine — composed of an adenine ring and a ribose sugar — and three phosphate groups (triphosphate). The phosphoryl groups, starting with the group closest to the ribose, are referred to as the alpha (α), beta (β), and gamma (γ) phosphates. Consequently, as a nucleotide, it (and its relatives ADP and AMP) is basically a monomer of RNA. ATP is highly soluble in water and is quite stable in solutions between pH 6.8–7.4, but is rapidly hydrolysed at extreme pH. Consequently, ATP is best stored as an anhydrous salt.

ATP is an unstable molecule in unbuffered water, in which it hydrolyses to ADP and phosphate. This is because the strength of the bonds between the phosphate groups in ATP are less than the strength of the hydrogen bonds (hydration bonds), between its products (ADP + phosphate), and water. Thus, if ATP and ADP are in chemical equilibrium in water, almost all of the ATP will eventually be converted to ADP. A system that is far from equilibrium contains Gibbs free energy, and is capable of doing work. Living cells maintain the ratio of ATP to ADP at a point ten orders of magnitude from equilibrium, with ATP concentrations a thousandfold higher than the concentration of ADP. This displacement from equilibrium means that the hydrolysis of ATP in the cell releases a large amount of free energy.

Two high-energy phosphate bonds (phosphoanhydride bonds) (those that connect adjacent phosphates) in an ATP molecule are responsible for the high energy content of this molecule. In the context of biochemical reactions, these anhydride bonds are frequently—and sometimes controversially—referred to as *high-energy bonds*. Energy stored in ATP may be released upon hydrolysis of the anhydride bonds. The bonds formed after hydrolysis—or the phosphorylation of a residue by ATP—are lower in energy than the phosphoanhydride bonds of ATP. During enzyme-catalyzed hydrolysis of ATP or phosphorylation by ATP, the available free energy can be harnessed by a living system to do work.

Any unstable system of potentially reactive molecules could potentially serve as a way of storing free energy, if the cell maintained their concentration far from the equilibrium point of the reaction. However, as is the case with most polymeric biomolecules, the breakdown of RNA, DNA, and ATP into simpler monomers is driven by both energy-release and entropy-increase considerations, in both standard concentrations, and also those concentrations encountered within the cell.

The standard amount of energy released from hydrolysis of ATP can be calculated from the changes in energy under non-natural (standard) conditions, then correcting to biological concentrations. The net change in heat energy (enthalpy) at standard temperature and pressure of the decomposition of ATP into hydrated ADP and hydrated inorganic phosphate is −20.5 kJ/mol, with a change in free energy of 3.4 kJ/mol. The

energy released by cleaving either a phosphate (P_i) or pyrophosphate (PP_i) unit from ATP at standard state of 1 M are:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G^\circ = -30.5 \text{ kJ/mol } (-7.3 \text{ kcal/mol})$$
$$ATP + H_2O \rightarrow AMP + PP_i \quad \Delta G^\circ = -45.6 \text{ kJ/mol } (-10.9 \text{ kcal/mol})$$

These values can be used to calculate the change in energy under physiological conditions and the cellular ATP/ADP ratio. However, a more representative value (which takes AMP into consideration) called the Energy charge is increasingly being employed. The values given for the Gibbs free energy for this reaction are dependent on a number of factors, including overall ionic strength and the presence of alkaline earth metal ions such as Mg^{2+} and Ca^{2+}. Under typical cellular conditions, ΔG is approximately −57 kJ/mol (−14 kcal/mol).

Ionization in biological systems

ATP has multiple ionizable groups with different acid dissociation constants. In neutral solution, ATP is ionized and exists mostly as ATP^{4-}, with a small proportion of ATP^{3-}. As ATP has several negatively charged groups in neutral solution, it can chelate metals with very high affinity. The binding constant for various metal ions are (given as per mole) as Mg^{2+} (9 554), Na^+ (13), Ca^{2+} (3 722), K^+ (8), Sr^{2+} (1 381) and Li^+ (25). Due to the strength of these interactions, ATP exists in the cell mostly in a complex with Mg^{2+}.

Biosynthesis

The ATP concentration inside the cell is typically 1–10 *mM*. ATP can be produced by redox reactions using simple and complex sugars (carbohydrates) or lipids as an energy source. For ATP to be synthesized from complex fuels, they first need to be broken down into their basic components. Carbohydrates are hydrolysed into simple sugars, such as glucose and fructose. Fats (triglycerides) are metabolised to give fatty acids and glycerol.

The overall process of oxidizing glucose to carbon dioxide is known as cellular respiration and can produce about 30 molecules of ATP from a single molecule of glucose. ATP can be produced by a number of distinct cellular processes; the three main pathways used to generate energy in eukaryotic organisms are glycolysis and the citric acid cycle/oxidative phosphorylation, both components of cellular respiration; and beta-oxidation. The majority of this ATP production by a non-photosynthetic aerobic eukaryote takes place in the mitochondria, which can make up nearly 25% of the total volume of a typical cell.

Glycolysis

In glycolysis, glucose and glycerol are metabolized to pyruvate via the glycolytic pathway. In most organisms, this process occurs in the cytosol, but in some protozoa such as the kinetoplastids, this is carried out in a specialized organelle called the glycosome. Glycolysis generates a net two molecules of ATP through substrate phosphorylation

catalyzed by two enzymes: PGK and pyruvate kinase. Two molecules of NADH are also produced, which can be oxidized via the electron transport chain and result in the generation of additional ATP by ATP synthase. The pyruvate generated as an end-product of glycolysis is a substrate for the Krebs Cycle.

Glucose

In the mitochondrion, pyruvate is oxidized by the pyruvate dehydrogenase complex to Acetyl group, which is fully oxidized to carbon dioxide by the citric acid cycle (also known as the Krebs Cycle). Every "turn" of the citric acid cycle produces two molecules of carbon dioxide, one molecule of the ATP equivalent guanosine triphosphate (GTP) through substrate-level phosphorylation catalyzed by succinyl-CoA synthetase, three molecules of the reduced coenzyme NADH, and one molecule of the reduced coenzyme $FADH_2$. Both of these latter molecules are recycled to their oxidized states (NAD^+ and FAD, respectively) via the electron transport chain, which generates additional ATP by oxidative phosphorylation. The oxidation of an NADH molecule results in the synthesis of between 2-3 ATP molecules, and the oxidation of one $FADH_2$ yields between 1-2 ATP molecules. The majority of cellular ATP is generated by this process. Although the citric acid cycle itself does not involve molecular oxygen, it is an obligately aerobic process because O_2 is needed to recycle the reduced NADH and $FADH_2$ to their oxidized states. In the absence of oxygen the citric acid cycle will cease to function due to the lack of available NAD^+ and FAD.

The generation of ATP by the mitochondrion from cytosolic NADH relies on the malate-aspartate shuttle (and to a lesser extent, the glycerol-phosphate shuttle) because the inner mitochondrial membrane is impermeable to NADH and NAD^+. Instead of transferring the generated NADH, a malate dehydrogenase enzyme converts oxaloacetate to malate, which is translocated to the mitochondrial matrix. Another malate dehydrogenase-catalyzed reaction occurs in the opposite direction, producing oxaloacetate and NADH from the newly transported malate and the mitochondrion's interior store of NAD^+. A transaminase converts the oxaloacetate to aspartate for transport back across the membrane and into the intermembrane space.

In oxidative phosphorylation, the passage of electrons from NADH and $FADH_2$ through the electron transport chain powers the pumping of protons out of the mitochondrial matrix and into the intermembrane space. This creates a proton motive force that is the net effect of a pH gradient and an electric potential gradient across the inner mitochondrial membrane. Flow of protons down this potential gradient — that is, from the intermembrane space to the matrix — provides the driving force for ATP synthesis by ATP synthase. This enzyme contains a rotor subunit that physically rotates relative to the static portions of the protein during ATP synthesis.

Most of the ATP synthesized in the mitochondria will be used for cellular processes in the cytosol; thus it must be exported from its site of synthesis in the mitochondrial matrix. The inner membrane contains an antiporter, the ADP/ATP translocase, which is an integral membrane protein used to exchange newly-synthesized ATP in the matrix for

ADP in the intermembrane space. This translocase is driven by the membrane potential, as it results in the movement of about 4 negative charges out of the mitochondrial membrane in exchange for 3 negative charges moved inside. However, it is also necessary to transport phosphate into the mitochondrion; the phosphate carrier moves a proton in with each phosphate, partially dissipating the proton gradient.

Beta oxidation

Fatty acids can also be broken down to acetyl-CoA by beta-oxidation. Each round of this cycle reduces the length of the acyl chain by two carbon atoms and produces one NADH and one $FADH_2$ molecule, which are used to generate ATP by oxidative phosphorylation. Because NADH and $FADH_2$ are energy-rich molecules, dozens of ATP molecules can be generated by the beta-oxidation of a single long acyl chain. The high energy yield of this process and the compact storage of fat explain why it is the most dense source of dietary calories.

Anaerobic respiration

Anaerobic respiration or fermentation entails the generation of energy via the process of oxidation in the absence of O_2 as an electron acceptor. In most eukaryotes, glucose is used as both an energy store and an electron donor. The equation for the oxidation of glucose to lactic acid is:

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH(OH)COOH + 2\ ATP$$

In prokaryotes, multiple electron acceptors can be used in anaerobic respiration. These include nitrate, sulfate or carbon dioxide. These processes lead to the ecologically-important processes of denitrification, sulfate reduction and acetogenesis, respectively.

ATP replenishment by nucleoside diphosphate kinases

ATP can also be synthesized through several so-called "replenishment" reactions catalyzed by the enzyme families of nucleoside diphosphate kinases (NDKs), which use other nucleoside triphosphates as a high-energy phosphate donor, and the ATP:guanido-phosphotransferase family,

ATP production during photosynthesis

In plants, ATP is synthesized in thylakoid membrane of the chloroplast during the light-dependent reactions of photosynthesis in a process called photophosphorylation. Here, light energy is used to pump protons across the chloroplast membrane. This produces a proton-motive force and this drives the ATP synthase, exactly as in oxidative phosphorylation. Some of the ATP produced in the chloroplasts is consumed in the Calvin cycle, which produces triose sugars.

ATP recycling

The total quantity of ATP in the human body is about 0.1 mole. The majority of ATP is not usually synthesised *de novo*, but is generated from ADP by the aforementioned processes. Thus, at any given time, the total amount of ATP + ADP remains fairly constant.

The energy used by human cells requires the hydrolysis of 100 to 150 moles of ATP daily which is around 50 to 75 kg. Typically, a human will use up their body weight of ATP over the course of the day. This means that each ATP molecule is recycled 1000 to 1500 times during a single day (100 / 0.1 = 1000). ATP cannot be stored, hence its consumption closely follows its synthesis.

Regulation of biosynthesis

ATP production in an aerobic eukaryotic cell is tightly regulated by allosteric mechanisms, by feedback effects, and by the substrate concentration dependence of individual enzymes within the glycolysis and oxidative phosphorylation pathways. Key control points occur in enzymatic reactions that are so energetically favorable that they are effectively irreversible under physiological conditions.

In glycolysis, hexokinase is directly inhibited by its product, glucose-6-phosphate, and pyruvate kinase is inhibited by ATP itself. The main control point for the glycolytic pathway is phosphofructokinase (PFK), which is allosterically inhibited by high concentrations of ATP and activated by high concentrations of AMP. The inhibition of PFK by ATP is unusual, since ATP is also a substrate in the reaction catalyzed by PFK; the biologically active form of the enzyme is a tetramer that exists in two possible conformations, only one of which binds the second substrate fructose-6-phosphate (F6P). The protein has two binding sites for ATP - the active site is accessible in either protein conformation, but ATP binding to the inhibitor site stabilizes the conformation that binds F6P poorly. A number of other small molecules can compensate for the ATP-induced shift in equilibrium conformation and reactivate PFK, including cyclic AMP, ammonium ions, inorganic phosphate, and fructose 1,6 and 2,6 biphosphate.

The citric acid cycle is regulated mainly by the availability of key substrates, particularly the ratio of NAD^+ to NADH and the concentrations of calcium, inorganic phosphate, ATP, ADP, and AMP. Citrate - the molecule that gives its name to the cycle - is a feedback inhibitor of citrate synthase and also inhibits PFK, providing a direct link between the regulation of the citric acid cycle and glycolysis.

In oxidative phosphorylation, the key control point is the reaction catalyzed by cytochrome c oxidase, which is regulated by the availability of its substrate—the reduced form of cytochrome c. The amount of reduced cytochrome c available is directly related to the amounts of other substrates:

$$\frac{1}{2}NADH + cyt\ c_{ox} + ADP + P_i \iff \frac{1}{2}NAD^+ + cyt\ c_{red} + ATP$$

which directly implies this equation:

$$\frac{cyt\ c_{red}}{cyt\ c_{ox}} = \left(\frac{[NADH]}{[NAD]^+}\right)^{\frac{1}{2}} \left(\frac{[ADP][Pi]}{[ATP]}\right) K_{eq}$$

Thus, a high ratio of [NADH] to [NAD^+] or a low ratio of [ADP] [P_i] to [ATP] imply a high amount of reduced cytochrome c and a high level of cytochrome c oxidase activity. An additional level of regulation is introduced by the transport rates of ATP and NADH between the mitochondrial matrix and the cytoplasm.

Functions in cells

Metabolism, synthesis, and active transport

ATP is consumed in the cell by energy-requiring (endothermic) processes and can be generated by energy-releasing (exothermic) processes. In this way ATP transfers energy between spatially-separate metabolic reactions. ATP is the main energy source for the majority of cellular functions. This includes the synthesis of macromolecules, including DNA and RNA, and proteins. ATP also plays a critical role in the transport of macromolecules across cell membranes, e.g. exocytosis and endocytosis.

Roles in cell structure and locomotion

ATP is critically involved in maintaining cell structure by facilitating assembly and disassembly of elements of the cytoskeleton. In a related process, ATP is required for the shortening of actin and myosin filament crossbridges required for muscle contraction. This latter process is one of the main energy requirements of animals and is essential for locomotion and respiration.

Cell signalling

Extracellular signalling

ATP is also a signalling molecule. ATP, ADP, or adenosine are recognised by purinergic receptors. Purinoreceptors might be the most abundant receptors in mammalian tissues (Abbracchio M.P. et al., 2008).

In humans, this signalling role is important in both the central and peripheral nervous system. Activity-dependent release of ATP from synapses, axons and glia activates purinergic membrane receptors known as P2. The *P2Y* receptors are *metabotropic*, i.e. G protein-coupled and modulate mainly intracellular calcium and sometimes cyclic AMP

levels. Though named between $P2Y_1$ and $P2Y_{15}$, only nine members of the P2Y family have been cloned, and some are only related through weak homology and several ($P2Y_5$, $P2Y_7$, $P2Y_9$, $P2Y_{10}$) do not function as receptors that raise cytosolic calcium. The *P2X ionotropic* receptor subgroup comprises seven members ($P2X_1$–$P2X_7$) which are ligand-gated Ca^{2+}-permeable ion channels that open when bound to an extracellular purine nucleotide. In contrast to P2 receptors (agonist order ATP > ADP > AMP > ADO), purinergic nucleotides like ATP are not strong agonists of P1 receptors which are strongly activated by adenosine and other nucleosides (ADO > AMP > ADP > ATP). P1 receptors have A1, A2a, A2b, and A3 subtypes ("A" as a remnant of old nomenclature of *adenosine receptor*), all of which are G protein-coupled receptors, A1 and A3 being coupled to Gi, and A2a and A2b being coupled to Gs. All adenosine receptors were shown to activate at least one subfamily of mitogen-activated protein kinases. The actions of adenosine are often antagonistic or synergistic to the actions of ATP. In the CNS, adenosine has multiple functions, such as modulation of neural development, neuron and glial signalling and the control of innate and adaptive immune systems (Abbracchio M.P. et al., 2008).

Intracellular signalling

ATP is critical in signal transduction processes. It is used by kinases as the source of phosphate groups in their phosphate transfer reactions. Kinase activity on substrates such as proteins or membrane lipids are a common form of signal transduction. Phosphorylation of a protein by a kinase can activate this cascade such as the mitogen-activated protein kinase cascade.

ATP is also used by adenylate cyclase and is transformed to the second messenger molecule cyclic AMP, which is involved in triggering calcium signals by the release of calcium from intracellular stores. This form of signal transduction is particularly important in brain function, although it is involved in the regulation of a multitude of other cellular processes.

DNA and RNA synthesis

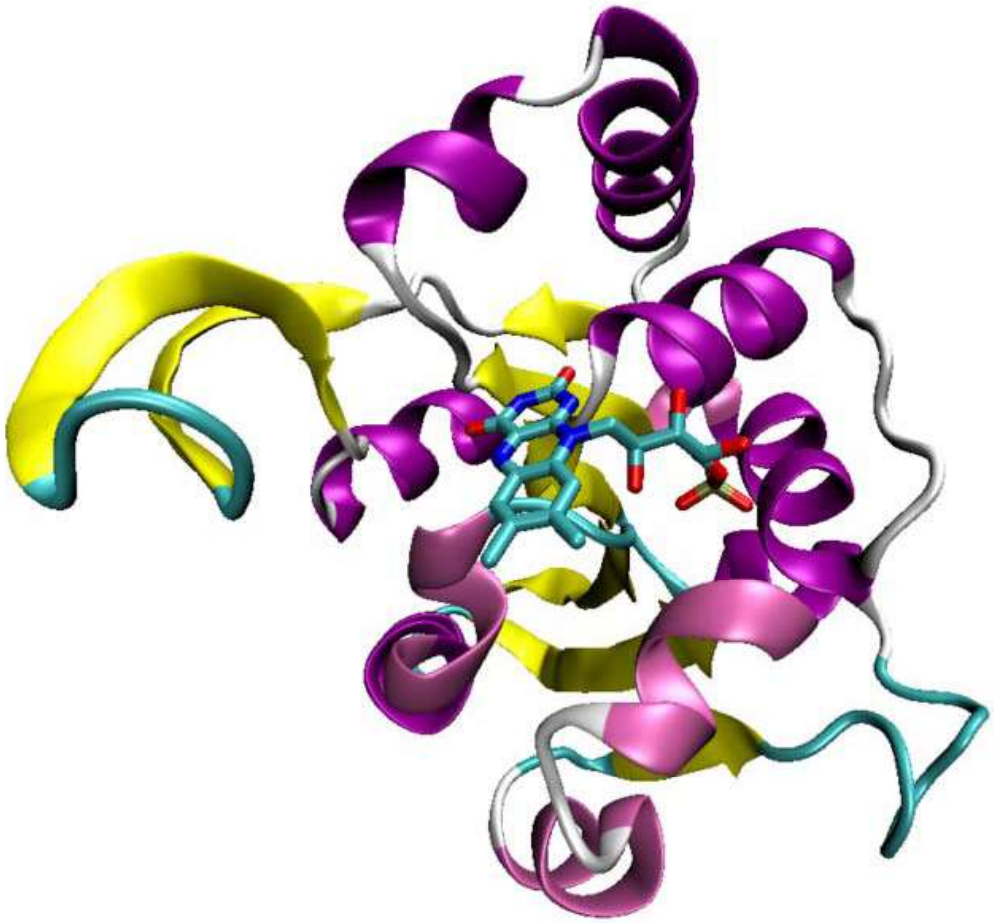
In all known organisms, the deoxyribonucleotides that make up DNA are synthesized by the action of ribonucleotide reductase (RNR) enzymes on their corresponding ribonucleotides. These enzymes reduce the sugar residue from ribose to deoxyribose by removing oxygen from the 2' hydroxyl group; the substrates are ribonucleoside diphosphates and the products deoxyribonucleoside diphosphates (the latter are denoted dADP, dCDP, dGDP, and dUDP respectively.) All ribonucleotide reductase enzymes use a common sulfhydryl radical mechanism reliant on reactive cysteine residues that oxidize to form disulfide bonds in the course of the reaction. RNR enzymes are recycled by reaction with thioredoxin or glutaredoxin.

The regulation of RNR and related enzymes maintains a balance of dNTPs relative to each other and relative to NTPs in the cell. Very low dNTP concentration inhibits DNA synthesis and DNA repair and is lethal to the cell, while an abnormal ratio of dNTPs is

mutagenic due to the increased likelihood of the DNA polymerase incorporating the wrong dNTP during DNA synthesis. Regulation of or differential specificity of RNR has been proposed as a mechanism for alterations in the relative sizes of intracellular dNTP pools under cellular stress such as hypoxia.

In the synthesis of the nucleic acid RNA, ATP is one of the four nucleotides incorporated directly into RNA molecules by RNA polymerases. The energy driving this polymerization comes from cleaving off a pyrophosphate (two phosphate groups). The process is similar in DNA biosynthesis, except that ATP is reduced to the deoxyribonucleotide dATP, before incorporation into DNA.

Binding to proteins



An example of the Rossmann fold, a structural domain of a decarboxylase enzyme from the bacterium *Staphylococcus epidermidis* (PDB ID 1G5Q) with a bound flavin mononucleotide cofactor.

Some proteins that bind ATP do so in a characteristic protein fold known as the Rossmann fold, which is a general nucleotide-binding structural domain that can also

bind the cofactor NAD. The most common ATP-binding proteins, known as kinases, share a small number of common folds; the protein kinases, the largest kinase superfamily, all share common structural features specialized for ATP binding and phosphate transfer.

ATP in complexes with proteins generally requires the presence of a divalent cation, almost always magnesium, which binds to the ATP phosphate groups. The presence of magnesium greatly decreases the dissociation constant of ATP from its protein binding partner without affecting the ability of the enzyme to catalyze its reaction once the ATP has bound. The presence of magnesium ions can serve as a mechanism for kinase regulation.

ATP analogues

Biochemistry laboratories often use *in vitro* studies to explore ATP-dependent molecular processes. Enzyme inhibitors of ATP-dependent enzymes such as kinases are needed to examine the binding sites and transition states involved in ATP-dependent reactions. ATP analogs are also used in X-ray crystallography to determine a protein structure in complex with ATP, often together with other substrates. Most useful ATP analogs cannot be hydrolyzed as ATP would be; instead they trap the enzyme in a structure closely related to the ATP-bound state. Adenosine 5'-(gamma-thiotriphosphate) is an extremely common ATP analog in which one of the gamma-phosphate oxygens is replaced by a sulfur atom; this molecule is hydrolyzed at a dramatically slower rate than ATP itself and functions as an inhibitor of ATP-dependent processes. In crystallographic studies, hydrolysis transition states are modeled by the bound vanadate ion. However, caution is warranted in interpreting the results of experiments using ATP analogs, since some enzymes can hydrolyze them at appreciable rates at high concentration.