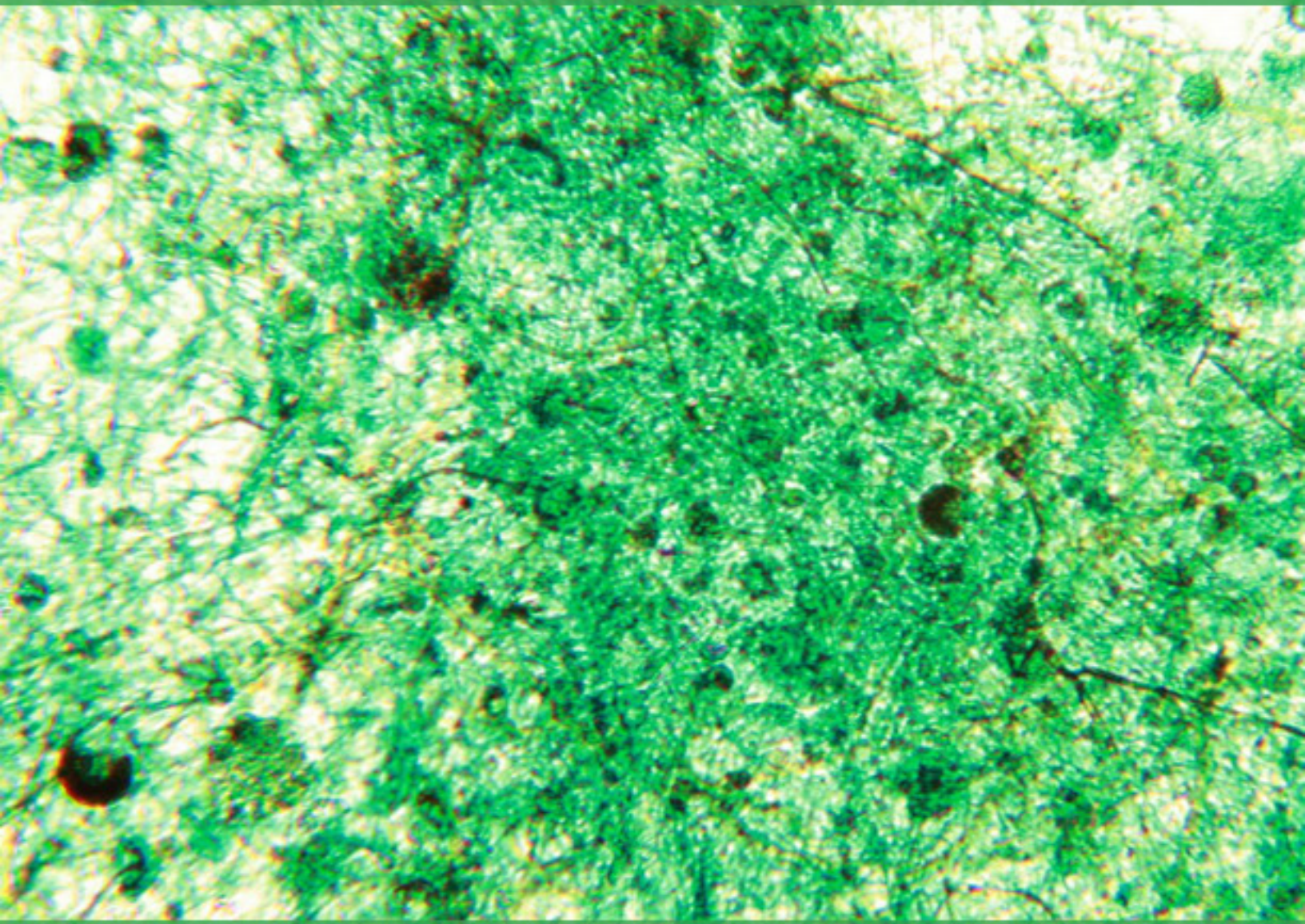
Plant Cell Biology



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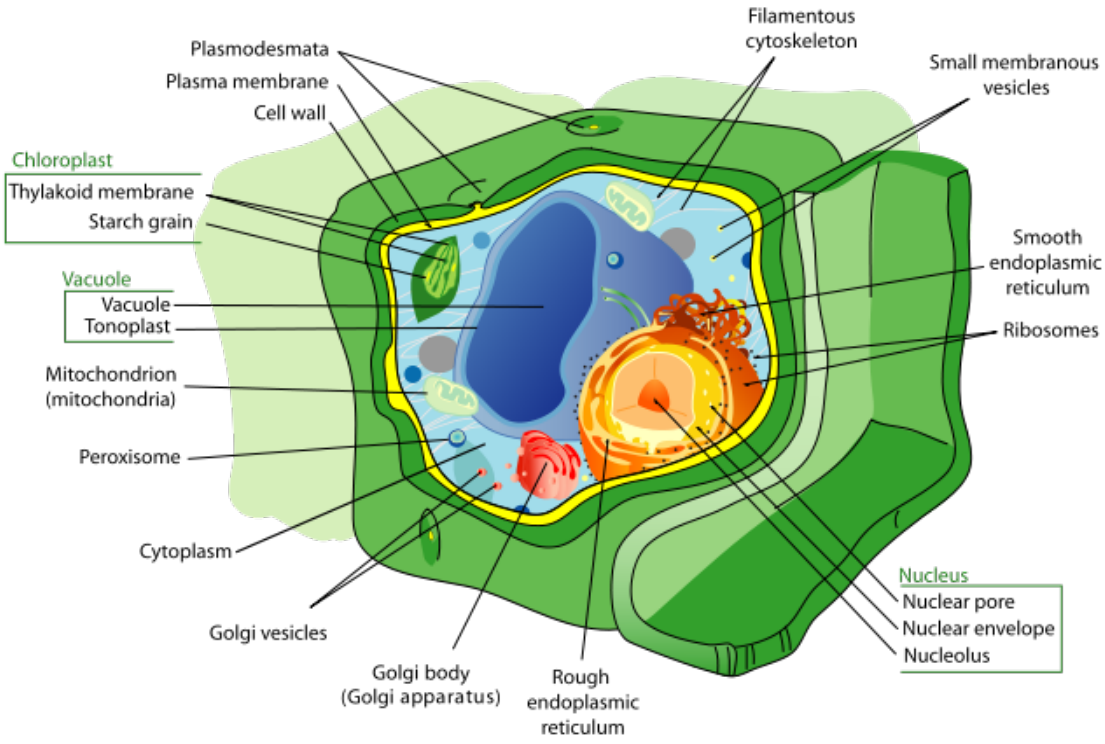
Plant Cell



Plant cell structure

Plant cells are eukaryotic cells that differ in several key respects from the cells of other eukaryotic organisms. Their distinctive features include:

- A large central vacuole, a water-filled volume enclosed by a membrane known as the *tonoplast* maintains the cell's turgor, controls movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.

- A cell wall composed of cellulose and hemicellulose, pectin and in many cases lignin, are secreted by the protoplast on the outside of the cell membrane. This contrasts with the cell walls of fungi (which are made of chitin), and of bacteria, which are made of peptidoglycan.

- Specialised cell-cell communication pathways known as plasmodesmata, pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

- Plastids, the notables one being the chloroplasts, which contain chlorophyll and the biochemical systems for light harvesting and photosynthesis, but also amyloplasts specialized for starch storage, elaioplasts specialized for fat storage, and chromoplasts specialized for synthesis and storage of pigments. As in mitochondria, which have a genome encoding 37 genes, plastids have their own genomes of about 100-120 unique genes and, it is presumed, arose as prokaryotic endosymbionts living in the cells of an early eukaryotic ancestor of the land plants and algae.

- Unlike animal cells, plant cells are stationary.

- Cell division by construction of a phragmoplast as a template for building a cell plate late in cytokinesis is characteristic of land plants and a few groups of algae, the notable one being the Charophytes and the Order Trentepohliales

- The sperm of bryophytes have flagellae similar to those in animals, but higher plants, (including Gymnosperms and flowering plants) lack the flagellae and centrioles that are present in animal cells.

Cell types

- Parenchyma cells are living cells that have diverse functions ranging from storage and support to photosynthesis and phloem loading (transfer cells). Apart from the xylem and phloem in its vascular bundles, leaves are composed mainly of parenchyma cells. Some parenchyma cells, as in the epidermis, are specialized for light penetration and focusing or regulation of gas exchange, but others are among the least specialized cells in plant tissue, and may remain totipotent, capable of dividing to produce new populations of undifferentiated cells, throughout their lives. Parenchyma cells have thin, permeable primary walls enabling the transport of small molecules between them, and their cytoplasm is responsible for a wide range of biochemical functions such as nectar secretion, or the manufacture of secondary products that discourage herbivory. Parenchyma cells that contain many chloroplasts and are concerned primarily with photosynthesis are called chlorenchyma cells. Others, such as the majority of the parenchyma cells in potato tubers and the seed cotyledons of legumes, have a storage function.

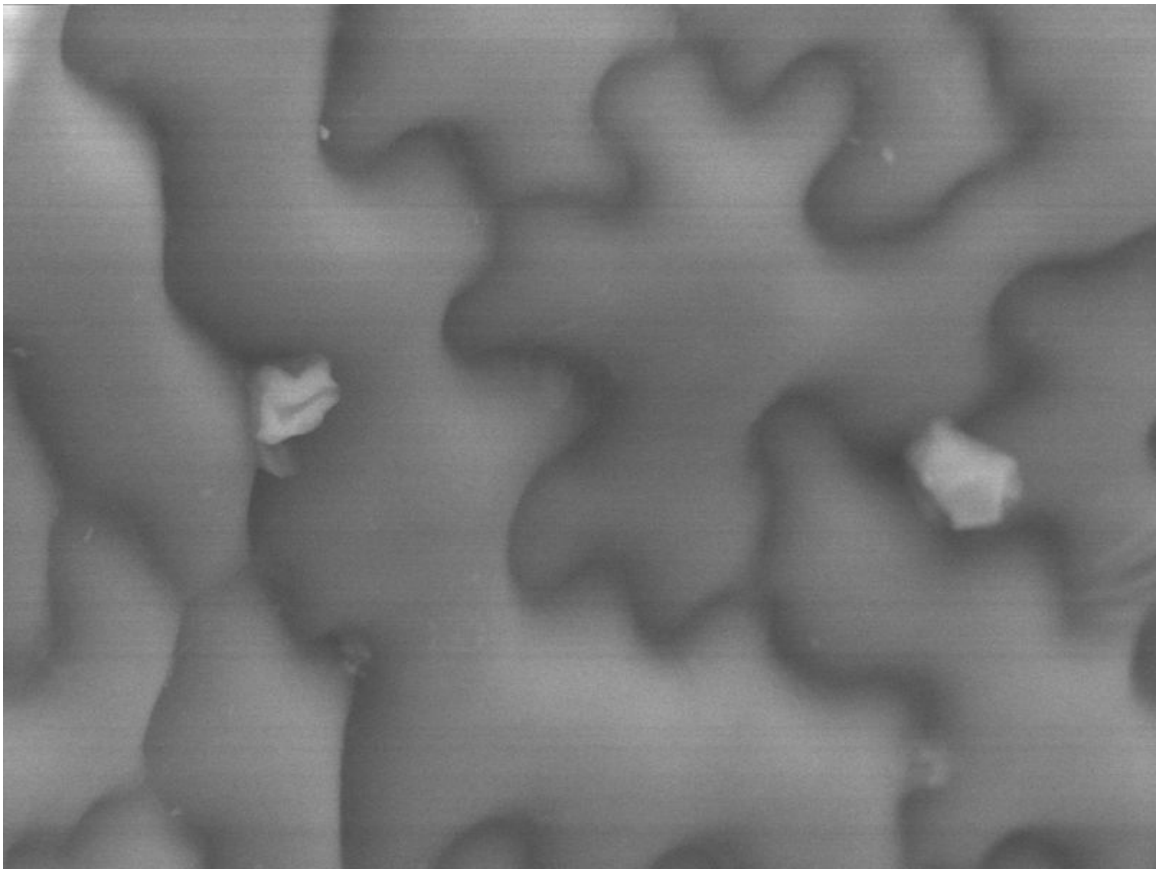
- Collenchyma cells - collenchyma cells are alive at maturity and have only a primary wall. These cells mature from meristem derivatives that initially resemble parenchyma, but differences quickly become apparent. Plastids do not develop, and the secretory apparatus (ER and Golgi) proliferates to secrete additional primary wall. The wall is most commonly thickest at the corners, where three or more cells come in contact, and thinnest where only two cells come in contact, though other arrangements of the wall thickening are possible.

Pectin and hemicellulose are the dominant constituents of collenchyma cell walls of dicotyledon angiosperms, which may contain as little as 20% of cellulose in *Petasites*. Collenchyma cells are typically quite elongated, and may divide transversely to give a septate appearance. The role of this cell type is to support the plant in axes still growing in length, and to confer flexibility and tensile strength on tissues. The primary wall lacks lignin that would make it tough and rigid, so this cell type provides what could be called plastic support - support that can hold a young stem or petiole into the air, but in cells that can be stretched as the cells around them elongate. Stretchable support (without elastic snap-back) is a good way to describe what collenchyma does. Parts of the strings in celery are collenchyma.

- Sclerenchyma cells - Sclerenchyma cells (from the Greek **skleros**, *hard*) are hard and tough cells with a function in mechanical support. They are of two broad types – sclereids or stone cells and fibres. The cells develop an extensive secondary cell wall that is laid down on the inside of the primary cell wall. The secondary wall is impregnated with lignin, making it hard and impermeable to water. Thus, these cells cannot survive for long' as they cannot exchange sufficient material to maintain active metabolism. Sclerenchyma cells are typically dead at functional maturity, and the cytoplasm is missing, leaving an empty central cavity.

Functions for sclereid cells (hard cells that give leaves or fruits a gritty texture) include discouraging herbivory, by damaging digestive passages in small insect larval stages, and physical protection (a solid tissue of hard sclereid cells form the pit wall in a peach and many other fruits). Functions of fibres include provision of load-bearing support and tensile strength to the leaves and stems of herbaceous plants. Sclerenchyma fibres are not involved in conduction, either of water and nutrients (as in the xylem) or of carbon compounds (as in the phloem), but it is likely that they may have evolved as modifications of xylem and phloem initials in early land plants.

Tissue types



cells of *Arabidopsis thaliana* epidermis

The major classes of cells differentiate from undifferentiated meristematic cells (analogous to the stem cells of animals) to form the tissue structures of roots, stems, leaves, flowers, and reproductive structures.

Xylem cells are elongated cells with lignified secondary thickening of the cell walls. Xylem cells are specialised for conduction of water, and first appeared in plants during their transition to land in the Silurian period more than 425 million years ago. The possession of xylem defines the vascular plants or Tracheophytes. Xylem tracheids are pointed, elongated xylem cells, the simplest of which have continuous primary cell walls and lignified secondary wall thickenings in the form of rings, hoops, or reticulate networks. More complex tracheids with valve-like perforations called bordered pits characterise the gymnosperms. The ferns and other pteridophytes and the gymnosperms have only xylem tracheids, while the angiosperms also have xylem vessels. Vessel members are hollow xylem cells aligned end-to-end, without end walls that are assembled into long continuous tubes. The bryophytes lack true xylem cells, but their sporophytes have a water-conducting tissue known as the hydrome that is composed of elongated cells of simpler construction.

Phloem is a specialised tissue for food conduction in higher plants. The conduction of food is a complex process that is carried in the plant with the help of special cell called phloem cells. These cells conduct inter- and intra-cellular fluid (food - proteins and other essential elements required by the plant for its metabolism) through the process of osmosis. This phenomenon is called ascent of sap in plants. Phloem consists of two cell types, the sieve tubes and the intimately-associated companion cells. The sieve tube elements lack nuclei and ribosomes, and their metabolism and functions are regulated by the adjacent nucleate companion cells. Sieve tubes are joined end-to-end with perforate end-plates between known as *sieve plates*, which allow transport of photosynthate between the sieve elements. The companion cells, connected to the sieve tubes via plasmodesmata, are responsible for loading the phloem with sugars. The bryophytes lack phloem, but moss sporophytes have a simpler tissue with analogous function known as the leptome.

Plant epidermal cells are specialised parenchyma cells covering the external surfaces of leaves, stems and roots. The epidermal cells of aerial organs arise from the superficial layer of cells known as the *tunica* (L1 and L2 layers) that covers the plant shoot apex, whereas the cortex and vascular tissues arise from innermost layer of the shoot apex known as the *corpus* (L3 layer). The epidermis of roots originates from the layer of cells immediately beneath the root cap.

The epidermis of all aerial organs, but not roots, is covered with a cuticle made of waxes and the polyester cutin. Several cell types may be present in the epidermis. Notable among these are the stomatal guard cells, glandular and clothing hairs or trichomes, and the root hairs of primary roots. In the shoot epidermis of most plants, only the guard cells have chloroplasts. The epidermal cells of the primary shoot are thought to be the only plant cells with the biochemical capacity to synthesize cutin.

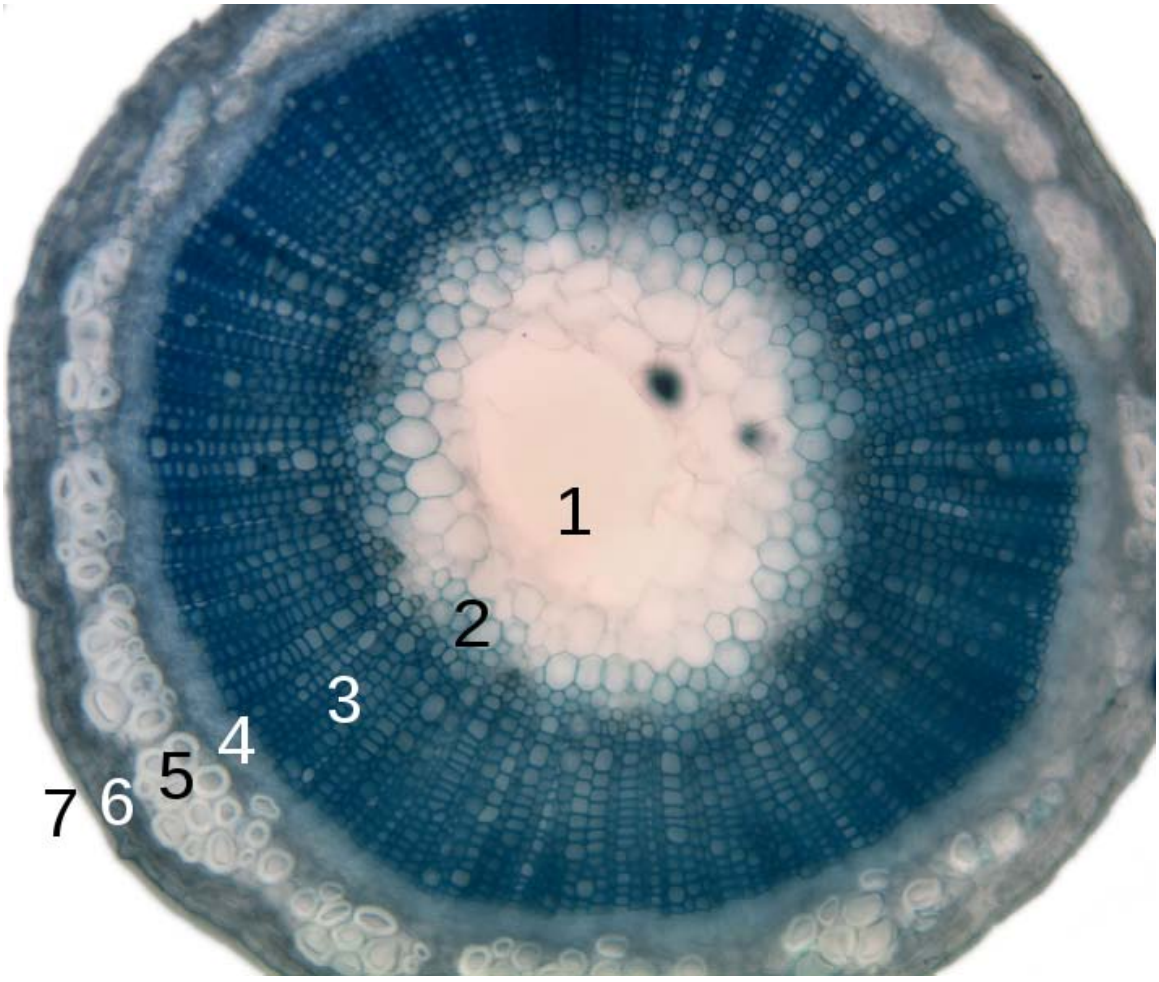
Organelles

- Cell membrane
- Cell wall
- Nuclear membrane
- Plasmodesma
- Vacuole
- Plastids
- Chloroplast
- Leucoplast
- Chromoplast
- Golgi Bodies
- Ribosome
- Endoplasmic reticulum
- Mitochondrion
- Lysosome
- Cytoplasm
- Nucleus

- DNA
- Chromatin
- RNA
- Cytoskeleton
- Nucleolus

Chapter 2

Ground Tissue



Cross-section of a flax plant stem:
1. Pith,
2. Protoxylem,
3. Xylem I,
4. Phloem I,
5. Sclerenchyma (bast fibre),

6. Cortex,
7. Epidermis

The types of **ground tissue** found in plants develop from *ground tissue* meristem and consists of three simple tissues:

- Parenchyma (cells with thin primary walls that retain their protoplasm)
- Collenchyma (cells with thick primary walls that retain their protoplasm)
- Sclerenchyma (cells with lignified secondary walls that have lost their protoplasm at maturity, i.e. are 'dead')

Parenchyma

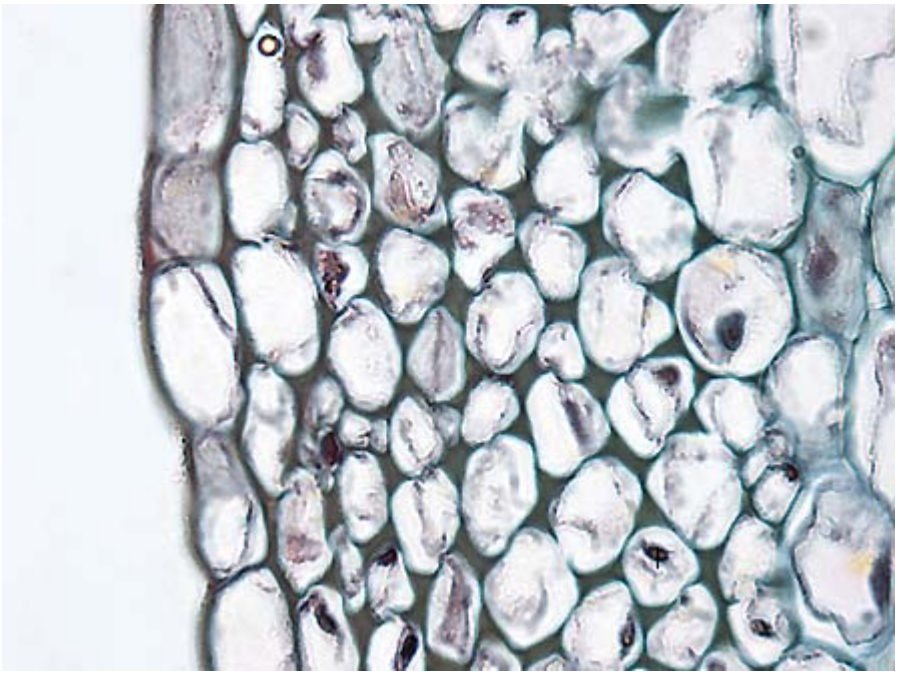
Parenchyma is the most common and versatile ground tissue. It forms, for example, the cortex and pith of stems, the cortex of roots, the mesophyll of leaves, the pulp of fruits, and the endosperm of seeds. Parenchyma cells are living cells and may remain meristematic at maturity, meaning that they are capable of cell division. They have thin but flexible cellulose cell walls, and are generally polygonal when close-packed, but approximately spherical when isolated from their neighbours. They have large central vacuoles, which allows the cells to store and regulate ions, waste products and water.

Parenchyma cells have a variety of functions:

- In leaves, they form the mesophyll and are responsible for photosynthesis and the exchange of gases, parenchyma cells in the mesophyll of leaves are a specialized parenchymatous tissue known as chlorenchyma (parenchyma with chloroplasts).
- Storage of starch, protein, fats and oils and water in roots, tubers (e.g. potato), seed endosperm (e.g. cereals) and cotyledons (e.g. pulses and groundnut)
- Secretion (e.g. hydathodes, nectaries and cells lining the inside of resin ducts)
- Wound repair and the potential for renewed meristematic activity
- Other specialized functions such as aeration (aerenchyma) and support

The form of parenchyma cells varies with their function. The epidermal parenchyma cells of a leaf are barrel shaped in cross section, but have a variety of outline shapes ranging from simple polygons to strongly branched and interlocked shapes resembling the pieces of a jigsaw puzzle, as in the leaves of *Arabidopsis thaliana*. In the epidermis of higher plants, only the guard cells have chloroplasts. This tissue serves as a barrier wall and protects the internal tissues from injury. In the spongy mesophyll of a leaf, parenchyma cells range from near-spherical and loosely arranged with large intercellular spaces to branched or stellate, mutually interconnected with their neighbours at the ends of the arms to form a three-dimensional network, as in the red kidney bean *Phaseolus vulgaris* and other mesophytes. These cells, with the epidermal guard cells of the stoma, form a system of air spaces and chambers that regulate the exchange of gases. They usually contain plastids.

Collenchyma



Cross section of collenchyma cells

The name 'collenchyma' derives from the Greek word κολλα ("kól-la"), meaning "glue", which refers to the thick, glistening appearance of the walls in fresh tissues. Collenchyma tissue is composed of elongated cells with unevenly thickened walls. They provide structural support, particularly in growing shoots and leaves. Collenchyma tissue composes, for example, the resilient strands in stalks of celery. Its growth is strongly affected by mechanical stress upon the plant. The walls of collenchyma in shaken plants (to mimic the effects of wind etc.), may be 40%-100% thicker than those not shaken. The wall is made up of cellulose and pectin.

There are three principal types of collenchyma:

- Angular collenchyma (thickened at intercellular contact points)
- Tangential collenchyma (cells arranged into ordered rows and thickened at the tangential face of the cell wall)
- Lacunar collenchyma (have intercellular space and thickening proximal to the intercellular space)

Collenchyma cells are most often found adjacent to outer growing tissues, the vascular cambium and are known for increasing structural support and integrity.

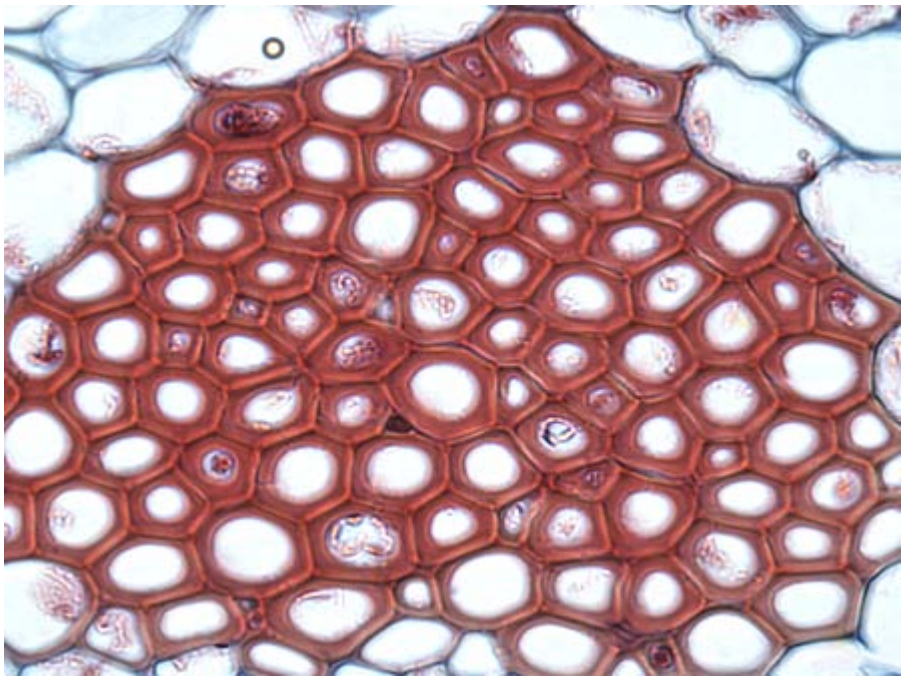
Sclerenchyma

Sclerenchyma is a supporting tissue in plants. Two groups of sclerenchyma cells exist: fibres and sclereids. Their walls consist of cellulose, hemicellulose and lignin.

Sclerenchyma cells are the principal supporting cells in plant tissues that have ceased elongation. Sclerenchyma fibres are of great economical importance, since they constitute the source material for many fabrics (flax, hemp, jute, ramie).

Unlike the collenchyma, mature sclerenchyma is composed of dead cells with extremely thick cell walls (secondary walls) that make up to 90% of the whole cell volume. The term "sclerenchyma" is derived from the Greek σκληρός ("sklē-rós"), meaning "hard". It is the hard, thick walls that make sclerenchyma cells important strengthening and supporting elements in plant parts that have ceased elongation. The difference between fibres and sclereids is not always clear. Transitions do exist, sometimes even within one and the same plant.

Fibres



Cross section of sclerenchyma fibers

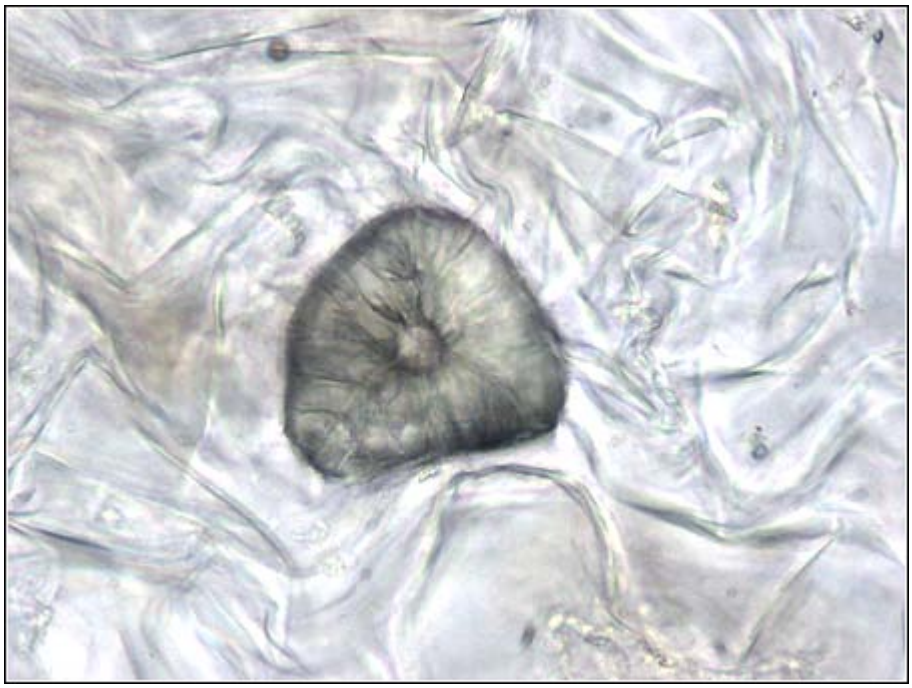
Fibres or bast are generally long, slender, so-called prosenchymatous cells, usually occurring in strands or bundles. Such bundles or the totality of a stem's bundles are colloquially called fibres. Their high load-bearing capacity and the ease with which they can be processed has since antiquity made them the source material for a number of things, like ropes, fabrics or mattresses. The fibres of flax (*Linum usitatissimum*) have been known in Europe and Egypt for more than 3000 years, those of hemp (*Cannabis sativa*) in China for just as long. These fibres, and those of jute (*Corchorus capsularis*) and ramie (*Boehmeria nivea*, a nettle), are extremely soft and elastic and are especially well suited for the processing to textiles. Their principal cell wall material is cellulose.

Contrasting are hard fibres that are mostly found in monocots. Typical examples are the fibres of many Gramineae, Agaves (sisal: *Agave sisalana*), lilies (*Yucca* or *Phormium*

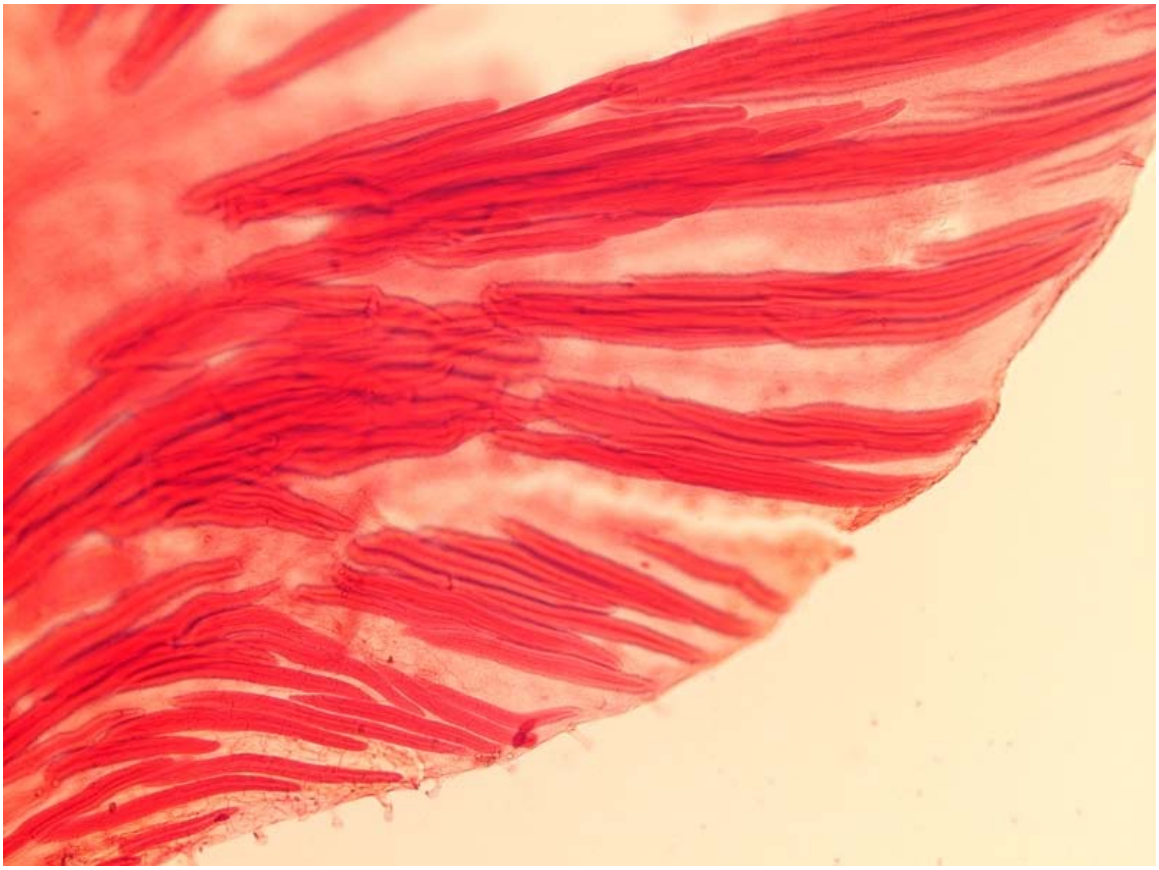
tenax), *Musa textilis* and others. Their cell walls contain, besides cellulose, a high proportion of lignin. The load-bearing capacity of *Phormium tenax* is as high as 20–25 kg/mm^2, the same as that of good steel wire (25 kg/ mm^2), but the fibre tears as soon as too great a strain is placed upon it, while the wire distorts and does not tear before a strain of 80 kg/mm^2. The thickening of a cell wall has been studied in *Linum*. Starting at the centre of the fibre are the thickening layers of the secondary wall deposited one after the other. Growth at both tips of the cell leads to simultaneous elongation. During development the layers of secondary material seem like tubes, of which the outer one is always longer and older than the next. After completion of growth the missing parts are supplemented, so that the wall is evenly thickened up to the tips of the fibres.

Fibres usually originate from meristematic tissues. Cambium and procambium are their main centers of production. They are usually associated with the xylem and phloem of the vascular bundles. The fibres of the xylem are always lignified, while those of the phloem are cellulosic. Reliable evidence for the fibre cells' evolutionary origin from tracheids exists. During evolution the strength of the tracheid cell walls was enhanced, the ability to conduct water was lost and the size of the pits reduced. Fibres that do not belong to the xylem are bast (outside the ring of cambium) and such fibres that are arranged in characteristic patterns at different sites of the shoot.

Sclereids



Fresh mount of a sclereid.



Long tapered sclereids supporting a leaf edge in *Dionysia kossinskyi*.

Sclereids are small bundles of sclerenchyma tissue in plants that form durable layers, such as the cores of apples and the gritty texture of pears. Sclereids are variable in shape. The cells can be isodiametric, prosenchymatic, forked or elaborately branched. They can be grouped into bundles, can form complete tubes located at the periphery or can occur as single cells or small groups of cells within parenchyma tissues. But compared with most fibres, sclereids are relatively short. Characteristic examples are brachysclereids or the stone cells (called stone cells because of their hardness) of pears (*Pyrus communis*) and quinces (*Cydonia oblonga*) and those of the shoot of the wax-plant (*Hoya carnosa*). The cell walls fill nearly all the cell's volume. A layering of the walls and the existence of branched pits is clearly visible. Branched pits such as these are called ramiform pits. The shell of many seeds like those of nuts as well as the stones of drupes like cherries or plums are made up from sclereids.

Chapter 3

Pectin

Polymer of D-Galacturonic Acid, Pectin. Shown here in powder form.

Pectin is a structural heteropolysaccharide contained in the primary cell walls of terrestrial plants. It was first isolated and described in 1825 by Henri Braconnot. It is produced commercially as a white to light brown powder, mainly extracted from citrus fruits, and is used in food as a gelling agent particularly in jams and jellies. It is also used in fillings, sweets, as a stabilizer in fruit juices and milk drinks and as a source of dietary fiber.

Biology

In plant cells, pectin consists of a complex set of polysaccharides (see below) that are present in most primary cell walls and particularly abundant in the non-woody parts of terrestrial plants. Pectin is present not only throughout primary cell walls but also in the middle lamella between plant cells where it helps to bind cells together.

The amount, structure and chemical composition of pectin differs between plants, within a plant over time and in different parts of a plant. During ripening, pectin is broken down by the enzymes pectinase and pectinesterase; in this process the fruit becomes softer as the middle lamella breaks down and cells become separated from each other. A similar process of cell separation caused by pectin breakdown occurs in the abscission zone of the petioles of deciduous plants at leaf fall.

Pectin is a natural part of human diet, but does not contribute significantly to nutrition. The daily intake of pectin from fruits and vegetables can be estimated to be around 5 g (assuming consumption of approximately 500 g fruits and vegetables per day).

In human digestion, pectin goes through the small intestine more or less intact. Pectin is thus a soluble dietary fiber.

Consumption of pectin has been shown to reduce blood cholesterol levels. The mechanism appears to be an increase of viscosity in the intestinal tract, leading to a reduced absorption of cholesterol from bile or food. In the large intestine and colon, microorganisms degrade pectin and liberate short-chain fatty acids that have positive influence on health (prebiotic effect).

Chemistry

Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic acid residues. Three pectic polysaccharides have been isolated from plant primary cell walls and structurally characterized. These are:

- Homogalacturonans
- Substituted galacturonans
- Rhamnogalacturonans

Homogalacturonans are linear chains of α-(1-4)-linked D-galacturonic acid. Substituted galacturonans are characterized by the presence of saccharide appendant residues (such as D-xylose or D-apiose in the respective cases of xylogalacturonan and apiogalacturonan) branching from a backbone of D-galacturonic acid residues. Rhamnogalacturonan I pectins (RG-I) contain a backbone of the repeating disaccharide: 4)-α-D-galacturonic acid-(1,2)-α-L-rhamnose-(1. From many of the rhamnose residues, sidechains of various neutral sugars branch off. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose, the types and proportions of neutral sugars varying with the origin of pectin.

Another structural type of pectin is rhamnogalacturonan II (RG-II), which is a less frequent complex, highly branched polysaccharide. Rhamnogalacturonan II is classified by some authors within the group of substituted galacturonans since the rhamnogalacturonan II backbone is made exclusively of D-galacturonic acid units.

Isolated pectin has a molecular weight of typically 60–130,000 g/mol, varying with origin and extraction conditions.

In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. This proportion is decreased more or less during pectin extraction. The ratio of esterified to non-esterified galacturonic acid determines the behavior of pectin in food applications. This is why pectins are classified as high- vs. low-ester pectins – or in short HM vs. LM-pectins, with more or less than half of all the galacturonic acid esterified.

The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts with sodium, potassium or calcium. The salts of partially esterified pectins are called pectinates, if the degree of esterification is below 5% the salts are called pectates, the insoluble acid form, pectic acid.

Some plants like sugar beet, potatoes and pears contain pectins with acetylated galacturonic acid in addition to methyl esters. Acetylation prevents gel-formation but increases the stabilising and emulsifying effects of pectin.

Amidated pectin is a modified form of pectin. Here, some of the galacturonic acid is converted with ammonia to carboxylic acid amide. These pectins are more tolerant of varying calcium concentrations that occur in use.

To prepare a pectin-gel, the ingredients are heated, dissolving the pectin. Upon cooling below gelling temperature, a gel starts to form. If gel formation is too strong, syneresis or a granular texture are the result, whilst weak gelling leads to excessively soft gels. In high-ester pectins at soluble solids content above 60% and a pH-value between 2.8 and 3.6, hydrogen bonds and hydrophobic interactions bind the individual pectin chains together. These bonds form as water is bound by sugar and forces pectin strands to stick together. These form a 3-dimensional molecular net that creates the macromolecular gel. The gelling-mechanism is called a low-water-activity gel or sugar-acid-pectin gel.

In low-ester pectins, ionic bridges are formed between calcium ions and the ionised carboxyl groups of the galacturonic acid. This is idealised in the so-called “egg box-model”. Low-ester pectins need calcium to form a gel, but can do so at lower soluble solids and higher pH-values than high-ester pectins.

Amidated pectins behave like low-ester pectins but need less calcium and are more tolerant of excess calcium. Also, gels from amidated pectin are thermo-reversible – they can be heated and after cooling solidify again, whereas conventional pectin-gels will afterwards remain liquid.

High-ester pectins set at higher temperatures than low-ester pectins. However, gelling reactions with calcium increase as the degree of esterification falls. Similarly, lower pH-values or higher soluble solids (normally sugars) increase gelling speed. Suitable pectins can therefore be selected for jams and for jellies, or for higher sugar confectionery jellies.

Sources and production

Apples, guavas, quince, plums, gooseberries, oranges and other citrus fruits, contain large amounts of pectin, while soft fruits like cherries, grapes and strawberries contain small amounts of pectin.

Typical levels of pectin in plants are (fresh weight):

- apples, 1–1.5%
- apricot, 1%
- cherries, 0.4%
- oranges 0.5–3.5%
- carrots approx. 1.4%
- citrus peels, 30%

The main raw-materials for pectin production are dried citrus peel or apple pomace, both by-products of juice production. Pomace from sugar-beet is also used to a small extent.

From these materials, pectin is extracted by adding hot dilute acid at pH-values from 1.5 – 3.5. During several hours of extraction, the protopectin loses some of its branching and chain-length and goes into solution. After filtering, the extract is concentrated in vacuum and the pectin then precipitated by adding ethanol or isopropanol. An old technique of precipitating pectin with aluminium salts is no longer used (apart from alcohols and polyvalent cations; pectin also precipitates with proteins and detergents).

Alcohol-precipitated pectin is then separated, washed and dried. Treating the initial pectin with dilute acid leads to low-esterified pectins. When this process includes ammonium hydroxide, amidated pectins are obtained. After drying and milling pectin is usually standardised with sugar and sometimes calcium-salts or organic acids to have optimum performance in a particular application.

Worldwide, approximately 40,000 metric tons of pectin are produced every year.

Uses

The main use for pectin is as a gelling agent, thickening agent and stabilizer in food. The classical application is giving the jelly-like consistency to jams or marmalades, which would otherwise be sweet juices. For household use, pectin is an ingredient in gelling sugar (also known as "Jam Sugar") where it is diluted to the right concentration with sugar and some citric acid to adjust pH. In some countries, pectin is also available as a solution or an extract, or as a blended powder, for home jam making. For conventional

jams and marmalades that contain above 60% sugar and soluble fruit solids, high-ester pectins are used. With low-ester pectins and amidated pectins less sugar is needed, so that diet products can be made. Pectin can also be used to stabilize acidic protein drinks, such as drinking yogurt, and as a fat substitute in baked goods. Typical levels of pectin used as a food additive are between 0.5 – 1.0% - this is about the same amount of pectin as in fresh fruit.

In medicine, pectin increases viscosity and volume of stool so that it is used against constipation and diarrhea. Until 2002, it was one of the main ingredients used in Kaopectate, along with kaolinite. Pectin is also used in throat lozenges as a demulcent. In cosmetic products, pectin acts as stabilizer. Pectin is also used in wound healing preparations and specialty medical adhesives, such as colostomy devices. Also, it is considered a natural remedy for nausea. Pectin rich foods are proven to help nausea.

In ruminant nutrition, depending on the extent of lignification of the cell wall, pectin is up to 90% digestible by bacterial enzymes. Ruminant nutritionists recommend that the digestibility and energy concentration in forages can be improved by increasing pectin concentration in the forage.

In the cigar industry, pectin is considered an excellent substitute for vegetable glue and many cigar smokers and collectors will use pectin for repairing damaged tobacco wrapper leaves on their cigars.

Pectin is also used in jellybeans.

Legal status

At the FAO/WHO joint Expert Committee on Food Additives and in the EU, no numerical acceptable daily intake (ADI) has been set, as pectin is considered safe.

In the US, pectin is GRAS – Generally recognized as safe. In most foods it can be used according to good manufacturing practices in the levels needed for its application, “quantum satis”.

In the International Numbering System (INS) pectin has the number 440. In Europe pectins are differentiated into the E numbers E440(i) for non-amidated pectins and E440 (ii) for amidated pectins. There are specifications in all national and international legislation defining its quality and regulating its use.

History

Pectin was first isolated and described in 1825 by Henri Braconnot, though the action of pectin to make jams and marmalades was known long before. To obtain well set jams from fruits that had little or only poor quality pectin, pectin-rich fruits or their extracts were mixed into the recipe.

During industrialization, the makers of fruit preserves soon turned to producers of apple juice to obtain dried apple pomace that was cooked to extract pectin.

Later, in the 1920s and 1930s, factories were built that commercially extracted pectin from dried apple pomace and later citrus-peel in regions that produced apple juice in both the USA and in Europe.

At first pectin was sold as a liquid extract, but nowadays pectin is often used as dried powder that is easier to store and handle than a liquid.

Chapter 4

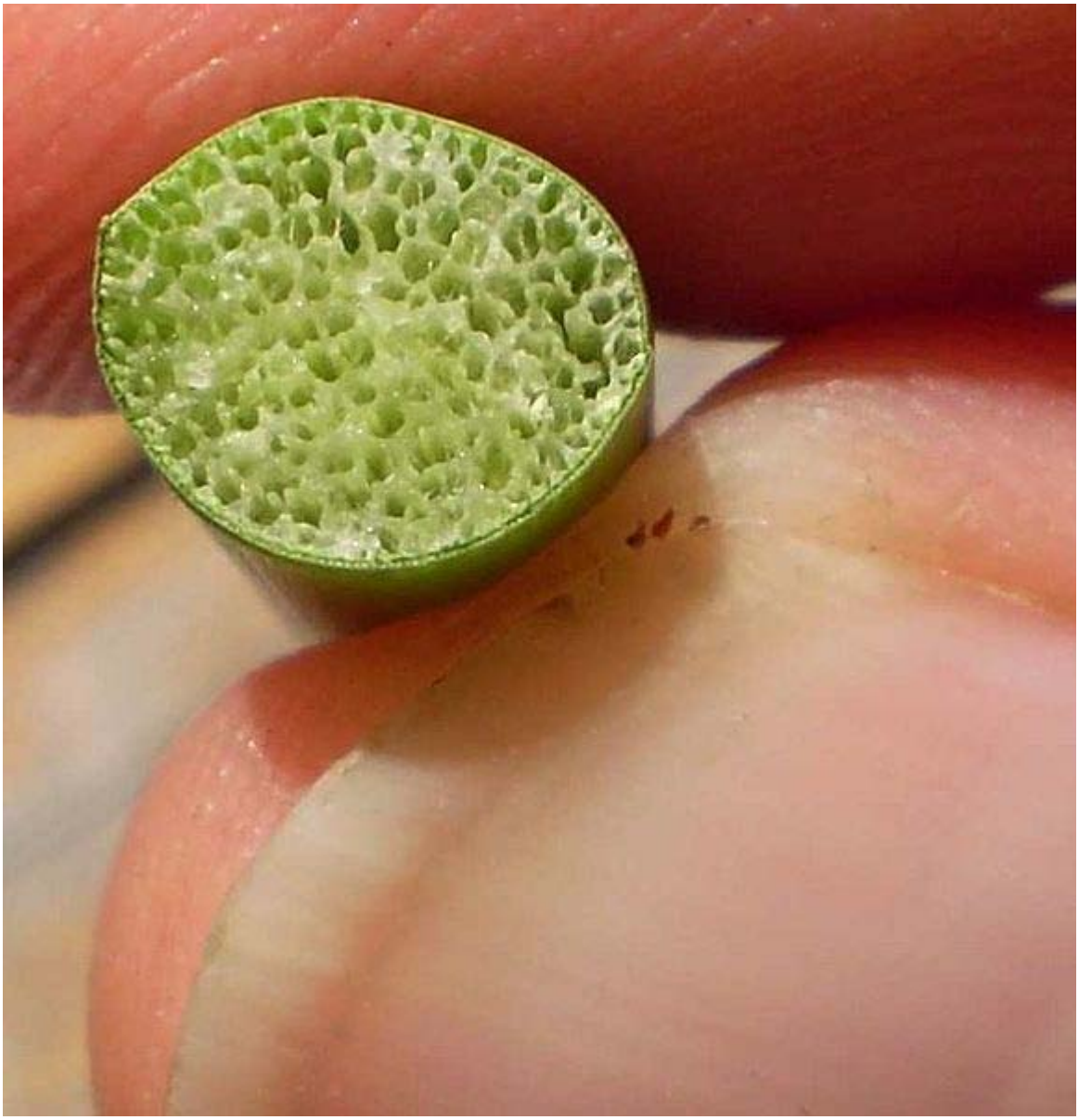
Aerenchyma and Amyloplast

Aerenchyma

Aerenchyma is an air channel in the roots of some plants, which allows exchange of gases between the shoot and the root. The channel of large air-filled cavities provides a low-resistance internal pathway for the exchange of gases such as oxygen and ethylene between the plant above the water and the submerged tissues.

Aerenchyma form in roots subject to anoxia such as what occurs during flooding of plants and soil . For example, Blom et al. (1994) investigated the adaptive responses of plants to flooding along the banks of the Rhine River, which included such morphological changes such as aerenchyma formation.

Aerenchyma formation



Aerenchyma of Schoenoplectus validus

In maize, an aerenchyma is formed from highly selective cell death and dissolution in the root cortex during anoxia in the roots . When plant roots are submerged or the surrounding soil flooded, hypoxia develops, as soil microorganisms consume oxygen faster than diffusion occurs. Nitrification is inhibited as low oxygen occurs and toxic compounds are formed, as anaerobic bacteria use nitrate, manganese, and sulfate as alternative electron acceptors . The reduction-oxidation potential of the rhizhosphere decreases and metal ions such as iron and manganese precipitate.

In general, low oxygen stimulates trees and plants to produce ethylene . Yet Visser *et al.*., in 1997, found that ethylene slows down primary and adventitious root elongation and formation. Thus, in addition to supplying root tissues with oxygen, aerenchymas assist in diffusing the accumulation of ethylene in order to prevent elongation inhibition (Visser et al. 1997).

Formation of Aerenchyma

Aerenchymas are formed by cell differentiation and collapse (lysigenous aerenchyma) or by cell separation without collapse (schizogenous aerenchyma). The differentiation or separation forms large continuous air spaces that allow diffusion of oxygen from shoot to root . Different experiments defined how cell collapse occurs. Cell death was blocked by antagonists of phospholipid metabolism, of cytolsolic Ca^{2+} or Ca-calmodulin, and of protein kinases. By contrast, reagents that activate G-proteins raise cytolsolic Ca^{2+} or inhibit phosphatases-promoted cell death (two references He *et al.*. 1996). An enzyme that was linked to this process is cellulase, which assists in cell wall breakage. In maize, a protein that is homologous to the enzyme XET (a protein that breaks the β-1,4 links between glucans and xyulosyl, the cross-linking molecule in plant cell walls) was found..

Advantages of aerenchyma

The large air-filled cavities provide a low-resistance internal pathway for the exchange of gases between the plant organs above the water and the submerged tissues. Some of the oxygen transported through the aerenchyma leaks through root pores into the surrounding soil. The resulting small rhizosphere of oxygenated soil around individual roots support microorganisms that prevent the influx of potentially toxic soil components such as sulfide, iron, and manganese. Nitrifying bacteria provide the roots with a favourable nitrogen source .

During drought, aerenchymas allow plant roots to grow deeper for water, even through compacted layers; thick and tough roots are formed. As the roots dieback and decay, the resulting voids are paths in which new roots can grow and elongate when resources are available.

Disadvantages of Aerenchyma

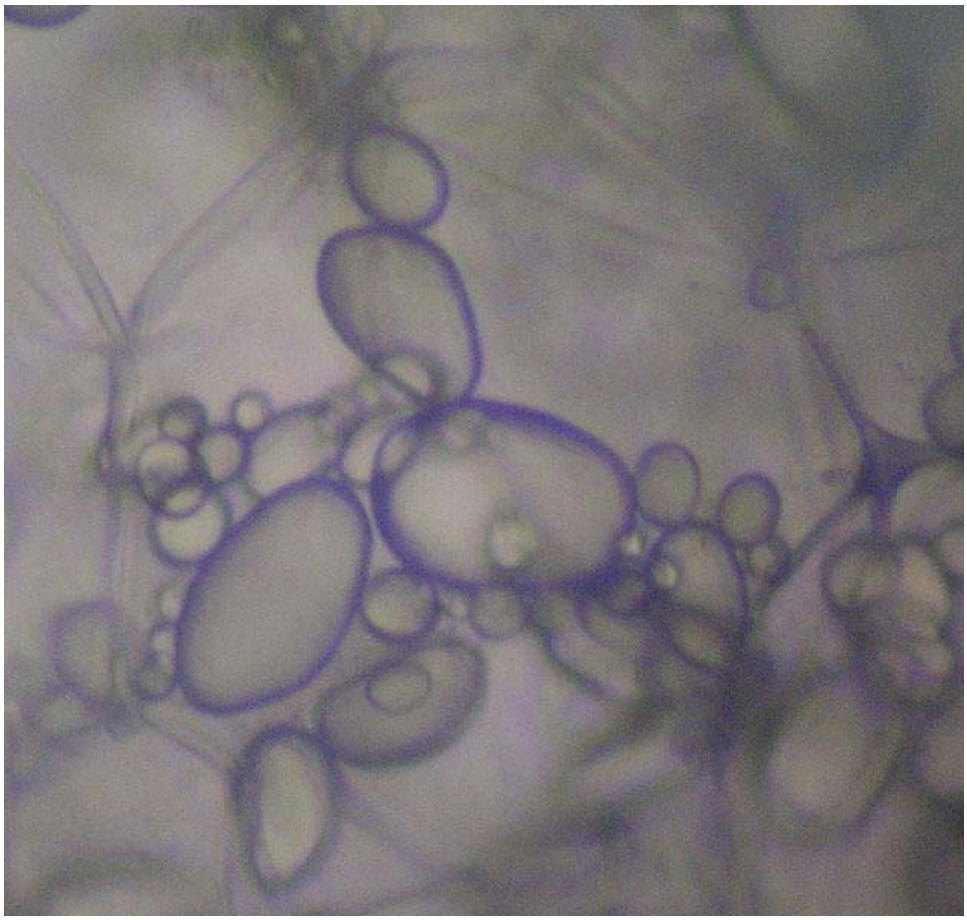
Not all plants are able to develop aerenchymas.

Aerenchymous roots may experience the following problems

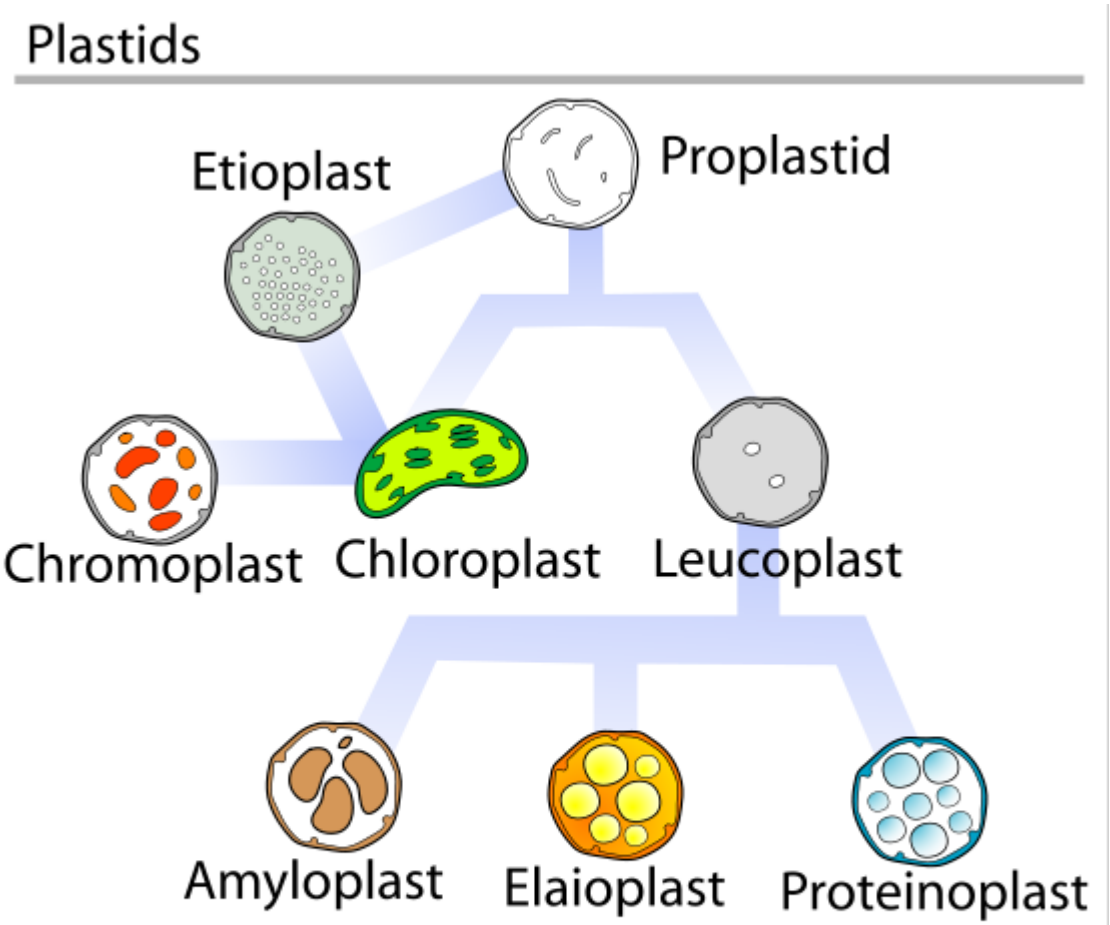
- Water and nutrient uptake may be less efficient; large intercellular spaces decrease the tissue available to transport water and nutrients from the root surface to the root xylem (Visser *et al.*. 1996, 2000a).
- Large root diameters reduce biomass-to-surface ratio, resulting in less uptake of water and nutrients and the reduced opportunity to explore all microzones for nutrients.

- Some roots with aerenchymas are less likely to resist the physical strain of compacted soils. Those roots that penetrate and survive dense and compact drained soils have a higher bulk density and a strongly lignified layer of cells surrounding the aerenchyma, which strengthens the root. This dense, lignified layer prevents radial leakage of oxygen from the aerenchyma and may block some water and nutrient uptake (Colmer *et al.*. 1998; Visser *et al.*. 2000).
- During drought, roots with aerenchyma may be less tolerant to water stress as the open structure of the cortex is probably a low-resistance pathway for water vapor, as well as for air, thereby increasing the susceptibility of the root to water loss.

Amyloplast



Amyloplasts in a potato cell



Types of plastid

Amyloplasts are non-pigmented organelles found in some plant cells. They are responsible for the synthesis and storage of starch granules, through the polymerization of glucose. Amyloplasts also convert this starch back into sugar when the plant needs energy. Large numbers of amyloplasts can be found in fruit and in underground storage tissues of some plants, such as in potato tubers.

Amyloplasts are plastids, specifically leucoplasts. Plastids are a specialized class of cellular organelles that carry their own genome and are believed to be descendants of cyanobacteria (blue-green algae) which formed a symbiotic relationship with the eukaryotic cell.

Starch synthesis and storage also takes place in chloroplasts, a type of pigmented plastid involved in photosynthesis. Amyloplasts and chloroplasts are closely related, and amyloplasts can turn into chloroplasts; this is for instance observed when potato tubers are exposed to light and turn green.

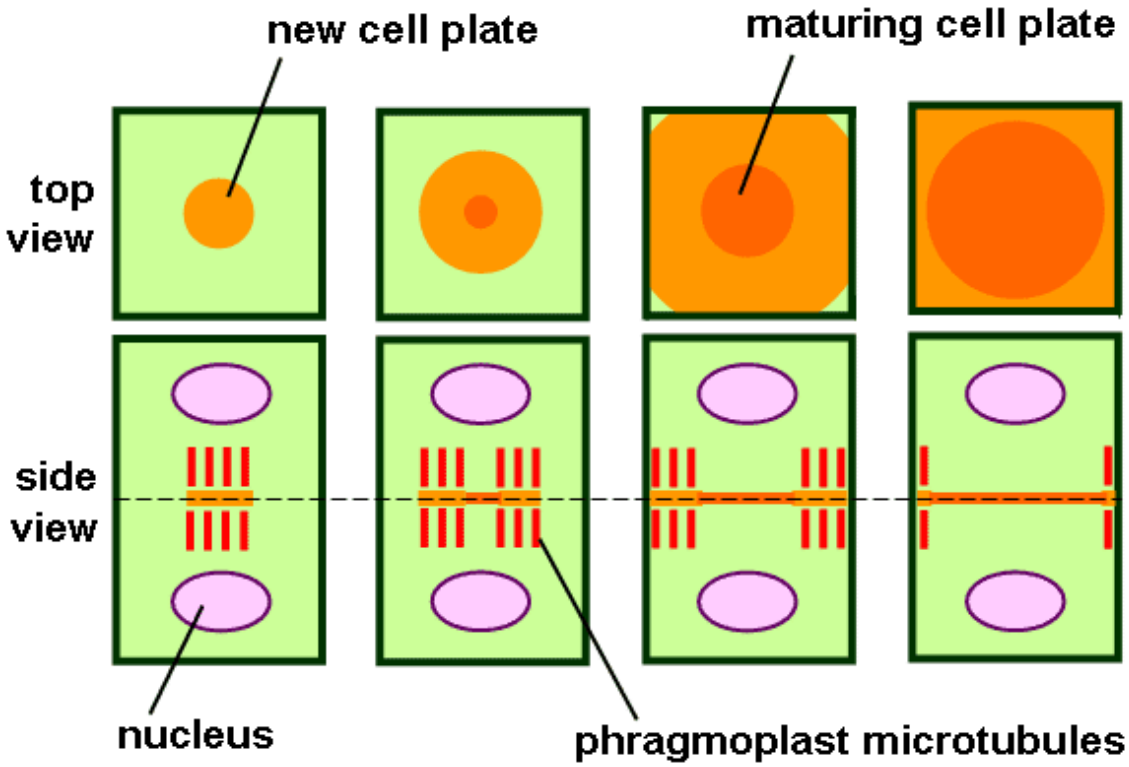
Sensing gravity

In the root cap (a tissue at the tip of the root) some specialized amyloplasts are involved in the perception of air by the plant (gravitropism). These specialized amyloplasts are denser than the cytoplasm and can sediment according to the gravity vector. They are found in a special subset of cells of the root cap (a tissue at the tip of the root) called statocytes. Statoliths are enmeshed in a web of actin and it is thought that their sedimentation transmits the gravitropic signal by activating mechanosensing channels. The gravitropic signal then leads to reorientation of auxin efflux carriers and subsequent redistribution of auxin streams in root cap and root as a whole. The changed relations in concentration of auxin leads to differential growth of the root tissues. Taken together, the root is then turning, following the gravity stimuli. They are also found in the endodermic layer of the inflorescence stem. The redistribution of auxin causes the shoot to turn in a direction opposite that of the gravity stimuli.

Chapter 5

Cell Plate, Leucoplast and Oleosin

Cell plate



Phragmoplast and cell plate formation in a plant cell during cytokinesis. Left side: Phragmoplast forms and cell plate starts to assemble in the center of the cell. Toawards the right: Phragmoplast enlarges in a donut-shape towards the outside of the cell, leaving behind mature cell plate in the center. The cell plate will transform into the new cell wall once cytokinesis is complete.

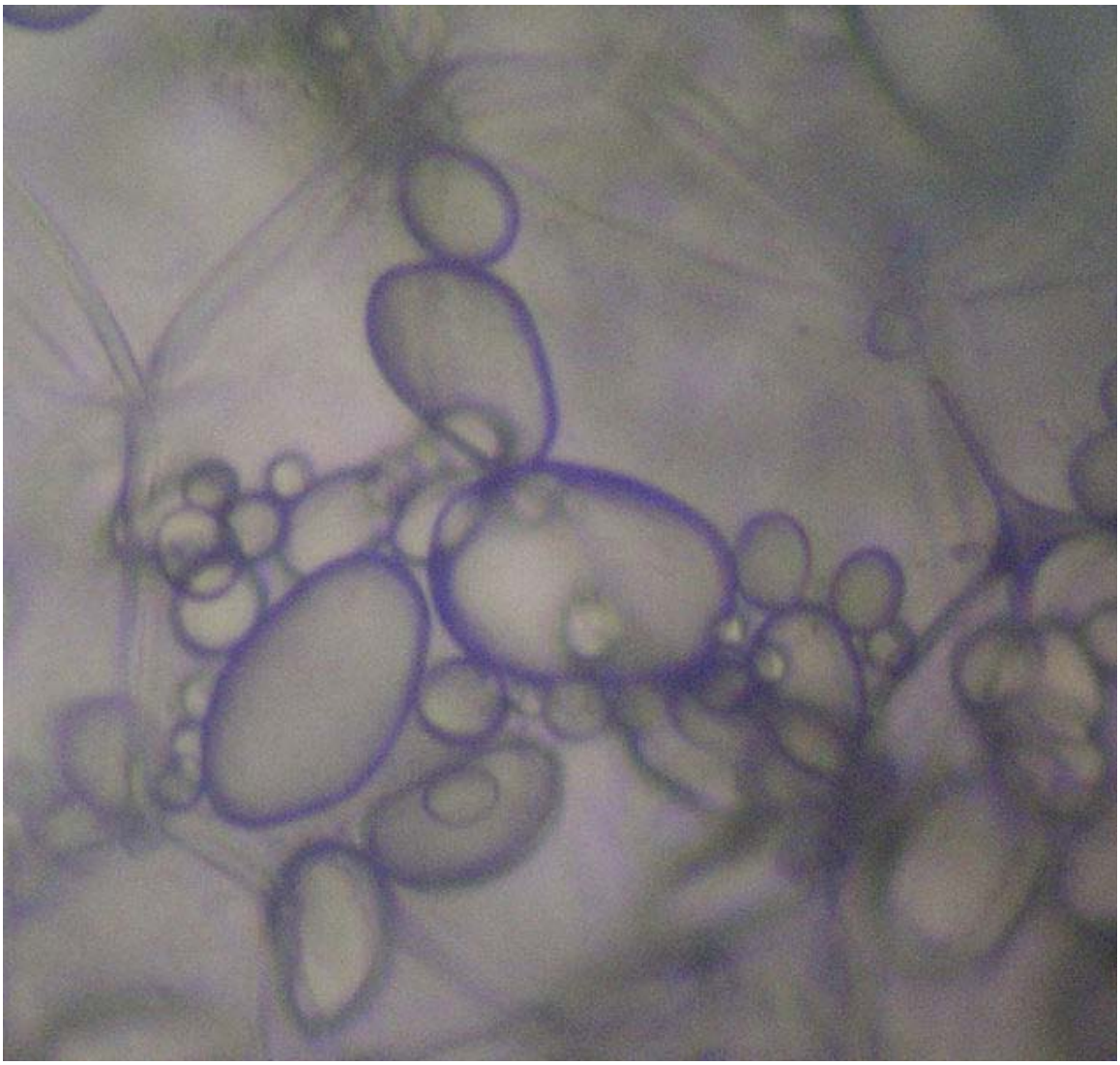
Cytokinesis in terrestrial plants occurs by **cell plate** formation. This process entails the delivery of Golgi-derived and endosomal vesicles carrying cell wall and cell membrane components to the plane of cell division and the subsequent fusion of these vesicles within this plane.

After formation of an early tubulo-vesicular network at the center of the cell, the initially labile cell plate consolidates into a tubular network and eventually a fenestrated sheet. The cell plate grows outward from the center of the cell to the parental plasma membrane with which it will fuse, thus completing cell division. Formation and growth of the cell plate is dependent upon the phragmoplast, which is required for proper targeting of Golgi-derived vesicles to the cell plate.

As the cell plate matures in the central part of the cell, the phragmoplast disassembles in this region and new elements are added on its outside. This process leads to a steady expansion of the phragmoplast, and concomitantly, to a continuous retargeting of Golgi-derived vesicles to the growing edge of the cell plate. Once the cell plate reaches and fuses with the plasma membrane the phragmoplast disappears. This event not only marks the separation of the two daughter cells, but also initiates a range of biochemical modifications that transform the callose-rich, flexible cell plate into a cellulose-rich, stiff primary cell wall.

The heavy dependence of cell plate formation on active Golgi stacks explains why plant cells, unlike mammalian cells, do not disassemble their secretion machinery during cell division.

Leucoplast



Leucoplasts, specifically, amyloplasts

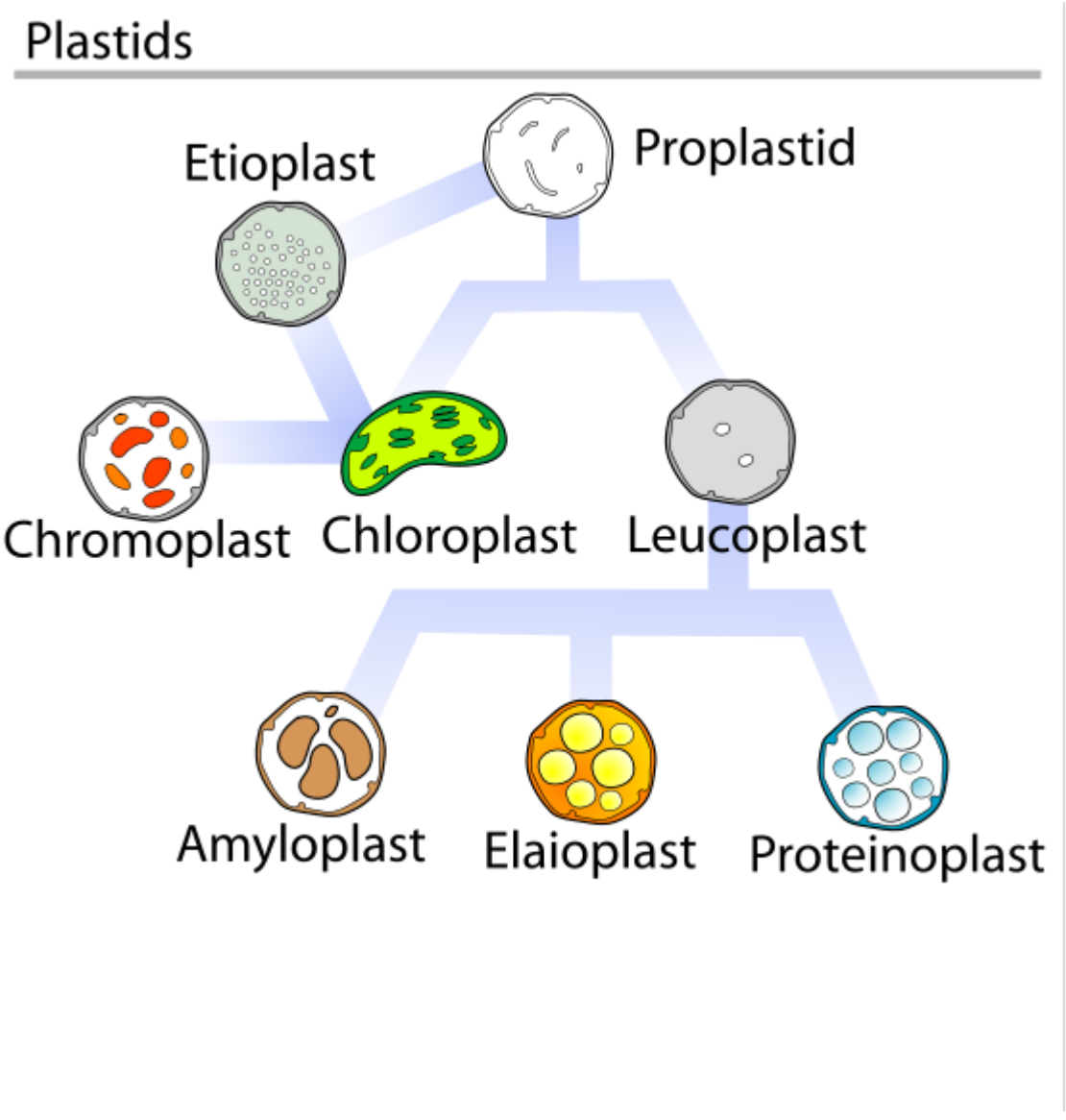
Leucoplasts are a category of plastid and as such are organelles found in plant cells. They are non-pigmented, in contrast to other plastids such as the chloroplast.

Lacking pigments, leucoplasts are not green, so they are predictably located in roots and non-photosynthetic tissues of plants. They may become specialized for bulk storage of starch, lipid or protein and are then known as amyloplasts, elaioplasts, or proteinoplasts respectively. However, in many cell types, leucoplasts do not have a major storage function and are present to provide a wide range of essential biosynthetic functions, including the synthesis of fatty acids, many amino acids, and tetrapyrrole compounds such as haem. In general, leucoplasts are much smaller than chloroplasts and have a variable morphology, often described as amoeboid. Extensive networks of stromules

interconnecting leucoplasts have been observed in epidermal cells of roots, hypocotyls and petals, and in callus and suspension culture cells of tobacco. In some cell types at certain stages of development, leucoplasts are clustered around the nucleus with stromules extending to the cell periphery, as observed for proplastids in the root meristem.

Etioplasts, which are pre-granal, immature chloroplasts but can also be chloroplasts which have been deprived of light, lack active pigment and can technically be considered leucoplasts. After several minutes exposure to light, etioplasts begin to transform into functioning chloroplasts and cease being leucoplasts.

Compare



- Plastid
  - Chloroplast and etioplast

- Chromoplast
- Leucoplast
  - Amyloplast
  - Elaioplast
  - Proteinoplast

Oleosin

Oleosins are structural proteins found in oil bodies and found in plant cells. Oil bodies are not considered organelles because they have a single layer membrane and lack the pre-requisite double layer membrane in order to be considered an organelle. They are found in plant parts with high oil content that undergo extreme desiccation as part of their maturation process, and help stabilize the bodies.

Oleosins comprise of three parts. The N- and C-terminal domains are amphipathic, whereas the middle part is strongly hydrophobic. Models show oleosins having a hairpin-like hydrophobic shape that is inserted inside the triacylglyceride (TAG), while the hydrophilic parts are left outside oil bodies.

Oleosins have been found on oil bodies of seeds, tapetum cells, and pollen but not fruits. Instead of a stabilizer of oil bodies, oleosins are believe to be involved in water-uptaking of pollen on stigma.

Use in Purification of Recombinant Protein

Oleosins provide an easy way of purifying proteins which have been produced recombinantly in plants. If the protein is made as a fusion protein with oleosin and a protease recognition site is incorporated between them, the fusion protein will sit in the membrane of the oil body, which can be easily isolated by centrifugation. The oil droplets can then be mixed with aqueous medium again, and oleosin cleaved from the protein of interest. Centrifugation will cause two phases to separate again, and the aqueous medium now contains the purified protein.

Chapter 6

Guard Cell

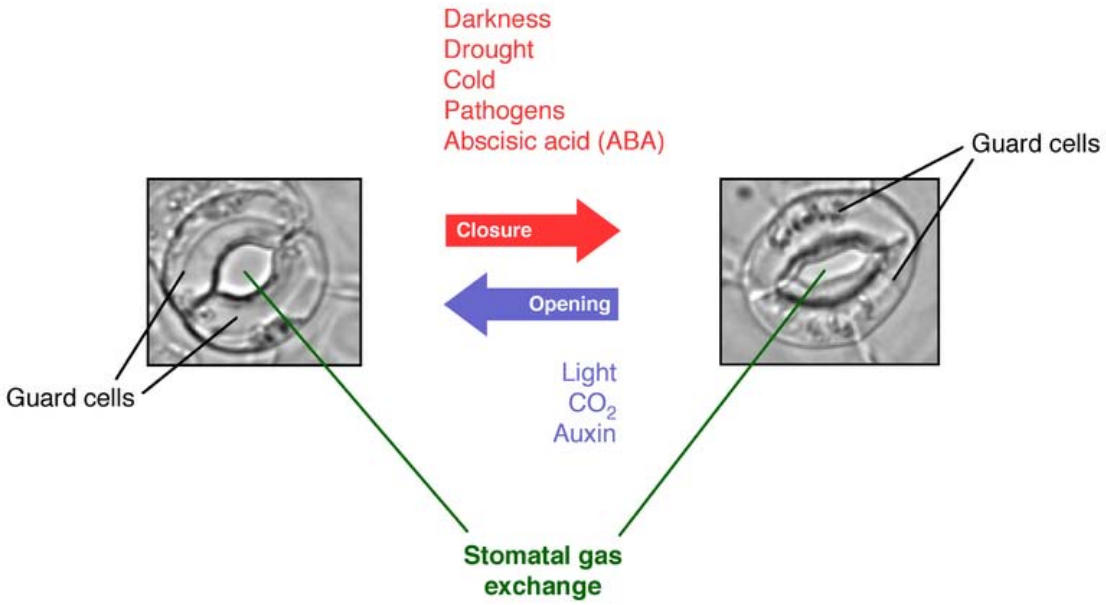


Figure 1. A stomatal pore in the surface (epidermis) of a leaf as viewed through a microscope. The central stomatal pore is formed by a pair of guard cells. The stomatal pore can either open (left) or close (right) depending on the environmental conditions.

Guard cells are specialized cells located in the leaf epidermis of plants. Pairs of guard cells surround tiny stomatal airway pores (Figure 1). These tiny holes in the surface of leaves are necessary for gas exchange into and out of the plant; carbon dioxide (CO_2) enters the plant allowing the carbon fixation reactions of photosynthesis to occur. Oxygen (O_2) exits the plant as a byproduct of photosynthesis. The opening and closing of the stomatal gas exchange holes is regulated by swelling and shrinking of the two surrounding guard cells (Figure 1). Due to the presence of the stomatal pores on plant leaf surfaces, water evaporates through the stomatal openings causing plants to lose water. Over 95% of water loss from plants can occur by evaporation (transpiration) through the stomatal pores. Therefore, it is important for plants to be able to balance the amount of CO_2 being brought into the plant with the amount of water escaping as a result of the open stomatal pores. Hence, the guard cells are the gate keepers of the plants ability to take in CO_2 from the atmosphere for photosynthesis – while regulating how much water

plants lose to the atmosphere. Opening and closure of the stomatal pore (Figure 1) is mediated by changes in the turgor pressure of the two guard cells. The turgor pressure of guard cells is controlled by movements of large quantities of ions and sugars into and out of the guard cells. When guard cells take up these solutes, the water potential (Ψ) inside the cells decreases, causing osmotic water flow into the guard cells. This leads to a turgor pressure increase causing swelling of the guard cells and the stomatal pores open (Figure 2). The ions that are taken up by guard cells are mainly potassium (K^+) ions and chloride (Cl^-) ions. In addition guard cells take up sugars that also contribute to opening of the stomatal pores.

Water loss and water use efficiency

Water stress (drought and salt stress) is one of the major environmental problems causing severe losses in agriculture and in nature. Drought tolerance of plants is mediated by several mechanisms that work together, including stabilizing and protecting the plant from damage caused by desiccation and also controlling how much water plants loose through the stomatal pores during drought. A plant hormone, abscisic acid (ABA), is produced in response to drought. A major type of ABA receptor has been identified. Future research is needed to test if these receptors can be used to engineer drought tolerance in plants. The plant hormone ABA causes the stomatal pores to close in response to drought, which reduces plant water loss via transpiration to the atmosphere and allows plants to avoid or slow down water loss during droughts. The use of drought tolerant crop plants would lead to a reduction in crop losses during droughts. Since guard cells control water loss of plants, the investigation on how stomatal opening and closure are regulated could lead to the development of plants with improved avoidance or slowing of desiccation and better water use efficiency.

Ion uptake and release

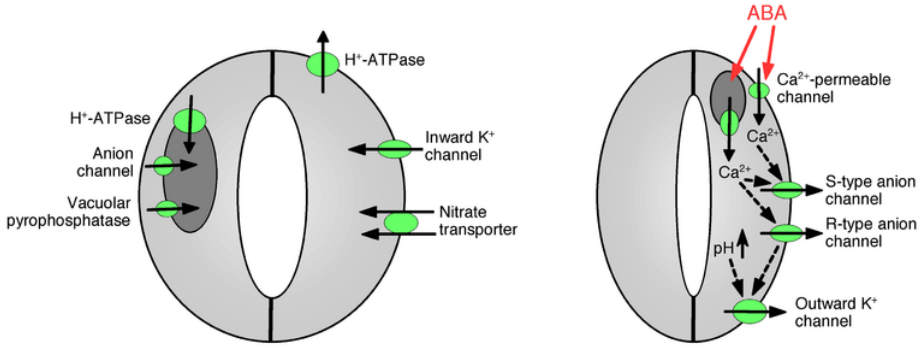


Figure 2. Ion channels and pumps regulating stomatal opening and closure.

Ion uptake into guard cells causes stomatal opening: The opening of gas exchange pores requires the uptake of potassium ions into guard cells. Potassium channels and pumps have been identified and shown to function in the uptake of ions and opening of stomatal apertures (Figure 2). Ion release from guard cells causes stomatal pore closing: Other ion channels have been identified that mediate release of ions from guard cells, which results

in osmotic water efflux from guard cells due to osmosis, shrinking of the guard cells, and closing of stomatal pores (Figures 1 and 2). Specialized potassium efflux channels participate in mediating release of potassium from guard cells. Anion channels were identified as important controllers of stomatal closing. Anion channels have several major functions in controlling stomatal closing: (a) They allow release of anions, such as chloride and malate from guard cells, which is needed for stomatal closing. (b) Anion channels are activated by signals that cause stomatal closing, for example by intracellular calcium and ABA. The resulting release of negatively charged anions from guard cells results in an electrical shift of the membrane to more positive voltages (depolarization) at the intracellular surface of the guard cell plasma membrane. This electrical depolarization of guard cells leads to activation of the outward potassium channels and the release of potassium through these channels (Figure 2). At least two major types of anion channels have been characterized in the plasma membrane: S-type anion channels and R-type anion channels.

Vacuolar ion transport

Vacuoles are large intracellular storage organelles in plants cells. In addition to the ion channels in the plasma membrane, vacuolar ion channels have important functions in regulation of stomatal opening and closure because vacuoles can occupy up to 90% of guard cell's volume. Therefore, a majority of ions are released from vacuoles when stomata are closed. Vacuolar K^+ (VK) channels and fast vacuolar channels can mediate K^+ release from vacuoles. Vacuolar K^+ (VK) channels are activated by elevation in the intracellular calcium concentration. Another type of calcium-activated channel, is the slow vacuolar (SV) channel. SV channels have been shown to function as cation channels that are permeable to Ca^{2+} ions, but their exact functions are not yet known in plants.

Signal transduction

Guard cells perceive and process environmental and endogenous stimuli such as light, humidity, CO_2, temperature, drought, and plant hormones to trigger cellular responses resulting in stomatal opening or closure. These signal transduction pathways determine for example how quickly a plant will loose water during a drought period. Guard cells have become a model for single cell signaling. Using Arabidopsis thaliana, the investigation of signal processing in single guard cells has become open to the power of genetics. Cytosolic and nuclear proteins and chemical messengers that function in stomatal movements have been identified that mediate the transduction of environmental signals thus controlling CO_2 intake into plants and plant water loss. Research on guard cell signal transduction mechanisms is producing an understanding of how plants can improve their response to drought stress by reducing plant water loss. Guard cells also provide an excellent model for basic studies on how a cell integrates numerous kinds of input signals to produce a response (stomatal opening or closing). These responses require coordination of numerous cell biological processes in guard cells, including signal reception, ion channel and pump regulation, membrane trafficking, transcription, cytoskeletal rearrangements and more. A challenge for future research is to assign the functions of some of the identified proteins to these diverse cell biological processes.

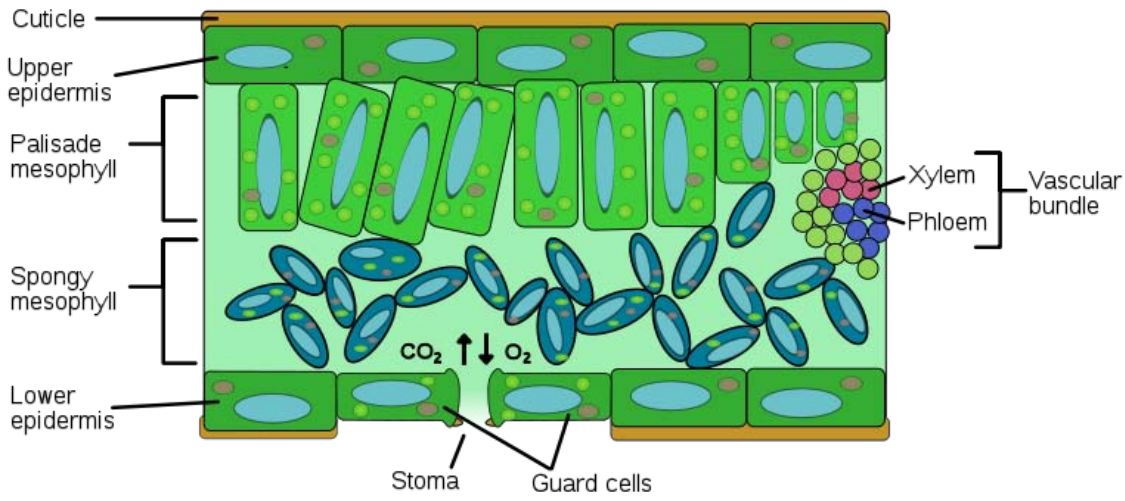
Development

During the development of plant leaves, the specialized guard cells differentiate from "guard mother cells". The density of the stomatal pores in leaves is regulated by environmental signals, including the continuingly increasing atmospheric CO_2 concentration, which reduces the density of stomatal pores in the surface of leaves in many plant species by presently unknown mechanisms. The genetics of stomatal development can be directly studied by imaging of the leaf epidermis using a microscope (Figure 1). Several major control proteins that function in a pathway mediating the development of guard cells and the stomatal pores have been identified.

Chapter 7

Palisade Cell, Phragmoplast and Phragmosome

Palisade cell

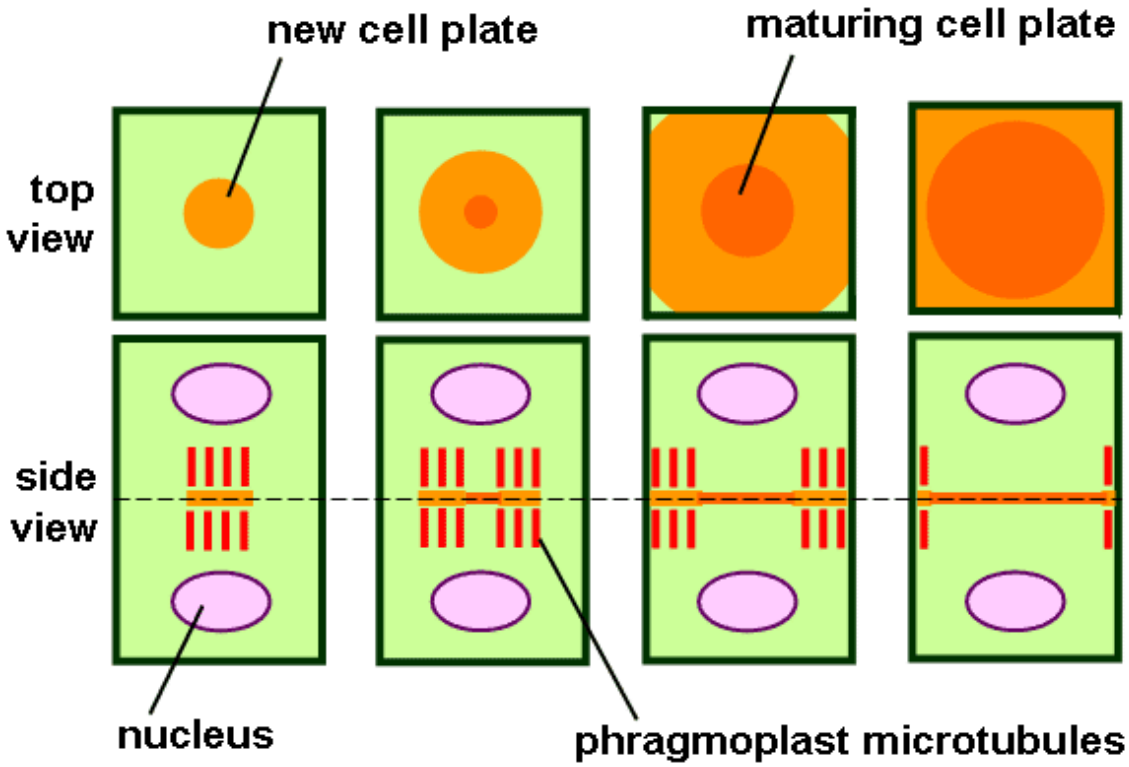


Cross-section of a leaf

Palisade cells are cells found within the mesophyll in leaves of dicotyledonous plants. They contain chloroplasts, which convert the energy stored in photons to chemical energy through photosynthesis, which is made up of two main stages; the light-dependent reactions and light-independent reactions. The cylindrical shape of palisade cells allows a large amount of light to be absorbed by the chloroplasts. Beneath the palisade mesophyll are the spongy mesophyll cells, irregularly-shaped cells that having many intercellular spaces to allow the passage of gases, such as the intake of carbon dioxide for photosynthesis to take place. The stomata is the way in which these gases are exchanged, as well as the transpiration of water from the xylem, either by the apoplast or symplast

pathway. Palisade cells are positioned towards the upper surface of the leaf and contain the largest number of chloroplasts per cell in plants. This makes them the primary site of photosynthesis in a plant's leaves. They have a very large surface area in order for them to absorb more light during photosynthesis.

Phragmoplast



Phragmoplast and cell plate formation in a plant cell during cytokinesis. Left side: Phragmoplast forms and cell plate starts to assemble in the center of the cell. Towards the right: Phragmoplast enlarges in a donut-shape towards the outside of the cell, leaving behind mature cell plate in the center. The cell plate will transform into the new cell wall once cytokinesis is complete.

The **phragmoplast** is a plant cell specific structure that forms during late cytokinesis. It serves as a scaffold for cell plate assembly and subsequent formation of a new cell wall separating the two daughter cells.

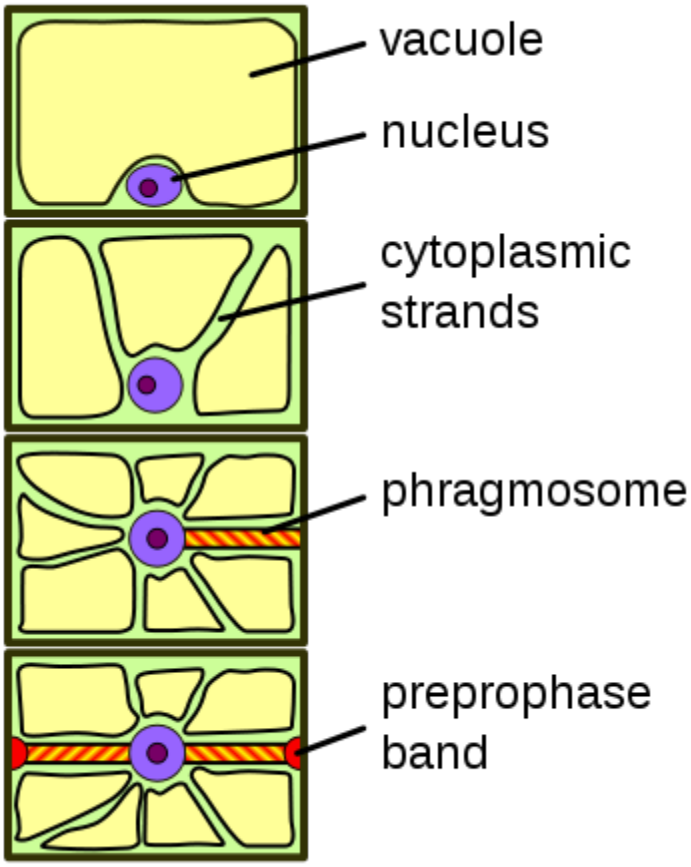
The phragmoplast is a complex assembly of microtubules (MTs), microfilaments (MFs), and endoplasmic reticulum (ER) elements, that assemble in two opposing sets perpendicular to the plane of the future cell plate during anaphase and telophase. It is initially barrel-shaped and forms from the mitotic spindle between the two daughter nuclei while nuclear envelopes reassemble around them. The cell plate initially forms as a disc between the two halves of the phragmoplast structure. While new cell plate material

is added to the edges of the growing plate, the phragmoplast microtubules disappear in the center and regenerate at the edges of the growing cell plate. The two structures grow outwards until they reach the outer wall of the dividing cell. If a phragmosome was present in the cell, the phragmoplast and cell plate will grow through the space occupied by the phragmosome. They will reach the parent cell wall exactly at the position formerly occupied by the preprophase band.

The microtubules and actin filaments within the phragmoplast serve to guide vesicles with cell wall material to the growing cell plate. Actin filaments are also possibly involved in guiding the phragmoplast to the site of the former preprophase band location at the parent cell wall. While the cell plate is growing, segments of smooth endoplasmic reticulum are trapped within it, later forming the plasmodesmata connecting the two daughter cells.

The phragmoplast can only be observed in bryophytes and vascular plants and a few algae. Some algae use another type of microtubule array, a phycoplast, during cytokinesis.

Phragmosome



Phragmosome formation in a highly vacuolated plant cell. From top to bottom: 1) Interphase cell with large central vacuole. 2) Cytoplasmic strands starting to penetrate vacuole. 3) Nucleus migration into center and formation of the phragmosome. 4) Phragmosome formation completed and formation of preprophase band marking future cell division plane.

The **phragmosome** is a sheet of cytoplasm forming in highly vacuolated plant cells in preparation for mitosis. In contrast to animal cells, plant cells often contain large central vacuoles occupying up to 90% of the total cell volume and pushing the nucleus against the cell wall. In order for mitosis to occur, the nucleus has to move into the center of the cell. This happens during G2 phase of the cell cycle just after DNA replication.

Initially, cytoplasmic strands form that penetrate the central vacuole and provide pathways for nuclear migration. Actin filaments along these cytoplasmic strands pull the nucleus into the center of the cell. These cytoplasmic strands fuse into a transverse sheet of cytoplasm along the plane of future cell division, forming the phragmosome.

Phragmosome formation is only clearly visible in dividing plant cells that are highly vacuolated.

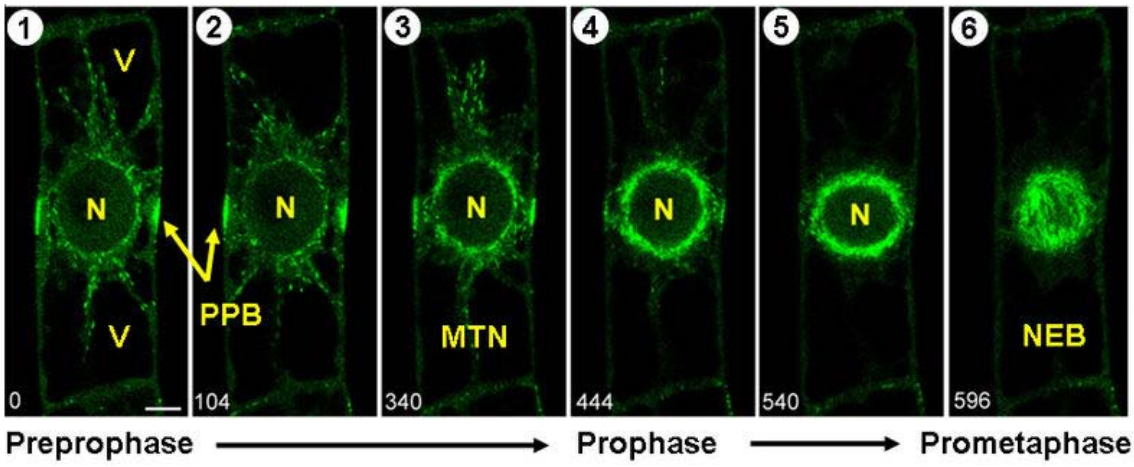
Just before mitosis, a dense band of microtubules appears around the phragmosome and the future division plane just below the plasma membrane. This preprophase band marks the equatorial plane of the future mitotic spindle as well as the future fusion sites for the new cell plate with the existing cell wall. It disappears as soon as the nuclear envelope breaks down and the mitotic spindle forms.

When mitosis is completed, the cell plate and new cell wall form starting from the center along the plane occupied by the phragmosome. The cell plate grows outwards until it fuses with the cell wall of the dividing cell at exactly the spots predicted by the preprophase band.

Chapter 8

Preprophase and Preprophase Band

Preprophase



Microtubule dynamics during preprophase and prophase in plant cell mitosis, modified from Donukshe et al. The images follow a tobacco BY-2 cell through the first stages of mitosis (c. 12 minutes). The growing ends of microtubules are shown in green (labeled with green fluorescent protein fused to the microtubule plus end binding protein EB1 of *Arabidopsis thaliana*). N = Nucleus, V = Vacuole, PPB = Preprophase band, MTN = Microtubule nucleation starts at the nuclear envelope, NEB = Nuclear envelope breakdown at the onset of prometaphase.

Preprophase is an additional phase during mitosis in plant cells that does not occur in other eukaryotes such as animals or fungi. It precedes prophase and is characterized by two distinct events:

- The formation of the preprophase band, a dense microtubule ring underneath the plasma membrane.
- The initiation of microtubule nucleation at the nuclear envelope.

Function of preprophase in the cell cycle

Plant cells are fixed with regards to their neighbor cells within the tissues they are growing in. In contrast to animals where certain cells can migrate within the embryo to form new tissues, the seedlings of higher plants grow entirely based on the orientation of cell division and subsequent elongation and differentiation of cells within their cell walls. Therefore, the accurate control of cell division planes and placement of the future cell wall in plant cells is crucial for the correct architecture of plant tissues and organs.

The preprophase stage of somatic plant cell mitosis serves to establish the precise location of the division plane and future cell wall before the cell enters prophase. This is achieved through the formation of a transient microtubule structure, the preprophase band, and a so far unknown mechanism by which the cell is able to "memorize" the position of the preprophase band to guide the new cell wall growing during cytokinesis to the correct location. In gametophyte tissues during the reproductive phase of the plant life cycle, cell division planes may be established without the use of a preprophase band.

In highly vacuolated plant cells, preprophase may be preceded by the formation of a phragmosome. The function of the phragmosome is to suspend the cell nucleus in the center of the cell in preparation for mitosis. If a phragmosome is visible, the preprophase band will appear at its outer edge.

Preprophase band formation

At the beginning of preprophase, the cortical microtubules of a plant cell disappear and aggregate into a dense ring underneath the plasma membrane. This preprophase band runs around the equatorial plane of the future mitotic spindle and marks the plane of cell division and future fusion site for the cell plate. It consists of microtubules and microfilaments (actin) and persists into prophase. Spindle formation occurs during prophase with the axis perpendicular to the plane surrounded by the preprophase band.

Microtubule nucleation

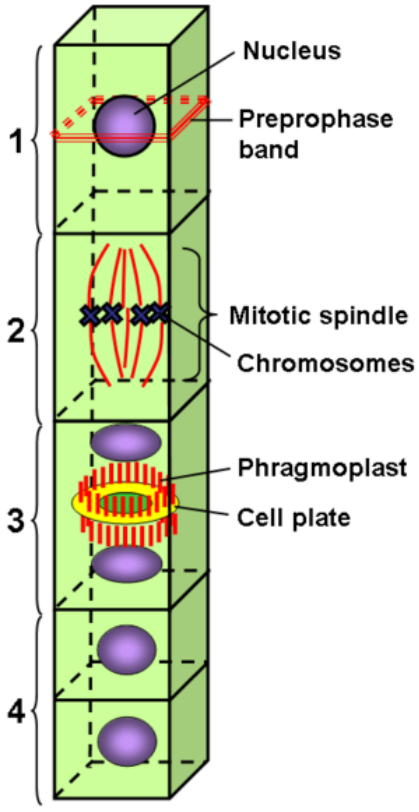
In contrast to animal cells, plant cells do not possess centrosomes to organize their mitotic spindles. Instead, the nuclear envelope acts as a microtubule organizing center (MTOC) for spindle formation during preprophase. The first sign is a clear, actin-free zone appearing around the nuclear envelope. This zone fills with microtubules nucleating on the surface of the nucleus. The preprophase spindle forms by self-assembly of these microtubules in the cytoplasm surrounding the nuclear envelope. It is reinforced through chromosome (kinetochore)-mediated spindle assembly after the nuclear envelope breaks down at the end of prophase.

Transition into prophase

During progression from preprophase into prophase, the randomly oriented microtubules align parallel along the nuclear surface according to the spindle axis. This structure is

called the *prophase spindle*. Triggered by nuclear envelope breakdown at the end of prophase, the preprophase band disappears and the prophase spindle matures into the metaphase spindle occupying the space of the former nucleus. Experiments with drugs destroying microfilaments indicate that actin may play a role in keeping the cellular "memory" of the position of the division plane after the preprophase band breaks down to direct cytokinesis in telophase.

Preprophase band



The preprophase band predicts the cell division plane: 1) Preprophase band formation during preprophase. 2) Metaphase spindle orients with the equator along the plane marked by preprophase band. 3) Phragmoplast and cell plate form along the plane marked by preprophase band. 4) The new cell wall of the daughter cells connects with the parent cell wall along the line of the former preprophase band location.

The **preprophase band** is a microtubule array found in plant cells that are about to undergo cell division and enter the preprophase stage of the plant cell cycle. Besides the phragmosome, it is the first microscopically visible sign that a plant cell is about to enter

mitosis. The preprophase band was first observed and described by Jeremy Pickett-Heaps and Donald Northcote at Cambridge University in 1966.

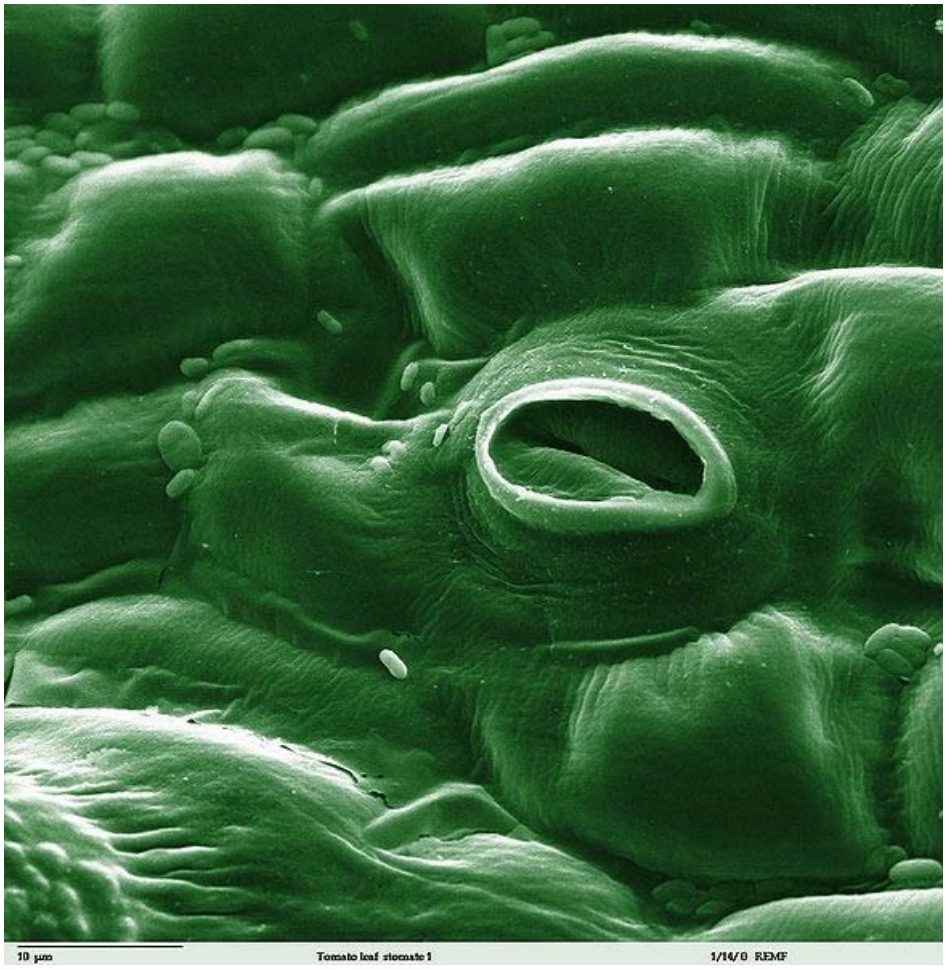
Just before mitosis starts, the preprophase band forms as a dense band of microtubules around the phragmosome and the future division plane just below the plasma membrane. It encircles the nucleus at the equatorial plane of the future mitotic spindle when dividing cells enter the G2 phase of the cell cycle after DNA replication is complete. The preprophase band consists mainly of microtubules and microfilaments (actin) and is generally 2-3 μm wide. When stained with fluorescent markers, it can be seen as two bright spots close to the cell wall on either side of the nucleus.

Plant cells lack centrosomes as microtubule organizing centers. Instead, the microtubules of the mitotic spindle aggregate on the nuclear surface and are reoriented to form the spindle at the end of prophase. The preprophase band also functions in properly orienting the mitotic spindle, and contributes to efficient spindle formation during prometaphase

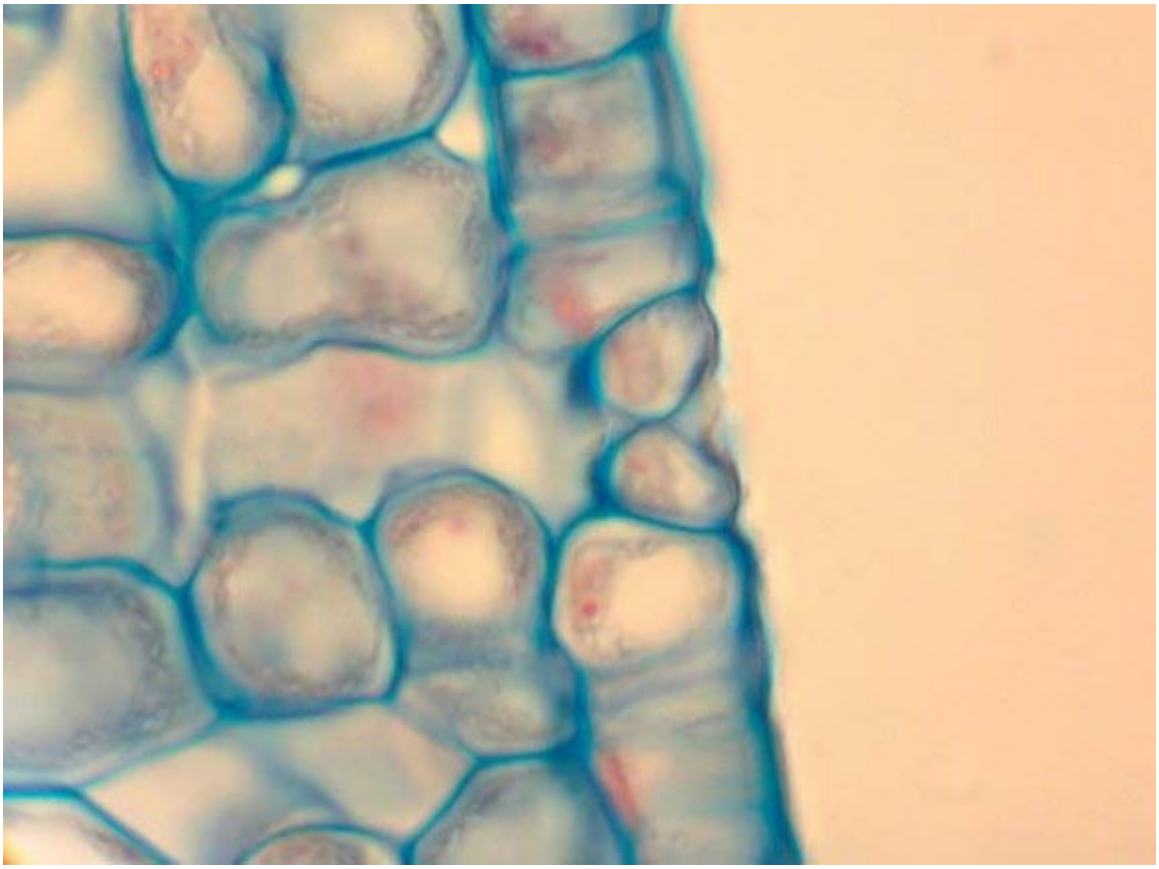
The preprophase band disappears as soon as the nuclear envelope breaks down and the mitotic spindle forms, leaving behind an actin-depleted zone. However, its position marks the future fusion sites for the new cell plate with the existing cell wall during telophase. When mitosis is completed, the cell plate and new cell wall form starting from the center along the plane occupied by the phragmosome. The cell plate grows outwards until it fuses with the cell wall of the dividing cell at exactly the spots predicted by the position of the preprophase band.

Chapter 9

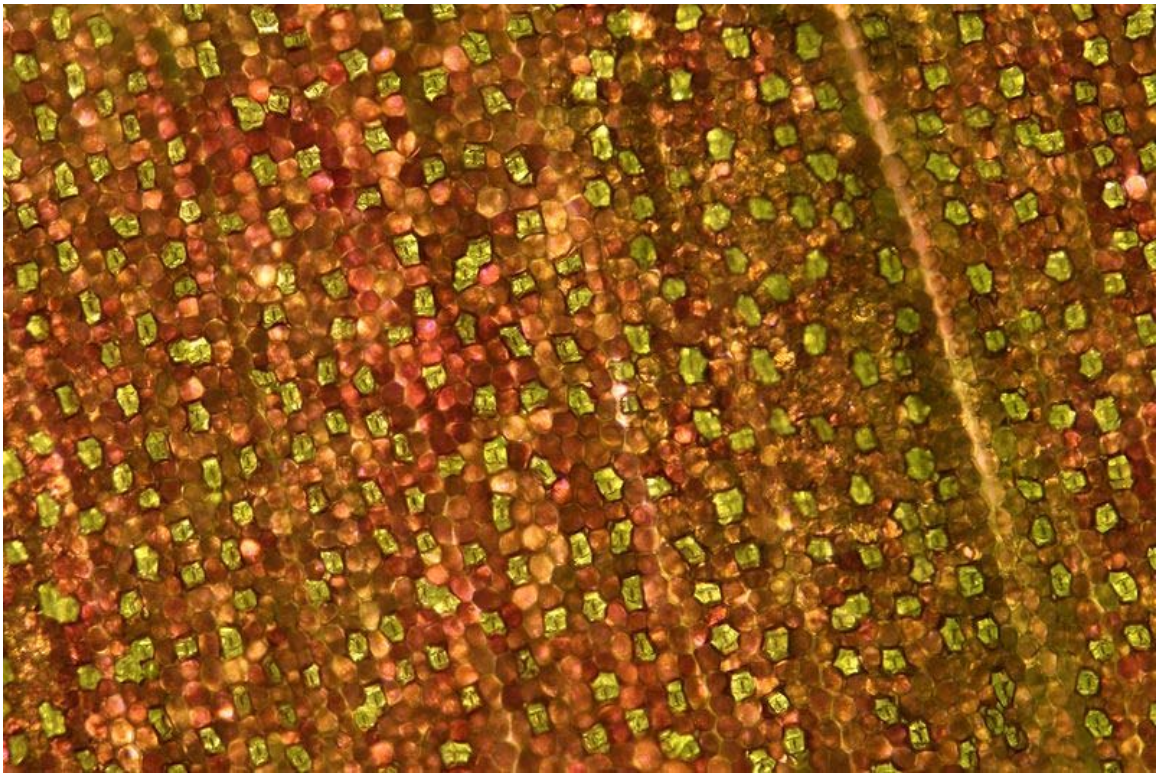
Stoma



Stoma in a tomato leaf shown via colorized scanning electron microscope image



A stoma in cross section



The underside of a leaf. In this species stomata appear green (due to chlorophyl) while the epidermal cells appear red due to additional pigmentation.

In botany, a **stoma** (also **stomate**; plural **stomata**) is a pore, found in the leaf and stem epidermis that is used for gas exchange. The pore is bordered by a pair of specialized parenchyma cells known as guard cells which are responsible for regulating the size of the opening. The term *stoma* is also used collectively to refer to an entire stomatal complex, both the pore itself and its accompanying guard cells. Air containing carbon dioxide and oxygen enters the plant through these openings where it is used in photosynthesis and respiration, respectively. Oxygen produced by photosynthesis in the **spongy layer** cells (parenchyma cells with pectin) of the leaf interior exits through these same openings. Also, water vapor is released into the atmosphere through these pores in a process called transpiration.

Stomata are present in the sporophyte generation of all land plant groups except liverworts. Dicotyledons usually have more stomata on the lower epidermis than the upper epidermis. Monocotyledons, on the other hand, usually have the same number of stomata on the two epidermes. In plants with floating leaves, stomata may be found only on the upper epidermis; submerged leaves may lack stomata entirely.

The word *stoma* derives from Greek στόμα, "mouth".

Function

Carbon gain and water loss

Carbon dioxide, a key reactant in photosynthesis, is present in the atmosphere at a concentration of about 384 ppm (as of March 2008). Most plants require the stomata to be open during daytime. The problem is that the air spaces in the leaf are saturated with water vapour, which exits the leaf through the stomata (this is known as transpiration). Therefore, plants cannot gain carbon dioxide without simultaneously losing water vapour.

Alternative approaches

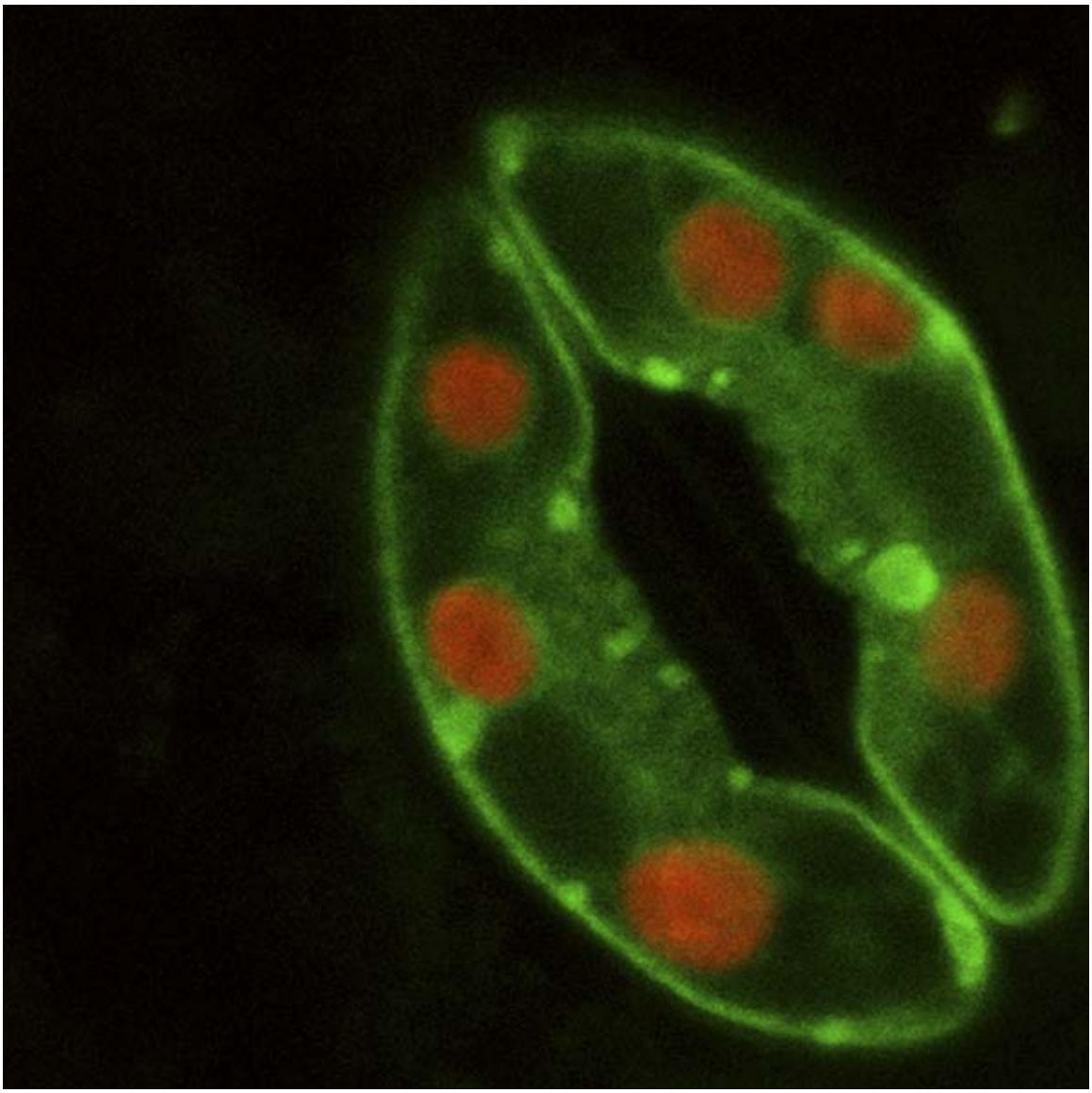
Ordinarily, carbon dioxide is fixed to ribulose-1,5-bisphosphate (BTAC) by the enzyme RuBisCO in mesophyll cells exposed directly to the air spaces inside the leaf. This exacerbates the carbon/water tradeoff for two reasons: first, Rubisco has a relatively low affinity for carbon dioxide and second, it fixes oxygen to RuBP, wasting energy and carbon in a process called photorespiration. For both of these reasons, Rubisco needs high carbon dioxide concentrations, which means high stomatal apertures and consequently high water loss.

However, plants possess another enzyme that can also fix carbon dioxide: PEP carboxylase or BTAC. This enzyme has high carbon dioxide affinity, so a given rate of carbon dioxide fixation can be achieved with less stomatal opening, and hence less water loss. However, the products of carbon fixation by PEPCase must be converted in an energy-intensive process to continue through the carbon reactions of photosynthesis. As a result, the PEPCase alternative is only preferable where water is more limiting but light — which provides the energy in this case — is plentiful, and/or where high temperatures increase the solubility of oxygen relative to that of carbon dioxide, magnifying Rubisco's oxygenation problem.

CAM plants

A group of mostly desert plants called "CAM" plants (Crassulacean acid metabolism, after the family Crassulaceae, which includes the species in which the CAM process was first discovered) open their stomata at night (when water evaporates more slowly from leaves for a given degree of stomatal opening), use PEPcarboxylase to fix carbon dioxide and store the products in large vacuoles. The following day, they close their stomata and release the carbon dioxide fixed the previous night into the presence of RuBisCO. This saturates RuBisCO with carbon dioxide, allowing minimal photorespiration. This approach, however, is severely limited by the capacity to store fixed carbon in the vacuoles, so it is preferable only when water is severely limiting.

Opening and closure



Confocal microscopy image of an *Arabidopsis thaliana* stoma showing two guard cells exhibiting fluorescence from green fluorescent protein and native chlorophyll (red)

However, most plants do not have the aforementioned facility and must therefore open and close their stomata during the daytime in response to changing conditions, such as light intensity, humidity, and carbon dioxide concentration. It is not entirely certain how these responses work. However, the basic mechanism involves regulation of osmotic pressure.

When conditions are conducive to stomatal opening (e.g., high light intensity and high humidity), a proton pump drives protons (H^+) from the guard cells. This means that the cells' electrical potential becomes increasingly negative. The negative potential opens potassium voltage - gated channels and so an uptake of potassium ions (K^+) occurs. To

maintain this internal negative voltage so that entry of potassium ions does not stop, negative ions balance the influx of potassium. In some cases chloride ions enter, while in other plants the organic ion malate is produced in guard cells. This increase in solute concentration lowers the water potential inside the cell, which results in water diffusing into the cell through osmosis. This increases the cell's volume and turgor pressure. Then, because of rings of cellulose microfibrils that prevent the width of the guard cells from swelling, and thus only allow the extra turgor pressure to elongate the guard cells, whose ends are held firmly in place by surrounding epidermal cells, the two guard cells lengthen by bowing apart from one another, creating an open pore through which gas can move.

When the roots begin to sense a water shortage in the soil, abscisic acid (ABA) is released. ABA binds to receptor proteins in the guard cells' plasma membrane and cytosol, which first raises the pH of the cytosol of the cells and cause the concentration of free Ca^{2+} to increase in the cytosol due to influx from outside the cell and release of Ca^{2+} from internal stores such as the endoplasmic reticulum and vacuoles. This causes the chloride (Cl^-) and inorganic ions to exit the cells. Secondly, this stops the uptake of any further K^+ into the cells and subsequently the loss of K^+. The loss of these solutes causes an increase in water potential, which results in water diffusing back out of the cell by osmosis. This makes the cell flaccid, which results in the closing of the stomatal pores.

However there is a another hypothesis--starch and sugar hypothesis. During the day, plant experience photosynthesis and cause partial pressure of Carbon Dioxide to drop and cause the pH value to rise. This cause the starch(almost insoluble) to convert to sugar and dissolve in the cytoplasm, causing the water potential in guard cell to decrease and draw in water from the neighboring cell. The guard cell become turgid and the stomata expend. When there is no light, photosynthesis stop and partial pressure of carbon dioxide increase, causing the pH value in guard cell to rise. Soluble sugar is converted to insoluble starch and the water potential in guard cell increase and water is drown out of the guard cell and the stomata close. Yet this hypothesis has some flaw. For example, the CAM plant undergo photosynthesis in day, but it's stomata open in the night for gases exchange and close in the day to avoid great lost of water.

Interestingly, guard cells have more chloroplasts than the other epidermal cells from which guard cells are derived. Their function is controversial.

Inferring stomatal behavior from gas exchange

The degree of stomatal resistance can be determined by measuring leaf gas exchange of a leaf. The transpiration rate is dependent on the diffusion resistance provided by the stomatal pores, and also on the humidity gradient between the leaf's internal air spaces and the outside air. Stomatal resistance (or its inverse, stomatal conductance) can therefore be calculated from the transpiration rate and humidity gradient. This allows scientists to investigate how stomata respond to changes in environmental conditions, such as light intensity and concentrations of gases such as water vapor, carbon dioxide, and ozone. Evaporation (E) can be calculated as;

$E = (e_i - e_a) / P\, r$

where *ei* and *ea* = partial pressures of water in the leaf and in the ambient air; P = atmospheric pressure; and r = stomatal resistance. The inverse of r is conductance to water vapor (g), so the equation can be rearranged to;

$E = (e_i - e_a)\, g / P$

and solved for g;

$g = EP / (e_i - e_a)$

The rate of evaporation from a leaf can be determined using a photosynthesis system. These scientific instruments measure the amount of water vapour leaving the leaf and the vapor pressure of the ambient air. Photosynthetic systems may calculate water use efficiency (A/E), stomatal conductance (gs), intrinsic water use efficiency (A/gs), and sub-stomatal CO_2 concentration (Ci). These scientific instruments are commonly used by plant physiologists to measure CO_2 uptake and thus measure photosynthetic rate.

Evolution

The fossil record has little to say about the evolution of stomata. They may have evolved by the modification of conceptacles from plants' alga-like ancestors. It is clear, however, that the evolution of stomata must have happened at the same time as the waxy cuticle was evolving - these two traits together constituted a major advantage for primitive terrestrial plants.

Development

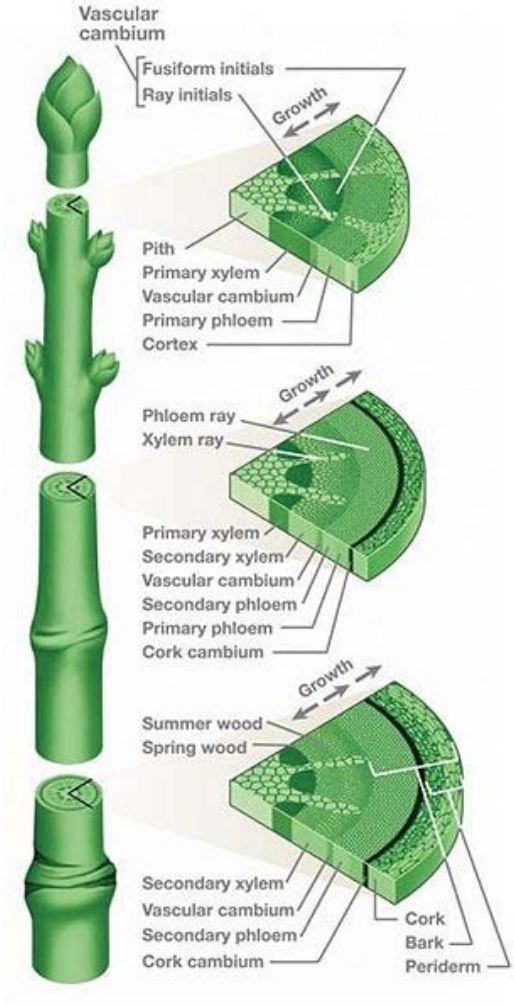
There are three major epidermal cell types which all ultimately derive from the L1 tissue layer of the shoot apical meristem, called protodermal cells: trichomes, pavement cells and guard cells, all of which are arranged in a nonrandom fashion. An asymmetrical cell division occurs in protodermal cells resulting in one large cell that is fated to become a pavement cell and a smaller cell called a meristemoid that will eventually differentiate into the guard cells that surround a stoma. This meristemoid then divides asymmetrically one to three times before differentiating into a guard mother cell. The guard mother cell then makes one symmetrical division, which forms a pair of guard cells.

Stomata as pathogenic pathways

Stomata are an obvious hole in the leaf by which, as was presumed for a while, pathogens can enter unchallenged. However, it has been recently shown that stomata do in fact sense the presence of some, if not all, pathogens. However, with the virulent bacteria applied to *Arabidopsis* plant leaves in the experiment, the bacteria released the chemical coronatine, which forced the stomata open again within a few hours.

Chapter 10

Xylem



Multiple cross sections of a flowering plant stem showing primary and secondary **xylem** and phloem

In vascular plants, **xylem** is one of the two types of transport tissue, phloem being the other. The word "xylem" is derived from classical Greek ξυλον (*xylon*), "wood", and indeed the best-known xylem tissue is wood, though it is found throughout the plant. Its basic function is to transport water but it also transports some nutrients through the plant.

Structure

The most distinctive xylem cells are the long tracheary elements that transport water. Tracheids and vessel elements are distinguished by the multitude of spots that occur on the vessel elements. In these spots both outer and inner walls are missing. The vessel elements are connected to each other in the ends, forming continuous tubes that are called *vessels*.

Xylem also contains other cell types, for example parenchyma.

Xylem can be found:

- in vascular bundles, present in non-woody plants and non-woody parts of plants with wood
- in secondary xylem, laid down by a meristem called the vascular cambium in woody plants
- as part of a stelar arrangement not divided into bundles, as in many ferns

In transitional stages of plants with secondary growth, the first two categories are not mutually exclusive, although usually a vascular bundle will contain *primary xylem* only.

The branching pattern exhibited by xylem follows Murray's law.

Primary and secondary xylem

Primary xylem is the xylem that is formed during primary growth from procambium. It includes protoxylem and metaxylem. Metaxylem develops after the protoxylem but before secondary xylem. It is distinguished by wider vessels and tracheids. Xylem development occurs in a number of patterns, which vary in the relative position of the protoxylem and metaxylem, e.g. endarch in which the protoxylem is towards the centre of the stem or root, or exarch in which the metaxylem is towards the centre.

Secondary xylem is the xylem that is formed during secondary growth from vascular cambium. Although secondary xylem is also found in members of the "gymnosperm" groups Gnetophyta and Ginkgophyta and to a lesser extent in members of the Cycadophyta, the two main groups in which secondary xylem can be found are:

1. conifers (*Coniferae*): there are some six hundred species of conifers. All species have secondary xylem, which is relatively uniform in structure throughout this group. Many conifers become tall trees: the secondary xylem of such trees is marketed as **softwood**.
2. angiosperms (*Angiospermae*): there are some quarter of a million to four hundred thousand species of angiosperms. Within this group secondary xylem has not been found in the monocots. In the remainder of the angiosperms, this secondary xylem may or may not be present; this may vary even within a species, depending on growing circumstances. In view of the size of this group, it will be no surprise that

no absolutes apply to the structure of secondary xylem within the angiosperms. Many non-monocot angiosperms become trees, and the secondary xylem of these is marketed as **hardwood**.

Main function - upwards water transport

The xylem transports water and soluble mineral nutrients from the roots throughout the plant. It is also used to replace water lost during transpiration and photosynthesis. Xylem sap consists mainly of water and inorganic ions, although it can contain a number of organic chemicals as well. This transport is not powered by energy spent by the tracheary elements themselves, which are dead by maturity and no longer have living contents. Two phenomena cause xylem sap to flow:

- **Transpirational pull**: the most important cause of xylem sap flow is the evaporation of water from the surfaces of mesophyll cells to the atmosphere. This transpiration causes millions of minute menisci to form in the mesophyll cell wall. The resulting surface tension causes a negative pressure or tension in the xylem that pulls the water from the roots and soil.
- **Root pressure**: If the water potential of the root cells is more negative than that of the soil, usually due to high concentrations of solute, water can move by osmosis into the root from the soil. This causes a positive pressure that forces sap up the xylem towards the leaves. In some circumstances, the sap will be forced from the leaf through a hydathode in a phenomenon known as guttation. Root pressure is highest in the morning before the stomata open and allow transpiration to begin. Different plant species can have different root pressures even in a similar environment; examples include up to 145 kPa in *Vitis riparia* but around zero in *Celastrus orbiculatus*.

The primary force that creates the capillary action movement of water upwards in plants is the adhesion between the water and the surface of the xylem conduits. Capillary action provides the force that establishes an equilibrium configuration, balancing gravity. When transpiration removes water at the top, the flow is needed to return to the equilibrium.

Transpirational pull results from the evaporation of water from the surfaces of cells in the leaves. This evaporation causes the surface of the water to recess into the pores of the cell wall. By capillary action, the water forms concave menisci inside the pores. The high surface tension of water pulls the concavity outwards, generating enough force to lift water as high as a hundred meters from ground level to a tree's highest branches.

Transpirational pull requires that the vessels transporting the water are very small in diameter, otherwise cavitation would break the water column. And as water evaporates from leaves, more is drawn up through the plant to replace it. When the water pressure within the xylem reaches extreme levels due to low water input from the roots (if, for example, the soil is dry), then the gases come out of solution and form a bubble - an embolism forms, which will spread quickly to other adjacent cells, unless *bordered pits*

are present (these have a plug-like structure called a torus, that seals off the opening between adjacent cells and stops the embolism from spreading).

Cohesion-tension theory

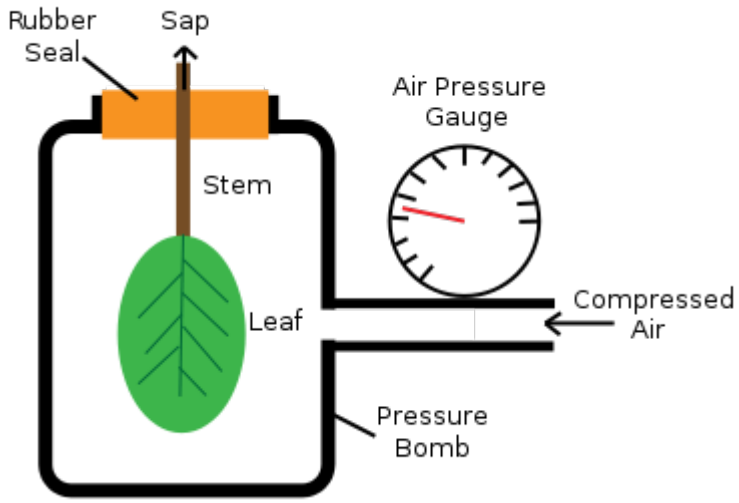
The *cohesion-tension theory* is a theory of intermolecular attraction commonly observed in the process of water traveling upwards (against the force of gravity) through the xylem of plants which was put forward by John Joly and Henry Horatio Dixon. Despite numerous objections, this is the most widely accepted theory for the transport of water through a plant's vascular system based on the classical research of Dixon-Joly (1894) , Askenasy (1895) , and Dixon (1914,1924) .

Water is a polar molecule due to the high electronegativity of the oxygen atom, which is an uncommon molecular configuration whereby the oxygen atom has two lone pairs of electrons. When two water molecules approach one another they form a hydrogen bond. The negatively charged oxygen atom of one water molecule forms a hydrogen bond with a positively charged hydrogen atom in another water molecule. This attractive force has several manifestations. Firstly, it causes water to be liquid at room temperature, while other lightweight molecules would be in a gaseous phase. Secondly, it (along with other intermolecular forces) is one of the principal factors responsible for the occurrence of surface tension in liquid water. This attractive force between molecules allows plants to draw water from the root (via osmosis) and then through the xylem to the leaf where photosynthesis converts water and carbon dioxide into glucose.

Water is constantly lost by transpiration in the leaf. When one water molecule is lost another is pulled along by the processes of cohesion and adhesion. Transpiration pull, utilizing capillary action and the inherent surface tension of water, is the primary mechanism of water movement in plants. However, it is not the only mechanism involved. Any use of water in leaves produces forces water to move into them.

Transpiration in leaves creates tension (negative pressure) in the mesophyll cells. Because of this tension, water is literally being pulled up from the roots into the leaves, helped by cohesion (the pull between individual water molecules, due to hydrogen bonds) and adhesion (the stickiness between water molecules and the hydrophilic cell walls of plants). This mechanism of water flow works because of water potential (water flows from high to low potential), and the rules of simple diffusion.

Measurement of pressure



A diagram showing the setup of a pressure bomb

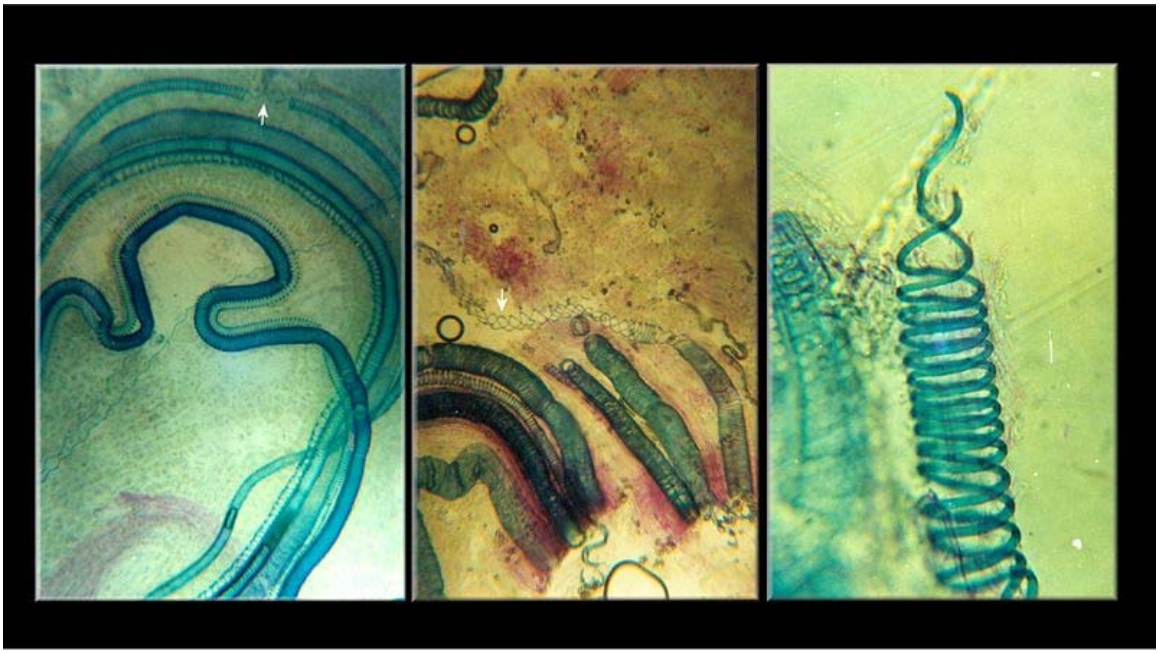
Until recently, the negative pressure (suction) of transpirational pull could only be measured indirectly, by applying external pressure with a pressure bomb to counteract it. When the technology to perform direct measurements with a pressure probe was developed, there was initially some controversy about whether the classic theory was correct, because some workers were unable to demonstrate negative pressures. More recent measurements do tend to validate the classic theory, for the most part. Xylem transport is driven by a combination of transpirational pull from above and root pressure from below, which makes the interpretation of measurements more complicated.

Evolution

Xylem appeared early in the history of terrestrial plant life. Fossil plants with anatomically preserved xylem are known from the Silurian (more than 400 million years ago), and trace fossils resembling individual xylem cells may be found in earlier Ordovician rocks. The earliest true and recognizable xylem consists of tracheids with a helical-annular reinforcing layer added to the cell wall. This is the only type of xylem found in the earliest vascular plants, and this type of cell continues to be found in the *protoxylem* (first-formed xylem) of all living groups of plants. Several groups of plants later developed pitted tracheid cells, it seems, through convergent evolution. In living plants, pitted tracheids do not appear in development until the maturation of the *metaxylem* (following the *protoxylem*).

In most plants, pitted tracheids function as the primary transport cells. The other type of tracheary element, besides the tracheid, is the vessel element. Vessel elements are joined by perforations into vessels. In vessels, water travels by *bulk flow*, as in a pipe, rather than by diffusion through cell membranes. The presence of vessels in xylem has been considered to be one of the key innovations that led to the success of the angiosperms.

However, the occurrence of vessel elements is not restricted to angiosperms, and they are absent in some archaic or "basal" lineages of the angiosperms: (e.g., Amborellaceae, Tetracentraceae, Trochodendraceae, and Winteraceae), and their secondary xylem is described by Arthur Cronquist as "primitively vesselless". Cronquist considered the vessels of *Gnetum* to be convergent with those of angiosperms. Whether the absence of vessels in basal angiosperms is a primitive condition is contested, the alternative hypothesis states that vessel elements originated in a precursor to the angiosperms and were subsequently lost.



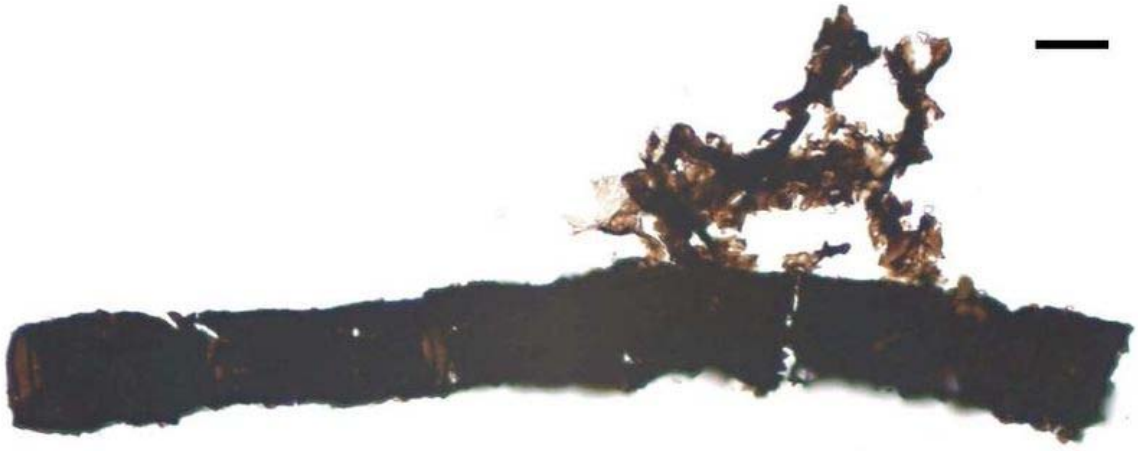
Photos showing xylem elements in the shoot of a fig tree (*Ficus alba*): crushed in hydrochloric acid, between slides and cover slips.

To photosynthesise, plants must absorb CO_2 from the atmosphere. However, this comes at a price: while stomata are open to allow CO_2 to enter, water can evaporate. Water is lost much faster than CO_2 is absorbed, so plants need to replace it, and have developed systems to transport water from the moist soil to the site of photosynthesis. Early plants sucked water between the walls of their cells, then evolved the ability to control water loss (and CO_2 acquisition) through the use of stomata. Specialised water transport tissues soon evolved in the form of hydroids, tracheids, then secondary xylem, followed by an endodermis and ultimately vessels.

The high CO_2 levels of Silurian-Devonian times, when plants were first colonising land, meant that the need for water was relatively low . As CO_2 was withdrawn from the atmosphere by plants, more water was lost in its capture, and more elegant transport mechanisms evolved. As water transport mechanisms, and waterproof cuticles, evolved, plants could survive without being continually covered by a film of water. This transition from poikilohydry to homoiohydry opened up new potential for colonisation. Plants then needed a robust internal structure that held long narrow channels for transporting water

from the soil to all the different parts of the above-soil plant, especially to the parts where photosynthesis occurred.

During the Silurian, CO_2 was readily available, so little water needed expending to acquire it. By the end of the Carboniferous, when CO_2 levels had lowered to something approaching today's, around 17 times more water was lost per unit of CO_2 uptake. However, even in these "easy" early days, water was at a premium, and had to be transported to parts of the plant from the wet soil to avoid desiccation. This early water transport took advantage of the **cohesion-tension** mechanism inherent in water. Water has a tendency to diffuse to areas that are drier, and this process is accelerated when water can be wicked along a fabric with small spaces. In small passages, such as that between the plant cell walls (or in tracheids), a column of water behaves like rubber – when molecules evaporate from one end, they literally pull the molecules behind them along the channels. Therefore transpiration alone provided the driving force for water transport in early plants. However, without dedicated transport vessels, the cohesion-tension mechanism cannot transport water more than about 2 cm, severely limiting the size of the earliest plants. This process demands a steady supply of water from one end, to maintain the chains; to avoid exhausing it, plants developed a waterproof cuticle. Early cuticle may not have had pores but did not cover the entire plant surface, so that gas exchange could continue. However, dehydration at times was inevitable; early plants cope with this by having a lot of water stored between their cell walls, and when it comes to it sticking out the tough times by putting life "on hold" until more water is supplied.



A banded tube from the late Silurian/early Devonian. The bands are difficult to see on this specimen, as an opaque carbonaceous coating conceals much of the tube. Bands are just visible in places on the left half of the image

To be free from the constraints of small size and constant moisture that the parenchymatic transport system inflicted, plants needed a more efficient water transport system. During the early Silurian, they developed specialized cells, which were lignified (or bore similar chemical compounds) to avoid implosion; this process coincided with cell death, allowing their innards to be emptied and water to be passed through them. These wider, dead, empty cells were a million times more conductive than the inter-cell

method, giving the potential for transport over longer distances, and higher CO_2 diffusion rates.

The first macrofossils to bear water-transport tubes *in situ* are the early Devonian pretracheophytes *Aglaophyton* and *Horneophyton*, which have structures very similar to the **hydroids** of modern mosses. Plants continued to innovate new ways of reducing the resistance to flow within their cells, thereby increasing the efficiency of their water transport. Bands on the walls of tubes, in fact apparent from the early Silurian onwards, are an early improvisation to aid the easy flow of water. Banded tubes, as well as tubes with pitted ornamentation on their walls, were lignified and, when they form single celled conduits, are considered to be **tracheids**. These, the "next generation" of transport cell design, have a more rigid structure than hydroids, allowing them to cope with higher levels of water pressure. Tracheids may have a single evolutionary origin, possibly within the hornworts, uniting all tracheophytes (but they may have evolved more than once).

Water transport requires regulation, and dynamic control is provided by stomata. By adjusting the amount of gas exchange, they can restrict the amount of water lost through transpiration. This is an important role where water supply is not constant, and indeed stomata appear to have evolved before tracheids, being present in the non-vascular hornworts.

An endodermis probably evolved during the Silu-Devonian, but the first fossil evidence for such a structure is Carboniferous. This structure in the roots covers the water transport tissue and regulates ion exchange (and prevents unwanted pathogens etc. from entering the water transport system). The endodermis can also provide an upwards pressure, forcing water out of the roots when transpiration is not enough of a driver.

Once plants had evolved this level of controlled water transport, they were truly homoiohydric, able to extract water from their environment through root-like organs rather than relying on a film of surface moisture, enabling them to grow to much greater size. As a result of their independence from their surroundings, they lost their ability to survive desiccation – a costly trait to retain.

During the Devonian, maximum xylem diameter increased with time, with the minimum diameter remaining pretty constant. By the middle Devonian, the tracheid diameter of some plant lineages had plateaued. Wider tracheids allow water to be transported faster, but the overall transport rate depends also on the overall cross-sectional area of the xylem bundle itself. The increase in vascular bundle thickness further seems to correlate with the width of plant axes, and plant height; it is also closely related to the appearance of leaves and increased stomatal density, both of which would increase the demand for water.

While wider tracheids with robust walls make it possible to achieve higher water transport pressures, this increases the problem of cavitation. Cavitation occurs when a bubble of air forms within a vessel, breaking the bonds between chains of water molecules and preventing them from pulling more water up with their cohesive tension.

A tracheid, once cavitated, cannot have its embolism removed and return to service (except in a few advanced angiosperms which have developed a mechanism of doing so). Therefore it is well worth plants' while to avoid cavitation occurring. For this reason, pits in tracheid walls have very small diameters, to prevent air entering and allowing bubbles to nucleate. Freeze-thaw cycles are a major cause of cavitation. Damage to a tracheid's wall almost inevitably leads to air leaking in and cavitation, hence the importance of many tracheids working in parallel.

Cavitation is hard to avoid, but once it has occurred plants have a range of mechanisms to contain the damage. Small pits link adjacent conduits to allow fluid to flow between them, but not air – although ironically these pits, which prevent the spread of embolisms, are also a major cause of them. These pitted surfaces further reduce the flow of water through the xylem by as much as 30%. Conifers, by the Jurassic, developed an ingenious improvement, using valve-like structures to isolate cavitated elements. These torus-margo structures have a blob floating in the middle of a donut; when one side depressurises the blob is sucked into the torus and blocks further flow. Other plants simply accept cavitation; for instance, oaks grow a ring of wide vessels at the start of each spring, none of which survive the winter frosts. Maples use root pressure each spring to force sap upwards from the roots, squeezing out any air bubbles.

Growing to height also employed another trait of tracheids – the support offered by their lignified walls. Defunct tracheids were retained to form a strong, woody stem, produced in most instances by a secondary xylem. However, in early plants, tracheids were too mechanically vulnerable, and retained a central position, with a layer of tough sclerenchyma on the outer rim of the stems. Even when tracheids do take a structural role, they are supported by sclerenchymatic tissue.

Tracheids end with walls, which impose a great deal of resistance on flow; vessel members have perforated end walls, and are arranged in series to operate as if they were one continuous vessel. The function of end walls, which were the default state in the Devonian, was probably to avoid embolisms. An embolism is where an air bubble is created in a tracheid. This may happen as a result of freezing, or by gases dissolving out of solution. Once an embolism is formed, it usually cannot be removed; the affected cell cannot pull water up, and is rendered useless.

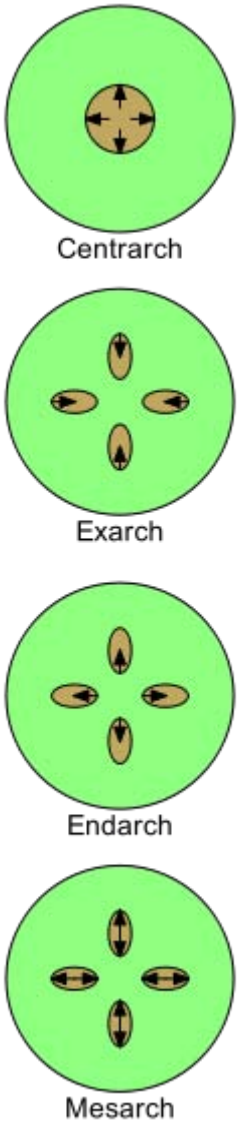
End walls excluded, the tracheids of prevascular plants were able to operate under the same hydraulic conductivity as those of the first vascular plant, *Cooksonia*.

The size of tracheids is limited as they comprise a single cell; this limits their length, which in turn limits their maximum useful diameter to 80 μm. Conductivity grows with the fourth power of diameter, so increased diameter has huge rewards; **vessel elements**, consisting of a number of cells, joined at their ends, overcame this limit and allowed larger tubes to form, reaching diameters of up to 500 μm, and lengths of up to 10 m.

Vessels first evolved during the dry, low CO_2 periods of the late Permian, in the horsetails, ferns and Selaginellales independently, and later appeared in the mid

Cretaceous in angiosperms and gnetophytes. Vessels allow the same cross-sectional area of wood to transport around a hundred times more water than tracheids! This allowed plants to fill more of their stems with structural fibres, and also opened a new niche to vines, which could transport water without being as thick as the tree they grew on. Despite these advantages, tracheid-based wood is a lot lighter, thus cheaper to make, as vessels need to be much more reinforced to avoid cavitation.

Development



Patterns of xylem development: xylem in brown; arrows show direction of development from protoxylem to metaxylem

Xylem development can be described by four terms: **centrarch**, **exarch**, **endarch** and **mesarch**. As it develops in young plants, its nature changes from *protoxylem* to

metaxylem (i.e. from *first xylem* to *after xylem*). The patterns in which protoxylem and metaxylem are arranged is important in the study of plant morphology.

Protoxylem and metaxylem

As a young vascular plant grows, one or more strands of primary xylem form in its stems and roots. The first xylem to develop is called 'protoxylem'. In appearance protoxylem is usually distinguished by narrower vessels formed of smaller cells. Some of these cells have walls which contain thickenings in the form of rings or helices. Functionally, protoxylem can extend: the cells are able to grow in size and develop while a stem or root is elongating. Later, 'metaxylem' develops in the strands of xylem. Metaxylem vessels and cells are usually larger; the cells have thickenings which are typically either in the form of ladderlike transverse bars (scalariform) or continuous sheets except for holes or pits (pitted). Functionally, metaxylem completes its development after elongation ceases when the cells no longer need to be able grow in size.

Patterns of protoxylem and metaxylem

There are four main patterns to the arrangement of protoxylem and metaxylem in stems and roots.

Centrarch refers to the case in which the primary xylem forms a single cylinder in the centre of the stem and develops from the centre outwards. The protoxylem is thus found in the central core and the metaxylem in a cylinder around it. This pattern was common in early land plants, such as "rhyniophytes".

The other three terms are used where there is more than one strand of primary xylem.

Exarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the outside inwards towards the centre, i.e. centripetally. The metaxylem is thus closest to the centre of the stem or root and the protoxylem closest to the periphery. The roots of vascular plants are normally considered to have exarch development.

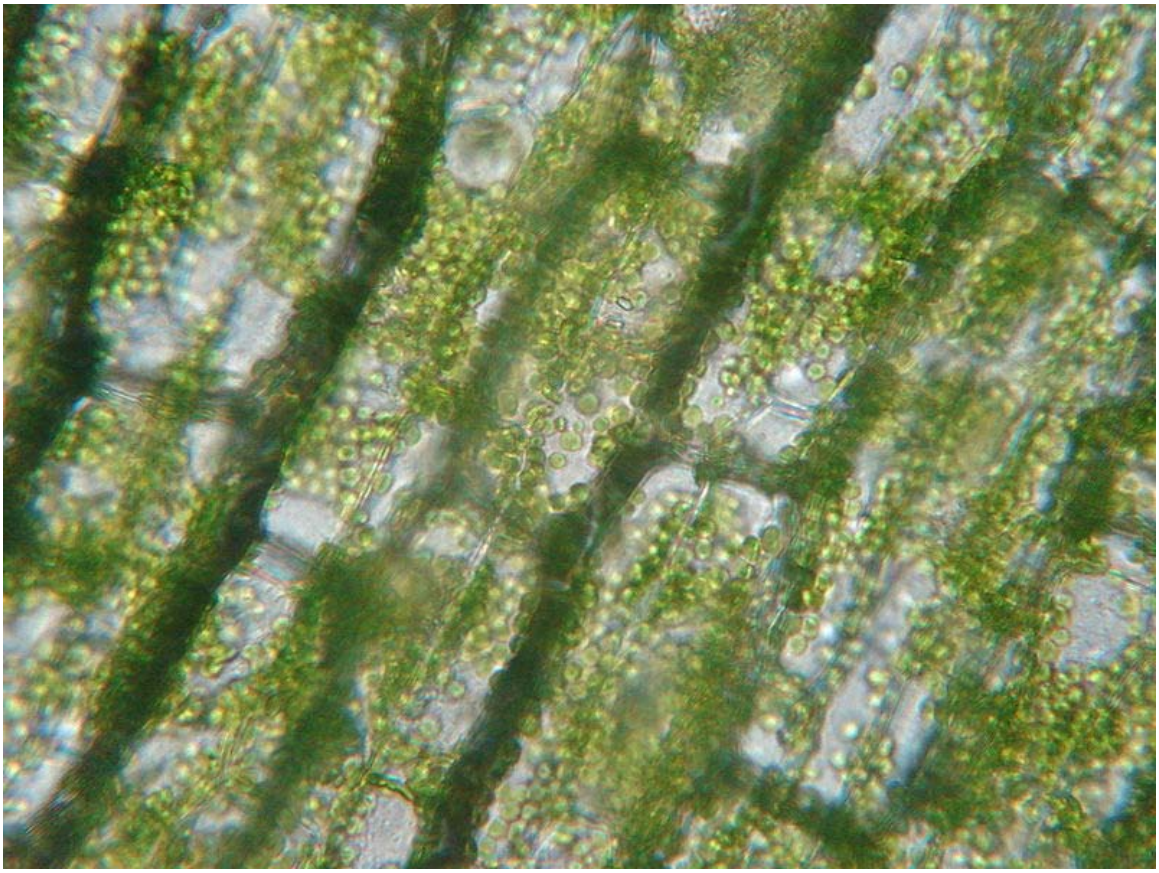
Endarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the inside outwards towards the periphery, i.e. centrifugally. The protoxylem is thus closest to the centre of the stem or root and the metaxylem closest to the periphery. The stems of seed plants typically have endarch development.

Mesarch is used when there is more than one strand of primary xylem in a stem or root, and the xylem develops from the middle of a strand in both directions. The metaxylem is thus on both the peripheral and central sides of the strand with the protoxylem between the metaxylem (possibly surrounded by it). The leaves and stems of many ferns have mesarch development.

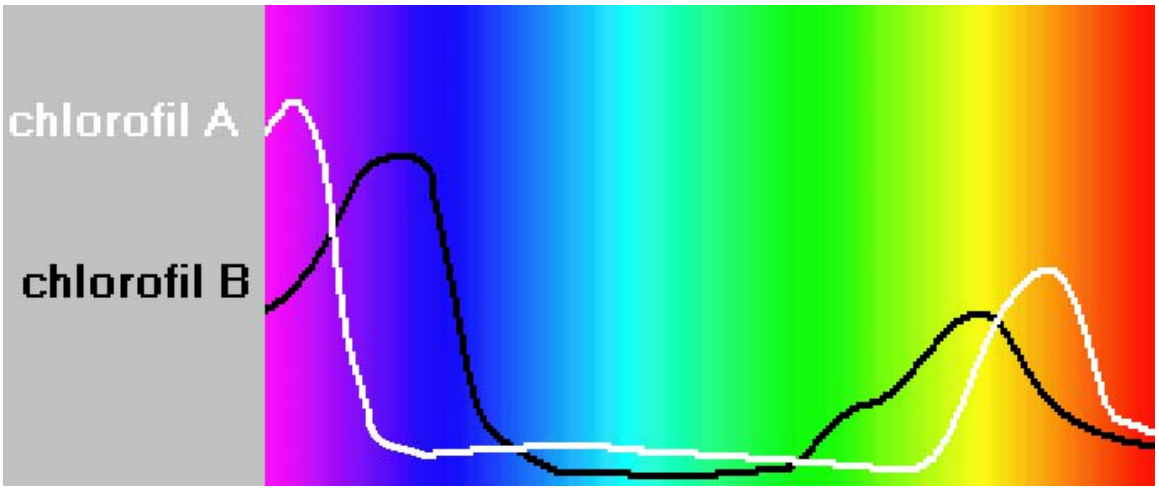
Chapter 11

Chlorophyll

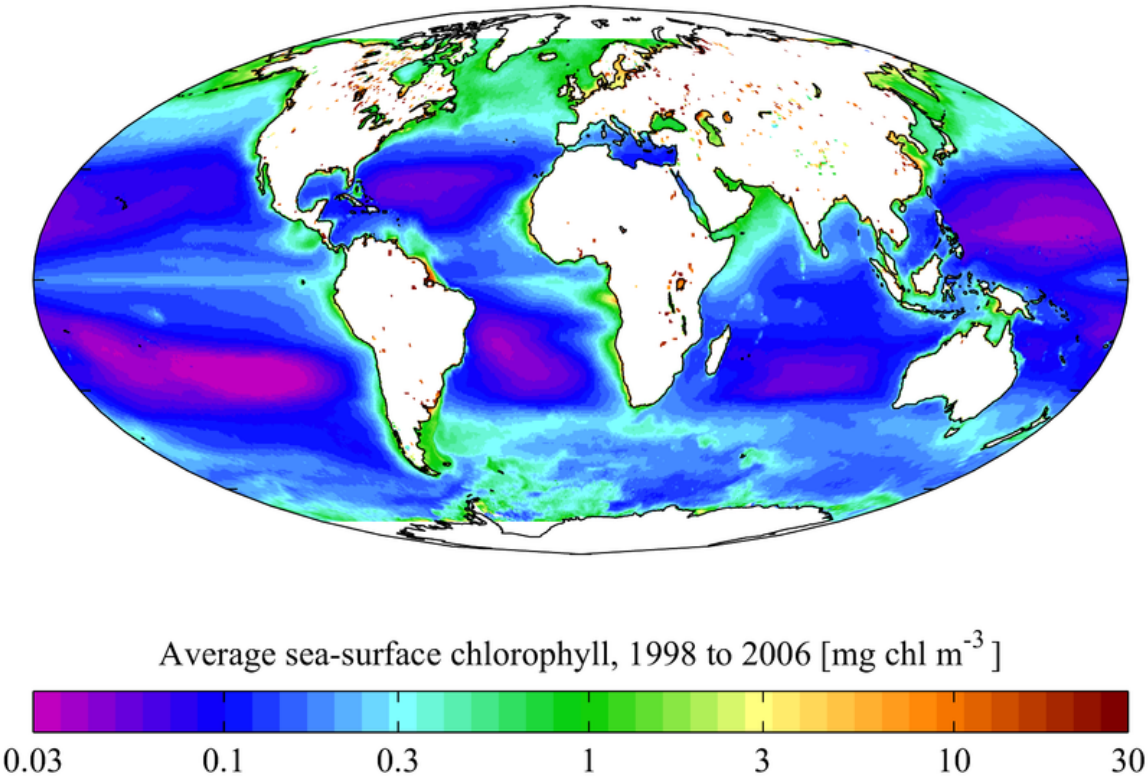
Chlorophyll gives leaves their green color and absorbs light that is used in photosynthesis.



Chlorophyll is found in high concentrations in chloroplasts of plant cells.



Absorption maxima of chlorophylls against the spectrum of white light.



SeaWiFS-derived average sea surface **chlorophyll** for the period 1998 to 2006.

Chlorophyll (also **chlorophyl**) is a green pigment found in almost all plants, algae, and cyanobacteria. Its name is derived from the Greek words *chloros* ("green") and *phyllon* ("leaf"). Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to obtain energy from light. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. However, it is a poor absorber of green and near-green portions of the spectrum; hence the green color of chlorophyll-containing tissues. Chlorophyll was first isolated by Joseph Bienaimé Caventou and Pierre Joseph Pelletier in 1817.

Chlorophyll and photosynthesis

Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light.

Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts. In these complexes, chlorophyll serves two primary functions. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystems.

The two currently accepted photosystem units are Photosystem II and Photosystem I, which have their own distinct reaction center chlorophylls, named P680 and P700,

respectively. These pigments are named after the wavelength (in nanometers) of their red-peak absorption maximum. The identity, function and spectral properties of the types of chlorophyll in each photosystem are distinct and determined by each other and the protein structure surrounding them. Once extracted from the protein into a solvent (such as acetone or methanol), these chlorophyll pigments can be separated in a simple paper chromatography experiment and, based on the number of polar groups between chlorophyll a and chlorophyll b, will chemically separate out on the paper.

The function of the reaction center chlorophyll is to use the energy absorbed by and transferred to it from the other chlorophyll pigments in the photosystems to undergo a charge separation, a specific redox reaction in which the chlorophyll donates an electron into a series of molecular intermediates called an electron transport chain. The charged reaction center chlorophyll ($P680^+$) is then reduced back to its ground state by accepting an electron. In Photosystem II, the electron that reduces $P680^+$ ultimately comes from the oxidation of water into O_2 and H^+ through several intermediates. This reaction is how photosynthetic organisms such as plants produce O_2 gas, and is the source for practically all the O_2 in Earth's atmosphere. Photosystem I typically works in series with Photosystem II; thus the $P700^+$ of Photosystem I is usually reduced, via many intermediates in the thylakoid membrane, by electrons ultimately from Photosystem II. Electron transfer reactions in the thylakoid membranes are complex, however, and the source of electrons used to reduce $P700^+$ can vary.

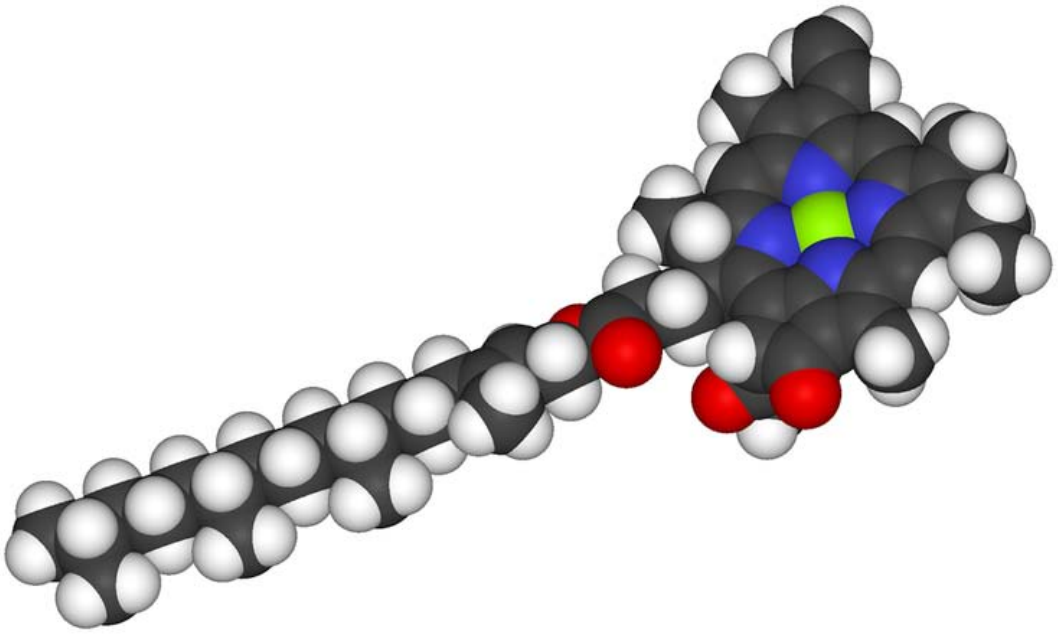
The electron flow produced by the reaction center chlorophyll pigments is used to shuttle H^+ ions across the thylakoid membrane, setting up a chemiosmotic potential used mainly to produce ATP chemical energy; and those electrons ultimately reduce $NADP^+$ to NADPH, a universal reductant used to reduce CO_2 into sugars as well as for other biosynthetic reductions.

Reaction center chlorophyll–protein complexes are capable of directly absorbing light and performing charge separation events without other chlorophyll pigments, but the absorption cross section (the likelihood of absorbing a photon under a given light intensity) is small. Thus, the remaining chlorophylls in the photosystem and antenna pigment protein complexes associated with the photosystems all cooperatively absorb and funnel light energy to the reaction center. Besides chlorophyll *a*, there are other pigments, called accessory pigments, which occur in these pigment–protein antenna complexes.

There is as yet no satisfactory scientific explanation as to why chlorophyll has evolved to "ignore" green and near-green light, which are a major part of the visible spectrum.

A green sea slug, *Elysia chlorotica*, has been found to use the chlorophyll it has eaten to perform photosynthesis for itself. This process is known as kleptoplasty, and no other animal has been found to have this ability.

Chemical structure



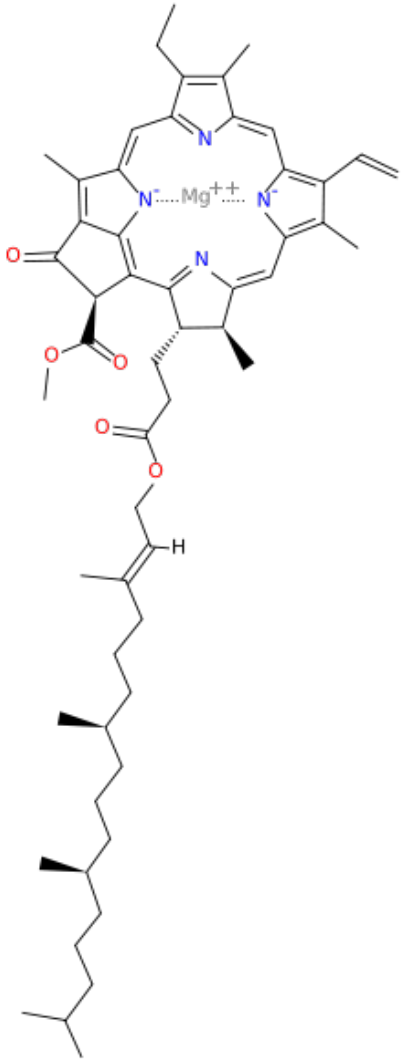
Space-filling model of the chlorophyll a molecule

Chlorophyll is a chlorin pigment, which is structurally similar to and produced through the same metabolic pathway as other porphyrin pigments such as heme. At the center of the chlorin ring is a magnesium ion. For the structures depicted here, some of the ligands attached to the Mg^{2+} center are omitted for clarity. The chlorin ring can have several different side chains, usually including a long phytol chain. There are a few different forms that occur naturally, but the most widely distributed form in terrestrial plants is chlorophyll *a*. The general structure of chlorophyll *a* was elucidated by Hans Fischer in 1940, and by 1960, when most of the stereochemistry of chlorophyll *a* was known, Robert Burns Woodward published a total synthesis of the molecule as then known. In 1967, the last remaining stereochemical elucidation was completed by Ian Fleming, and in 1990 Woodward and co-authors published an updated synthesis. In 2010, a near-infrared light photosynthetic pigment called Chlorophyll f may have been discovered in cyanobacteria and other oxygenic microorganisms that form stromatolites. Based on NMR data, optical and mass spectra, it is thought to have a structure of $C_{55}H_{70}O_6N_4Mg$ or [2-formyl]-chlorophyll a.

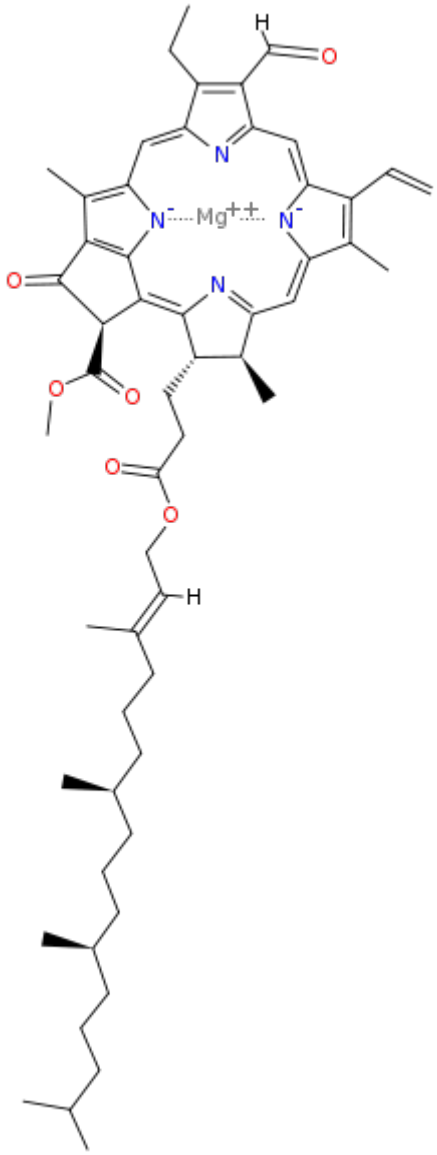
The different structures of chlorophyll are summarized below:

	Chlorophyll a	**Chlorophyll b**	**Chlorophyll c1**	**Chlorophyll c2**	**Chlorophyll d**	**Chlorophyll f**
Molecular formula	$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$	$C_{35}H_{30}O_5N_4Mg$	$C_{35}H_{28}O_5N_4Mg$	$C_{54}H_{70}O_6N_4Mg$	$C_{55}H_{70}O_6N_4Mg$

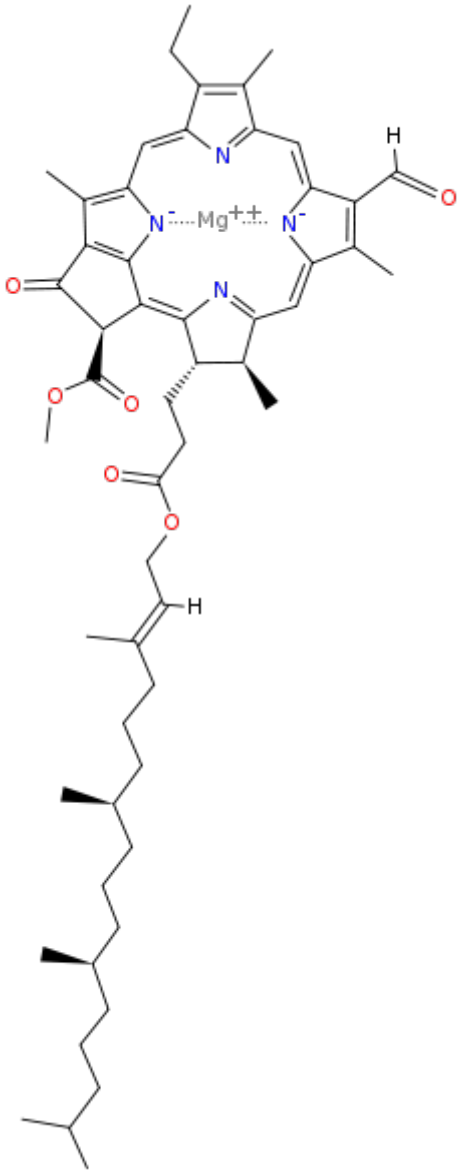
C2 group	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$	-CHO
C3 group	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_2$	$-CH=CH_2$	-CHO	$-CH=CH_2$
C7 group	$-CH_3$	-CHO	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
C8 group	$-CH_2CH_3$	$-CH_2CH_3$	$-CH_2CH_3$	$-CH=CH_2$	$-CH_2CH_3$	$-CH_2CH_3$
C17 group	$-CH_2CH_2COO-$ Phytyl	$-CH_2CH_2COO-$ Phytyl	$-CH=CHCOOH$	$-CH=CHCOOH$	$-CH_2CH_2COO-$ Phytyl	$-CH_2CH_2COO-$ Phytyl
C17-C18 bond	Single (chlorin)	Single (chlorin)	Double (porphyrin)	Double (porphyrin)	Single (chlorin)	Single (chlorin)
Occurrence	Universal	Mostly plants	Various algae	Various algae	Cyanobacteria	Cyanobacteria



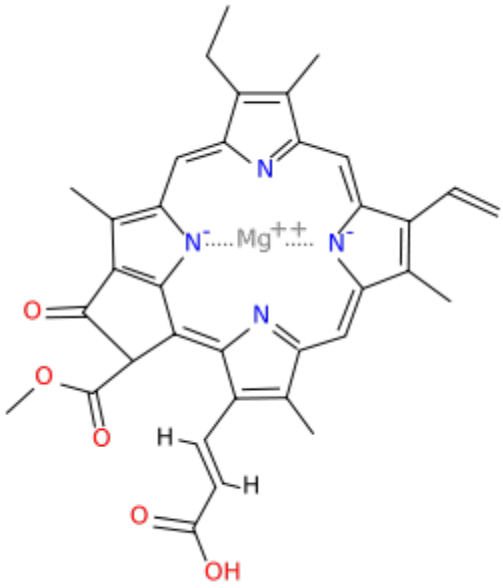
Structure of chlorophyll *a*



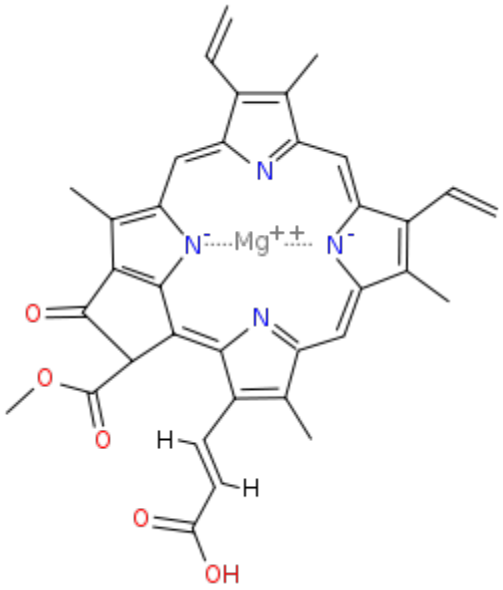
Structure of chlorophyll *b*



Structure of chlorophyll *d*

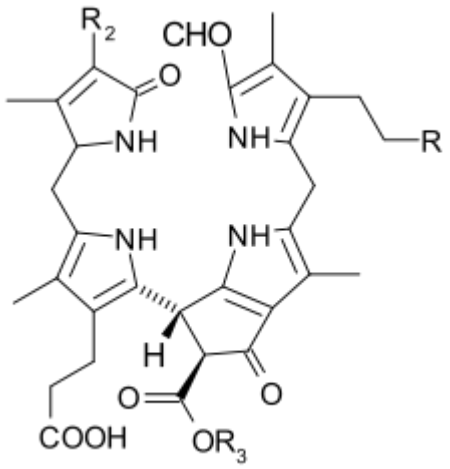


Structure of chlorophyll *c1*



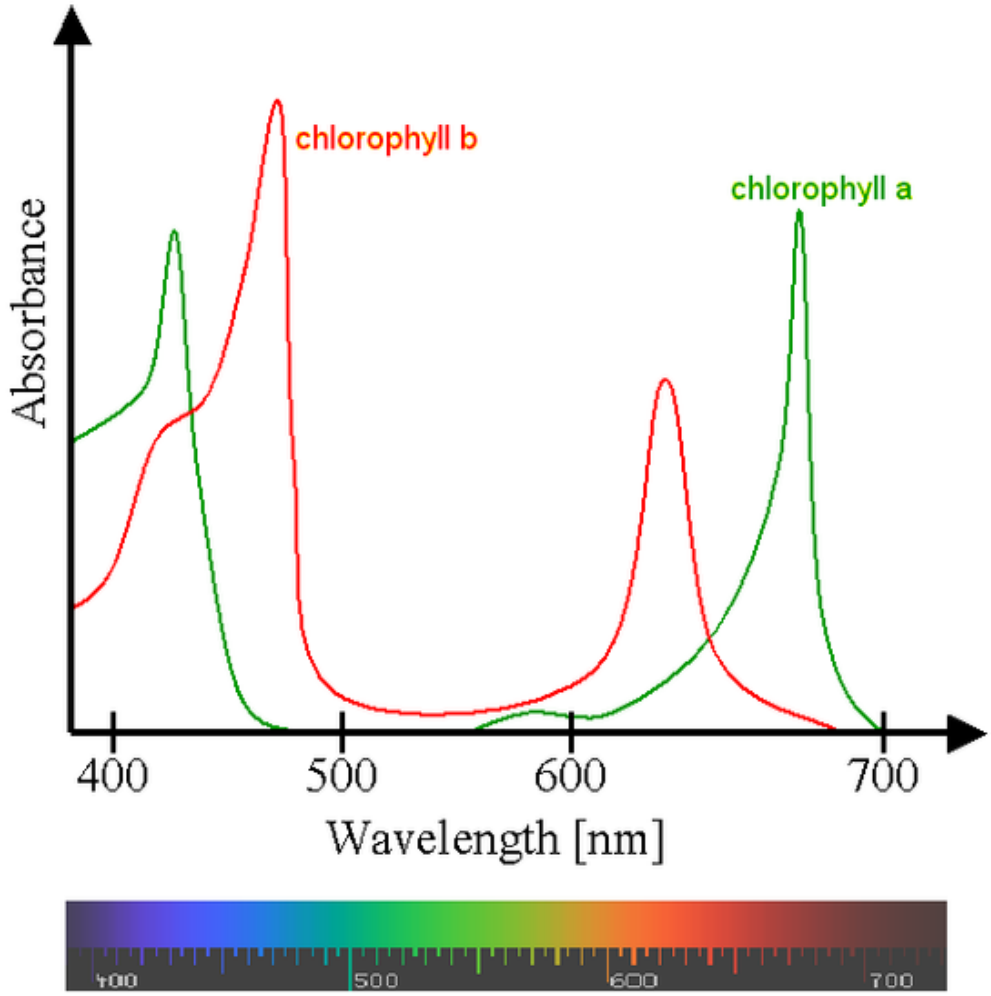
Structure of chlorophyll *c2*

When leaves degreen in the process of plant senescence, chlorophyll is converted to a group of colourless tetrapyrroles known as **nonfluorescent chlorophyll catabolites** (NCC's) with the general structure:



These compounds have also been identified in several ripening fruits.

Spectrophotometry



Absorbance spectra of free chlorophyll *a* (green) and *b* (red) in a solvent. The spectra of chlorophyll molecules are slightly modified *in vivo* depending on specific pigment-protein interactions.

Measurement of the absorption of light is complicated by the solvent used to extract it from plant material, which affects the values obtained,

- In diethyl ether, chlorophyll *a* has approximate absorbance maxima of 430 nm and 662 nm, while chlorophyll *b* has approximate maxima of 453 nm and 642 nm.
- The absorption peaks of chlorophyll *a* are at 665 nm and 465 nm. Chlorophyll *a* fluoresces at 673 nm (maximum) and 726 nm. The peak molar absorption coefficient of chlorophyll *a* exceeds 10^5 M^{-1} cm^{-1}, which is among the highest for small-molecule organic compounds.

By measuring chlorophyll fluorescence, plant ecophysiology can be investigated. Chlorophyll fluorometers are used by plant researchers to assess plant stress.

Biosynthesis

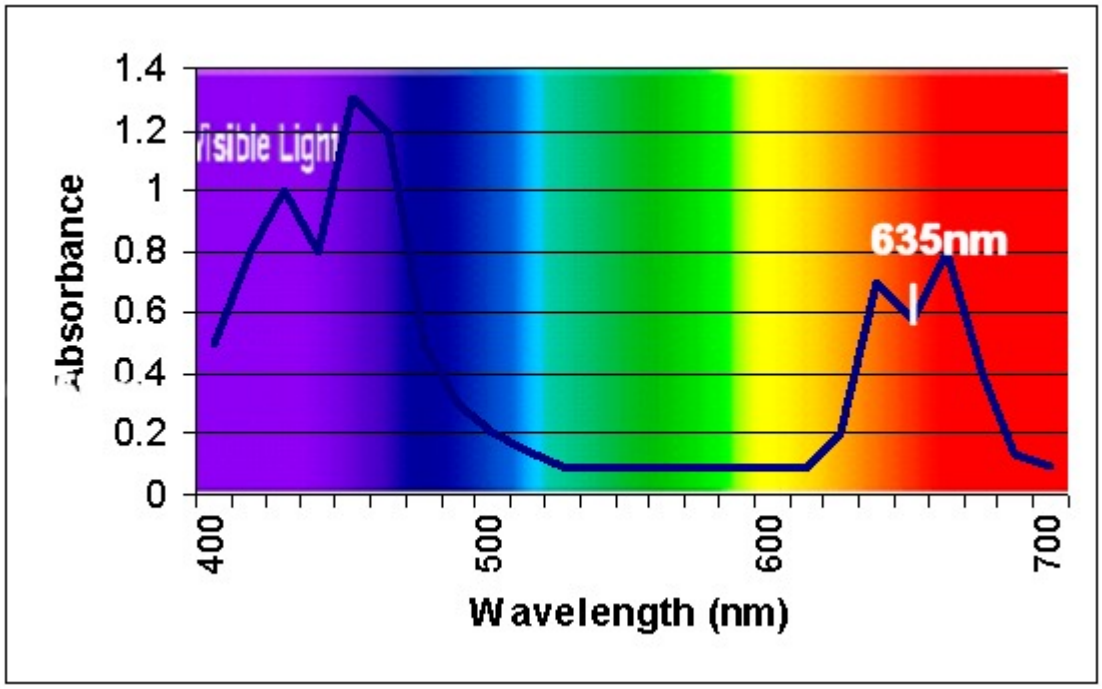
In plants, chlorophyll may be synthesized from succinyl-CoA and glycine, although the immediate precursor to chlorophyll *a* and *b* is protochlorophyllide. In Angiosperm plants, the last step, conversion of protochlorophyllide to chlorophyll, is light-dependent and such plants are pale (etiolated) if grown in the darkness. Non-vascular plants and green algae have an additional light-independent enzyme and grow green in the darkness as well.

Chlorophyll itself is bound to proteins and can transfer the absorbed energy in the required direction. Protochlorophyllide occurs mostly in the free form and, under light conditions, acts as a photosensitizer, forming highly toxic free radicals. Hence, plants need an efficient mechanism of regulating the amount of chlorophyll precursor. In angiosperms, this is done at the step of aminolevulinic acid (ALA), one of the intermediate compounds in the biosynthesis pathway. Plants that are fed by ALA accumulate high and toxic levels of protochlorophyllide; so do the mutants with the damaged regulatory system.

Chlorosis is a condition in which leaves produce insufficient chlorophyll, turning them yellow. Chlorosis can be caused by a nutrient deficiency of iron--called iron chlorosis—or by a shortage of magnesium or nitrogen. Soil pH sometimes plays a role in nutrient-caused chlorosis; many plants are adapted to grow in soils with specific pH levels and their ability to absorb nutrients from the soil can be dependent on this. Chlorosis can also be caused by pathogens including viruses, bacteria and fungal infections, or sap-sucking insects.

Measuring chlorophyll

The chlorophyll content of leaves can be non-destructively measured using hand-held, battery-powered meters.



The absorption spectrum of chlorophyll, showing the transmittance band measured by a CCM200 Chlorophyll Meter to calculate the relative chlorophyll content

Chlorophyll Content meters measure the optical absorption of a leaf to estimate it's chlorophyll content. Chlorophyll molecules absorb in the blue and red bands, but not the green and infra-red bands. Chlorophyll content meters measure the amount of absorption at the red band to estimate the amount of chlorophyll present in the leaf. To compensate for varying leaf thickness, Chlorophyll Meters also measure absorption at the infrared band which is not significantly affected by chlorophyll. For instance, the CCM200plus Chlorophyll Meter measures the transmittance at 653 nm (in the red band) and transmittance at 931 nm (in the infrared band). The percentage of transmittance at 931 nm, relative to the percentage of transmittance at 653 nm, estimates the relative chlorophyll content of the leaf.

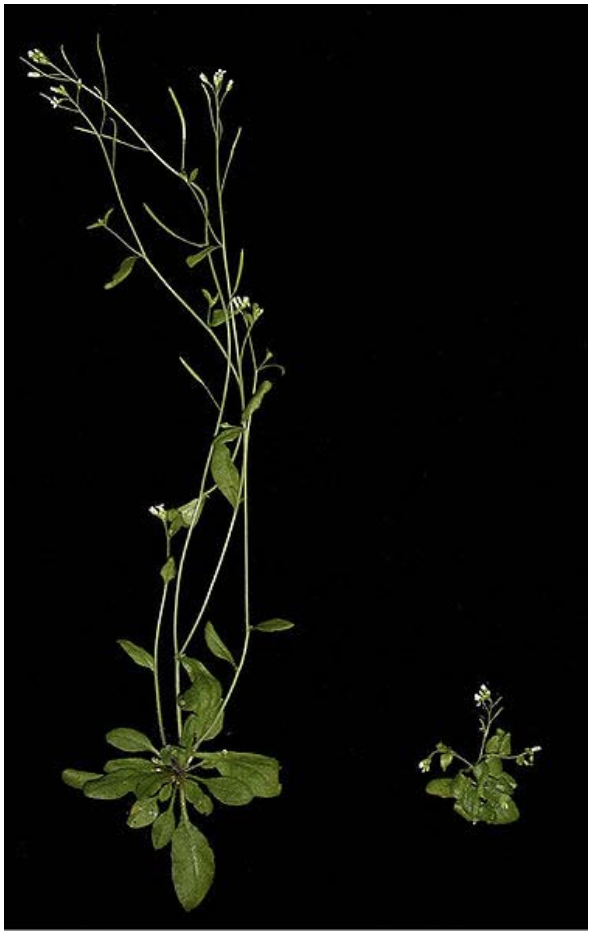
The measurements made by these devices are simple, quick and relatively inexpensive. They now, typically, have large data storage capacity, averaging and graphical displays.

Culinary use

Chlorophyll is registered as a food additive (colorant), and its E number is **E140**. Chefs use chlorophyll to color a variety of foods and beverages green, such as pasta and absinthe. Chlorophyll is not soluble in water and is first mixed with a small quantity of oil to obtain the desired result. Extracted Liquid Chlorophyll was considered unstable and always denatured, until 1997 when Frank S. & Lisa Sagliano used freeze-drying of liquid chlorophyll at the University of Florida and stabilized it as a powder, preserving it for future use.

Chapter 12

Plant Hormone



Lack of the plant hormone auxin can cause abnormal growth (right)

Plant hormones (also known as **phytohormones**) are chemicals that regulate plant growth, which, in the UK, are termed 'plant growth substances'. Plant hormones are signal molecules produced within the plant, and occur in extremely low concentrations. Hormones regulate cellular processes in targeted cells locally and when moved to other locations, in other locations of the plant. Hormones also determine the formation of

flowers, stems, leaves, the shedding of leaves, and the development and ripening of fruit. Plants, unlike animals, lack glands that produce and secrete hormones, instead each cell is capable of producing hormones. Plant hormones shape the plant, affecting seed growth, time of flowering, the sex of flowers, senescence of leaves and fruits. They affect which tissues grow upward and which grow downward, leaf formation and stem growth, fruit development and ripening, plant longevity, and even plant death. Hormones are vital to plant growth and lacking them, plants would be mostly a mass of undifferentiated cells.

Characteristics

The word hormone is derived from Greek, meaning 'set in motion.' Plant hormones affect gene expression and transcription levels, cellular division, and growth. They are naturally produced within plants, though very similar chemicals are produced by fungi and bacteria that can also affect plant growth. A large number of related chemical compounds are synthesized by humans, they are used to regulate the growth of cultivated plants, weeds, and in vitro-grown plants and plant cells; these manmade compounds are called **Plant Growth Regulators** or **PGRs** for short. Early in the study of plant hormones, "phytohormone" was the commonly used term, but its use is less widely applied now.

Plant hormones are not nutrients, but chemicals that in small amounts promote and influence the growth, development, and differentiation of cells and tissues. The biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. Plants lack glands to produce and store hormones, because, unlike animals, which have two circulatory systems (lymphatic and cardiovascular) powered by a heart that moves fluids around the body, plants use more passive means to move chemicals around the plant. Plants utilize simple chemicals as hormones, which move more easily through the plant's tissues. They are often produced and used on a local basis within the plant body, plant cells even produce hormones that affect different regions of the cell producing the hormone.

Hormones are transported within the plant by utilizing four types of movements. For localized movement, cytoplasmic streaming within cells and slow diffusion of ions and molecules between cells are utilized. Vascular tissues are used to move hormones from one part of the plant to another; these include sieve tubes that move sugars from the leaves to the roots and flowers, and xylem that moves water and mineral solutes from the roots to the foliage.

Not all plant cells respond to hormones, but those cells that do are programmed to respond at specific points in their growth cycle. The greatest effects occur at specific stages during the cell's life, with diminished effects occurring before or after this period. Plants need hormones at very specific times during plant growth and at specific locations. They also need to disengage the effects that hormones have when they are no longer needed. The production of hormones occurs very often at sites of active growth within the meristems, before cells have fully differentiated. After production they are sometimes moved to other parts of the plant where they cause an immediate effect or they can be stored in cells to be released later. Plants use different pathways to regulate internal

hormone quantities and moderate their effects; they can regulate the amount of chemicals used to biosynthesize hormones. They can store them in cells, inactivate them, or cannibalise already-formed hormones by conjugating them with carbohydrates, amino acids or peptides. Plants can also break down hormones chemically, effectively destroying them. Plant hormones frequently regulate the concentrations of other plant hormones. Plants also move hormones around the plant diluting their concentrations.

The concentration of hormones required for plant responses are very low (10^{-6} to 10^{-5} mol/L). Because of these low concentrations, it has been very difficult to study plant hormones, and only since the late 1970s have scientists been able to start piecing together their effects and relationships to plant physiology. Much of the early work on plant hormones involved studying plants that were genetically deficient in one or involved the use of tissue-cultured plants grown *in vitro* that were subjected to differing ratios of hormones, and the resultant growth compared. The earliest scientific observation and study dates to the 1880s; the determination and observation of plant hormones and their identification was spread-out over the next 70 years.

Classes of plant hormones

In general, it is accepted that there are five major classes of plant hormones, some of which are made up of many different chemicals that can vary in structure from one plant to the next. The chemicals are each grouped together into one of these classes based on their structural similarities and on their effects on plant physiology. Other plant hormones and growth regulators are not easily grouped into these classes; they exist naturally or are synthesized by humans or other organisms, including chemicals that inhibit plant growth or interrupt the physiological processes within plants. Each class has positive as well as inhibitory functions, and most often work in tandem with each other, with varying ratios of one or more interplaying to affect growth regulation.

The five major classes are:

Abscisic acid

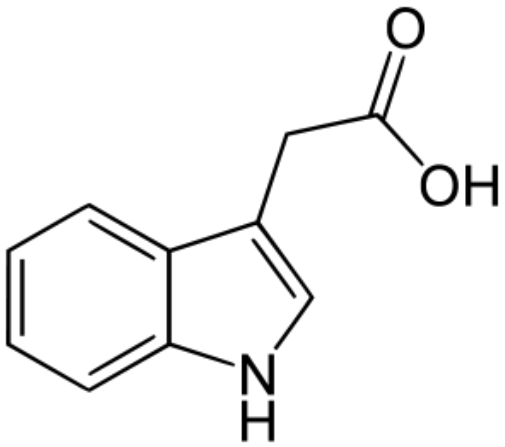
Abscisic acid also called ABA, was discovered and researched under two different names before its chemical properties were fully known, it was called *dormin* and *abscicin II*. Once it was determined that the two latter compounds were the same; it was named abscisic acid. The name "abscisic acid" was given because it was found in high concentrations in newly abscissed or freshly fallen leaves.

This class of PGR is composed of one chemical compound normally produced in the leaves of plants, originating from chloroplasts, especially when plants are under stress. In general, it acts as an inhibitory chemical compound that affects bud growth, seed and bud dormancy. It mediates changes within the apical meristem causing bud dormancy and the alteration of the last set of leaves into protective bud covers. Since it was found in freshly abscissed leaves, it was thought to play a role in the processes of natural leaf drop but further research has disproven this. In plant species from temperate parts of the world it

plays a role in leaf and seed dormancy by inhibiting growth, but, as it is dissipated from seeds or buds, growth begins. In other plants, as ABA levels decrease, growth then commences as gibberellin levels increase. Without ABA, buds and seeds would start to grow during warm periods in winter and be killed when it froze again. Since ABA dissipates slowly from the tissues and its effects take time to be offset by other plant hormones, there is a delay in physiological pathways that provide some protection from premature growth. It accumulates within seeds during fruit maturation, preventing seed germination within the fruit, or seed germination before winter. Abscisic acid's effects are degraded within plant tissues during cold temperatures or by its removal by water washing in out of the tissues, releasing the seeds and buds from dormancy.

In plants under water stress, ABA plays a role in closing the stomata. Soon after plants are water-stressed and the roots are deficient in water, a signal moves up to the leaves, causing the formation of ABA precursors there, which then move to the roots. The roots then release ABA, which is translocated to the foliage through the vascular system and modulates the potassium and sodium uptake within the guard cells, which then lose turgidity, closing the stomata. ABA exists in all parts of the plant and its concentration within any tissue seems to mediate its effects and function as a hormone; its degradation, or more properly catabolism, within the plant affects metabolic reactions and cellular growth and production of other hormones. Plants start life as a seed with high ABA levels, just before the seed germinates ABA levels decrease; during germination and early growth of the seedling, ABA levels decrease even more. As plants begin to produce shoots with fully functional leaves - ABA levels begin to increase, slowing down cellular growth in more "mature" areas of the plant. Stress from water or predation affects ABA production and catabolism rates, mediating another cascade of effects that trigger specific responses from targeted cells. Scientists are still piecing together the complex interactions and effects of this and other phytohormones.

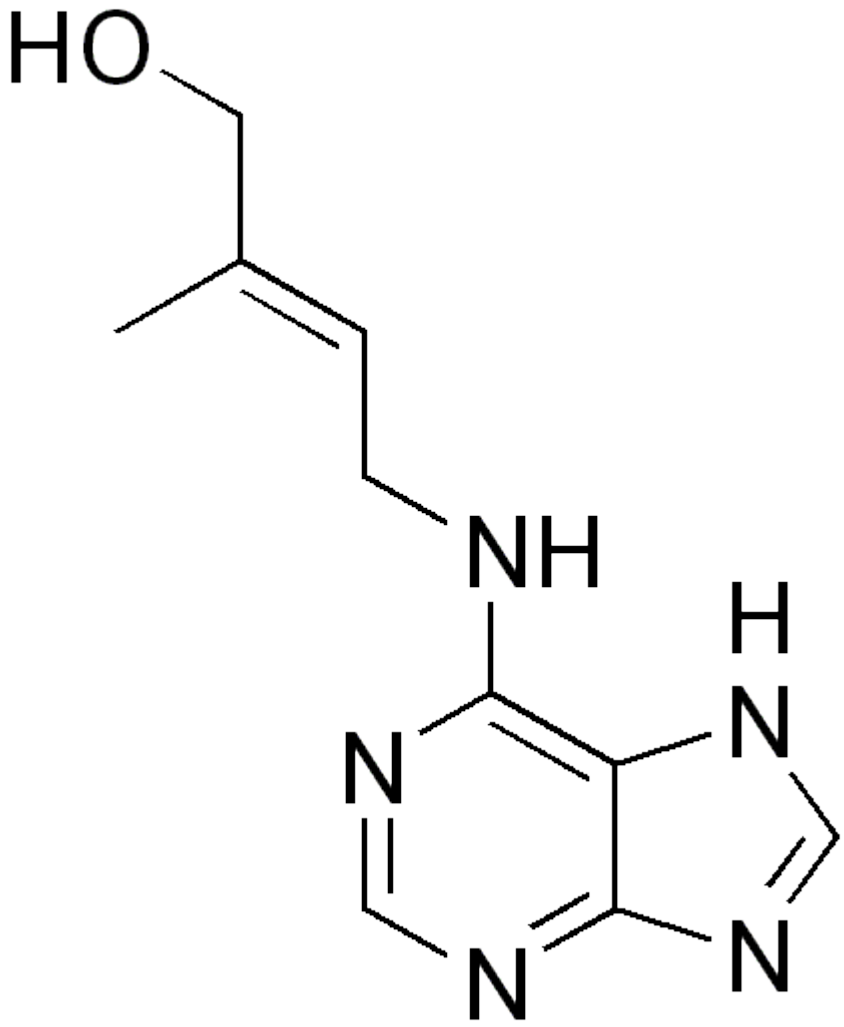
Auxins



The auxin indoleacetic acid

Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinins, they control the growth of stems, roots, and fruits, and convert stems into flowers. Auxins were the first class of growth regulators discovered. They affect cell elongation by altering cell wall plasticity. Auxins decrease in light and increase where it is dark. They stimulate cambium cells to divide and in stems cause secondary xylem to differentiate. Auxins act to inhibit the growth of buds lower down the stems (apical dominance), and also to promote lateral and adventitious root development and growth. Leaf abscission is initiated by the growing point of a plant ceasing to produce auxins. Auxins in seeds regulate specific protein synthesis, as they develop within the flower after pollination, causing the flower to develop a fruit to contain the developing seeds. Auxins are toxic to plants in large concentrations; they are most toxic to dicots and less so to monocots. Because of this property, synthetic auxin herbicides including 2,4-D and 2,4,5-T have been developed and used for weed control. Auxins, especially 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA), are also commonly applied to stimulate root growth when taking cuttings of plants. The most common auxin found in plants is indoleacetic acid or IAA. The correlation of auxins and cytokinins in the plants is a constant (A/C = const.).

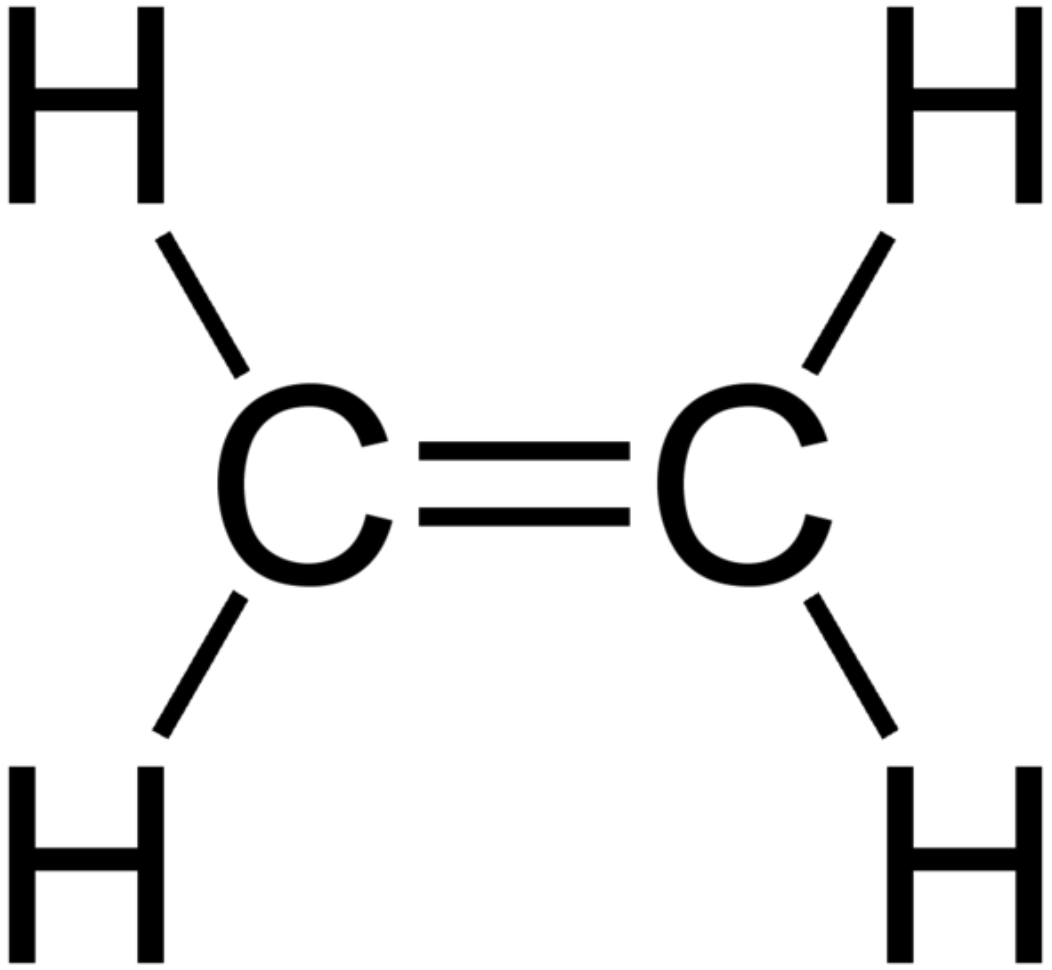
Cytokinins



The cytokinin zeatin , *Zea*, in which it was first discovered in immature kernels.

Cytokinins or CKs are a group of chemicals that influence cell division and shoot formation. They were called kinins in the past when the first cytokinins were isolated from yeast cells. They also help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth. They have a highly synergistic effect in concert with auxins and the ratios of these two groups of plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins; they in conjunction with ethylene promote abscission of leaves, flower parts and fruits. The correlation of auxins and cytokinins in the plants is a constant (A/C = const.).

Ethylene

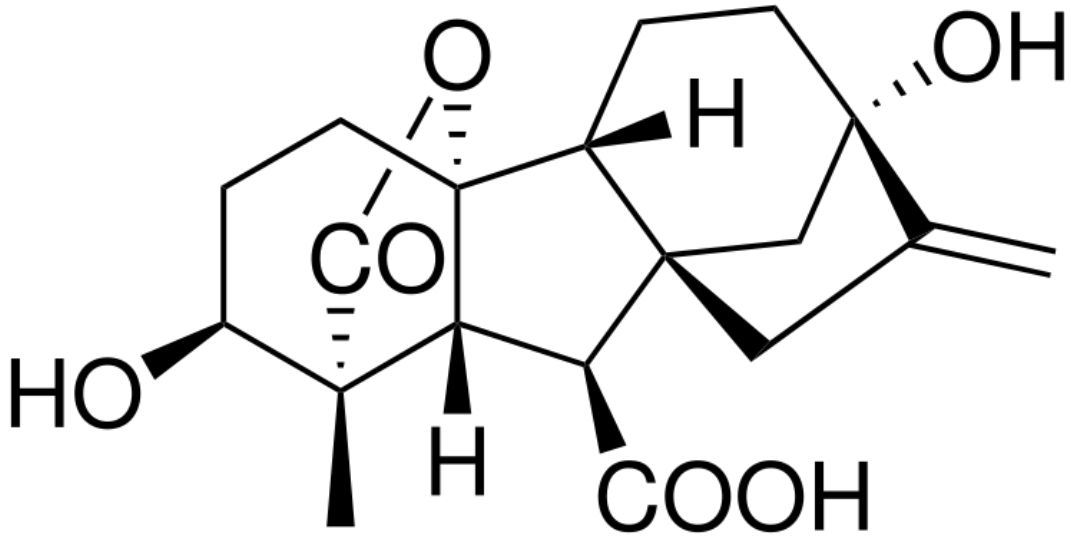


Ethylene

Ethylene is a gas that forms through the Yang Cycle from the breakdown of methionine, which is in all cells. Ethylene has very limited solubility in water and does not accumulate within the cell but diffuses out of the cell and escapes out of the plant. Its effectiveness as a plant hormone is dependent on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by phytochrome in the plant's cells produce a signal for ethylene production to decrease, allowing leaf expansion. Ethylene affects cell growth and cell shape; when a growing shoot hits an obstacle while underground, ethylene production greatly increases, preventing cell elongation and causing the stem to swell. The resulting thicker stem can exert more pressure against the object impeding its path to the surface. If the shoot does

not reach the surface and the ethylene stimulus becomes prolonged, it affects the stems natural geotropic response, which is to grow upright, allowing it to grow around an object. Studies seem to indicate that ethylene affects stem diameter and height: When stems of trees are subjected to wind, causing lateral stress, greater ethylene production occurs, resulting in thicker, more sturdy tree trunks and branches. Ethylene affects fruit-ripening: Normally, when the seeds are mature, ethylene production increases and builds-up within the fruit, resulting in a climacteric event just before seed dispersal. The nuclear protein Ethylene Insensitive2 (EIN2) is regulated by ethylene production, and, in turn, regulates other hormones including ABA and stress hormones.

Gibberellins



Gibberellin A1

Gibberellins, or GAs, include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers, including Eiichi Kurosawa, noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. Gibberellins are important in seed germination, affecting enzyme production that mobilizes food production used for growth of new cells. This is done by modulating chromosomal transcription. In grain (rice, wheat, corn, etc.) seeds, a layer of cells called the aleurone layer wraps around the endosperm tissue. Absoption of water by the seed causes production of GA. The GA is transported to the aleurone layer, which responds by producing enzymes that break down stored food reserves within the endosperm, which are utilized by the growing seedling. GAs produce bolting of rosette-forming plants, increasing internodal length. They promote flowering, cellular division, and in seeds growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA.

Other known hormones

Other identified plant growth regulators include:

- Brassinosteroids, are a class of polyhydroxysteroids, a group of plant growth regulators. Brassinosteroids have been recognized as a sixth class of plant hormones which stimulate cell elongation and division, gravitropism, resistance to stress and xylem differentiation. They inhibit root growth and leaf abscission. Brassinolide was the first identified brassinosteroid and was isolated from organic extracts of rapeseed (*Brassica napus*) pollen in 1970.
- Salicylic acid - activates genes in some plants that produce chemicals that aid in the defense against pathogenic invaders.
- Jasmonates - are produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are believed to also have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth.
- Plant peptide hormones - encompasses all small secreted peptides that are involved in cell-to-cell signaling. These small peptide hormones play crucial roles in plant growth and development, including defense mechanisms, the control of cell division and expansion, and pollen self-incompatibility .
- Polyamines - are strongly basic molecules with low molecular weight that have been found in all organisms studied thus far. They are essential for plant growth and development and affect the process of mitosis and meiosis.
- Nitric oxide (NO) - serves as signal in hormonal and defense responses.
- Strigolactones, implicated in the inhibition of shoot branching.
- Karrikins, a group of plant growth regulators found in the smoke of burning plant material that have the ability to stimulate the germination of seeds

Potential medical applications

Plant stress hormones activate cellular responses, including cell death, to diverse stress situations in plants. Researchers have found that some plant stress hormones share the ability to adversely affect human cancer cells . For example, sodium salicylate has been found to suppress proliferation of lymphoblastic leukemia, prostate, breast, and melanoma human cancer cells. Jasmonic acid, a plant stress hormone that belongs to the jasmonate family, induced death in lymphoblastic leukemia cells. Methyl jasmonate has been found to induce cell death in a number of cancer cell lines.

Hormones and plant propagation

Synthetic plant hormones or PGRs are commonly used in a number of different techniques involving plant propagation from cuttings, grafting, micropropagation, and tissue culture.

The propagation of plants by cuttings of fully developed leaves, stems, or roots is performed by gardeners utilizing auxin as a rooting compound applied to the cut surface;

the auxins are taken into the plant and promote root initiation. In grafting, auxin promotes callus tissue formation, which joins the surfaces of the graft together. In micropropagation, different PGRs are used to promote multiplication and then rooting of new plantlets. In the tissue-culturing of plant cells, PGRs are used to produce callus growth, multiplication, and rooting.

Seed dormancy

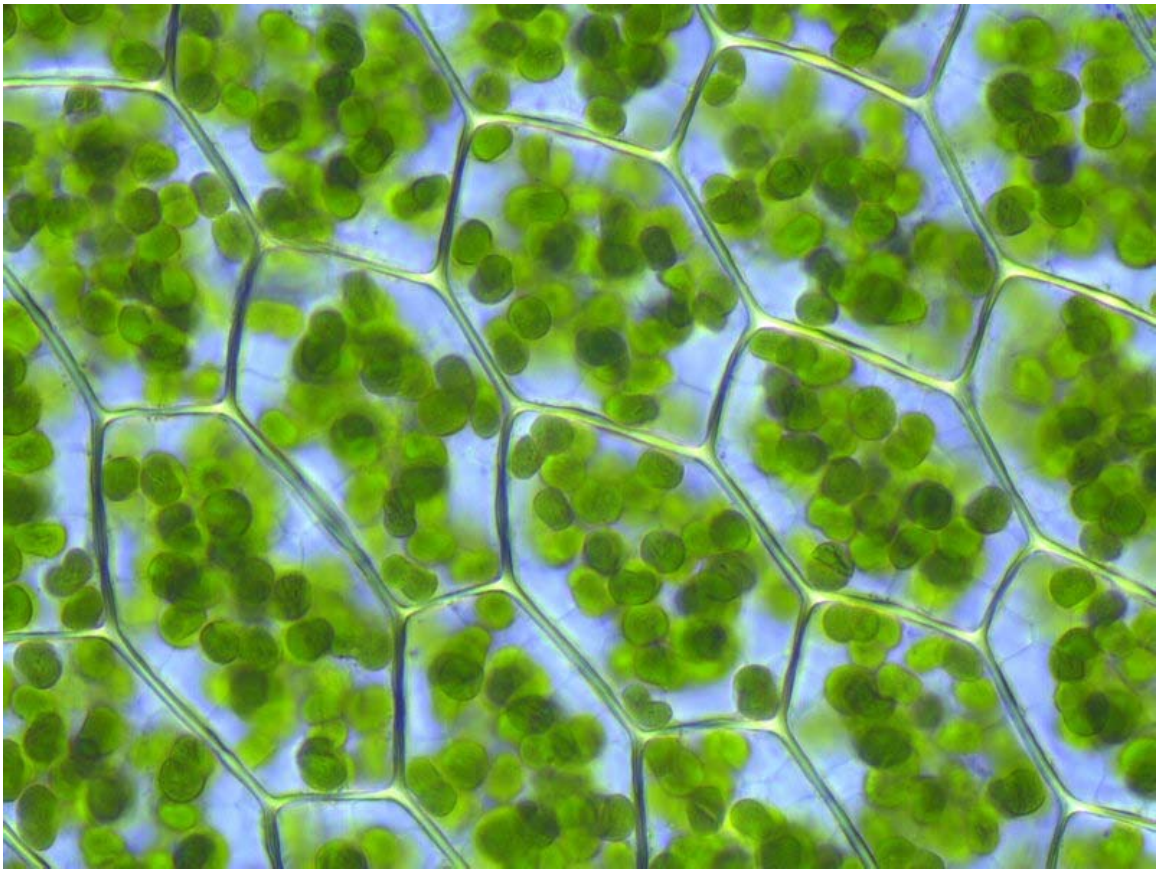
Plant hormones affect seed germinations and dormancy by affecting different parts of the seed.

Embryo dormancy is characterized by a high ABA/GA ratio, whereas the seed has a high ABA sensitivity and low GA sensitivity. To release the seed from this type of dormancy and initiate seed germination, an alteration in hormone biosynthesis and degradation towards a low ABA/GA ratio, along with a decrease in ABA sensitivity and an increase in GA sensitivity needs to occur.

ABA controls embryo dormancy, and GA embryo germination. Seed coat dormancy involves the mechanical restriction of the seed coat, this along with a low embryo growth potential, effectively produces seed dormancy. GA releases this dormancy by increasing the embryo growth potential, and/or weakening the seed coat so the radical of the seedling can break through the seed coat. Different types of seed coats can be made up of living or dead cells and both types can be influenced by hormones; those composed of living cells are acted upon after seed formation while the seed coats composed of dead cells can be influenced by hormones during the formation of the seed coat. ABA affects testa or seed coat growth characteristics, including thickness, and effects the GA-mediated embryo growth potential. These conditions and effects occur during the formation of the seed, often in response to environmental conditions. Hormones also mediate endosperm dormancy: Endosperm in most seeds is composed of living tissue that can actively respond to hormones generated by the embryo. The endosperm often acts as a barrier to seed germination, playing a part in seed coat dormancy or in the germination process. Living cells respond to and also affect the ABA/GA ratio, and mediate cellular sensitivity; GA thus increases the embryo growth potential and can promote endosperm weakening. GA also affects both ABA-independent and ABA-inhibiting processes within the endosperm.

Chapter 13

Plastid

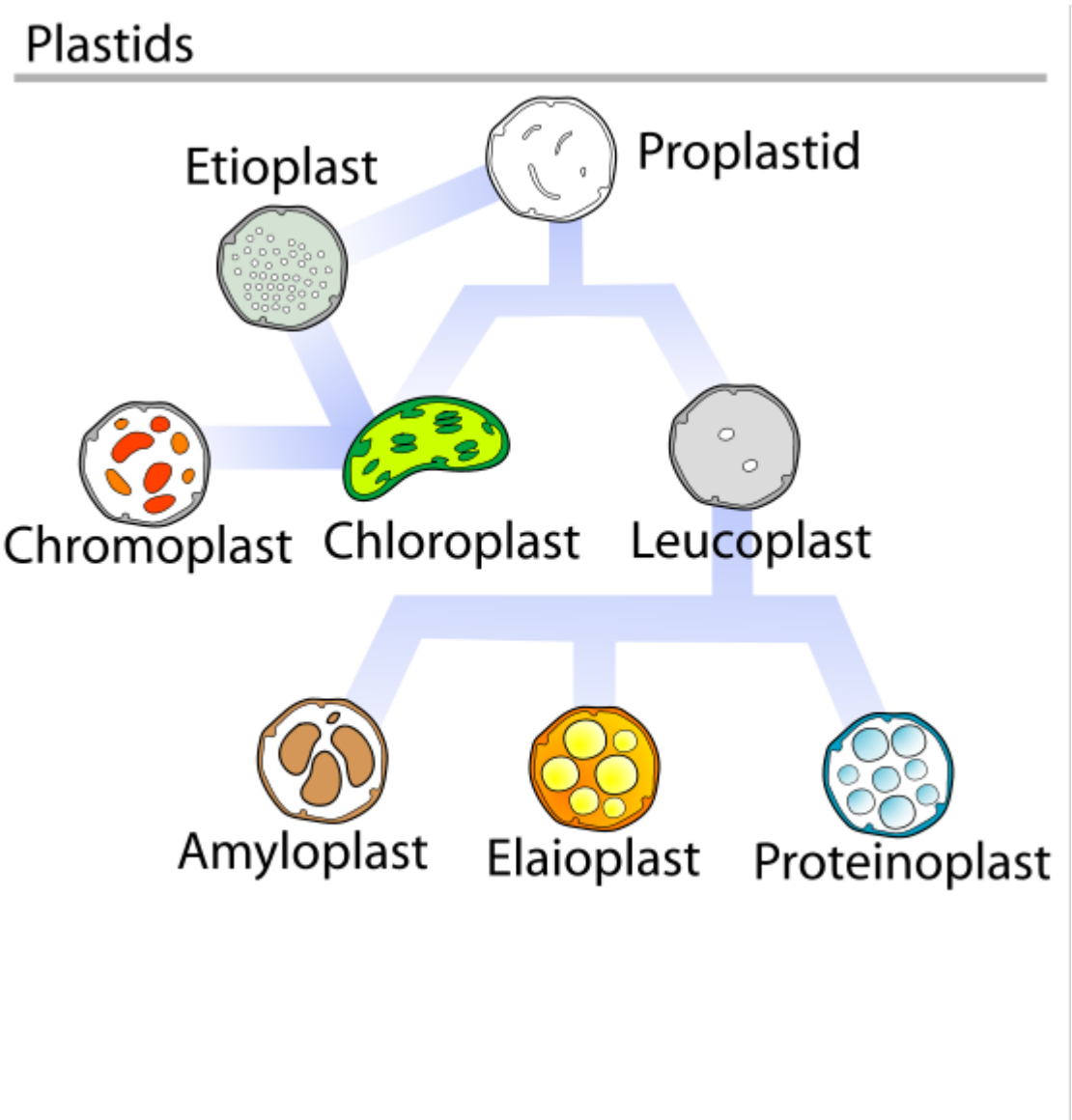


Plant cells with visible chloroplasts.

Plastids are major organelles found in the cells of plants and algae. Plastids are the site of manufacture and storage of important chemical compounds used by the cell. Plastids often contain pigments used in photosynthesis, and the types of pigments present can change or determine the cell's color.

Plastids in plants

Plastids are responsible for photosynthesis, storage of products like starch and for the synthesis have the ability to differentiate, or redifferentiate, between these and other forms. All plastids are derived from proplastids (formerly "eoplasts", *eo-*: dawn, early), which are present in the meristematic regions of the plant. Proplastids and young chloroplasts commonly divide, but more mature chloroplasts also have this capacity.



In plants, plastids may differentiate into several forms, depending upon which function they need to play in the cell. Undifferentiated plastids (*proplastids*) may develop into any of the following plastids:

- Chloroplasts: for photosynthesis;

- Chromoplasts: for pigment synthesis and storage
- Gerontoplasts: control the dismantling of the photosynthetic apparatus during senescence
- Leucoplasts: for monoterpene synthesis; *leucoplasts sometimes differentiate into more specialized plastids:*
    - Amyloplasts: for starch storage and detecting gravity
    - Elaioplasts: for storing fat
    - Proteinoplasts: for storing and modifying protein

Each plastid creates multiple copies of the circular 75–250 kilobase plastome. The number of genome copies per plastid is flexible, ranging from more than 1000 in rapidly dividing cells, which generally contain few plastids, to 100 or fewer in mature cells, where plastid divisions has given rise to a large number of plastids. The plastome contains about 100 genes encoding ribosomal and transfer ribonucleic acids (rRNAs and tRNAs) as well as proteins involved in photosynthesis and plastid gene transcription and translation. However, these proteins only represent a small fraction of the total protein set-up necessary to build and maintain the structure and function of a particular type of plastid. Nuclear genes encode the vast majority of plastid proteins, and the expression of plastid genes and nuclear genes is tightly co-regulated to allow proper development of plastids in relation to cell differentiation.

Plastid DNA exists as large protein-DNA complexes associated with the inner envelope membrane and called 'plastid nucleoids'. Each nucleoid particle may contain more than 10 copies of the plastid DNA. The proplastid contains a single nucleoid located in the centre of the plastid. The developing plastid has many nucleoids, localized at the periphery of the plastid, bound to the inner envelope membrane. During the development of proplastids to chloroplasts, and when plastids convert from one type to another, nucleoids change in morphology, size and location within the organelle. The remodelling of nucleoids is believed to occur by modifications to the composition and abundance of nucleoid proteins.

Many plastids, particularly those responsible for photosynthesis, possess numerous internal membrane layers.

In plant cells, long thin protuberances called stromules sometimes form and extend from the main plastid body into the cytosol and interconnect several plastids. Proteins, and presumably smaller molecules, can move within stromules. Most cultured cells that are relatively large compared to other plant cells have very long and abundant stromules that extend to the cell periphery.

Plastids in algae

In algae, the term leucoplast is used for all unpigmented plastids and their function differs from the leucoplasts of plants. Etioplasts, amyloplasts and chromoplasts are plant-specific

and do not occur in algae. Plastids in algae and hornworts may also differ from plant plastids in that they contain pyrenoids.

Glaucocystophytic algae contain muroplasts which are similar to chloroplasts except that they have a cell wall that is similar to that of prokaryotes. Rhydophytic algae contain rhydoplasts which are red chloroplasts which allow the algae to photosynthesise to a depth of up to 268m.

Inheritance of plastids

Most plants inherit the plastids from only one parent. Angiosperms generally inherit plastids from the female gamete, while many gymnosperms inherit plastids from the male pollen. Algae also inherit plastids from only one parent. The plastid DNA of the other parent is thus completely lost.

In normal intraspecific crossings (resulting in normal hybrids of one species), the inheritance of plastid DNA appears to be quite strictly 100% uniparental. In interspecific hybridisations, however, the inheritance of plastids appears to be more erratic. Although plastids inherit mainly maternally in interspecific hybridisations, there are many reports of hybrids of flowering plants that contain plastids of the father. Approximately ~20% of angiosperms, including alfalfa (Medicago), normally show biparental inheritance of plastids.

Origin of plastids

Plastids are thought to have originated from endosymbiotic cyanobacteria. They developed around 1500 million years ago and allowed eukaryotes to carry out oxygenic photosynthesis. Due to a split-up into three evolutionary lineages, the plastids are named differently: chloroplasts in green algae and plants, rhodoplasts in red algae and cyanelles in the glaucophytes. The plastids differ by their pigmentation, but also in ultrastructure. The chloroplasts e.g. have lost all phycobilisomes, the light harvesting complexes found in cyanobacteria, red algae and glaucophytes, but — only in plants and in closely related green algae — contain stroma and grana thylakoids. The glaucocystophycean plastid — in contrast to the chloroplasts and the rhodoplasts — is still surrounded by the remains of the cyanobacterial cell wall. All these primary plastids are surrounded by two membranes.

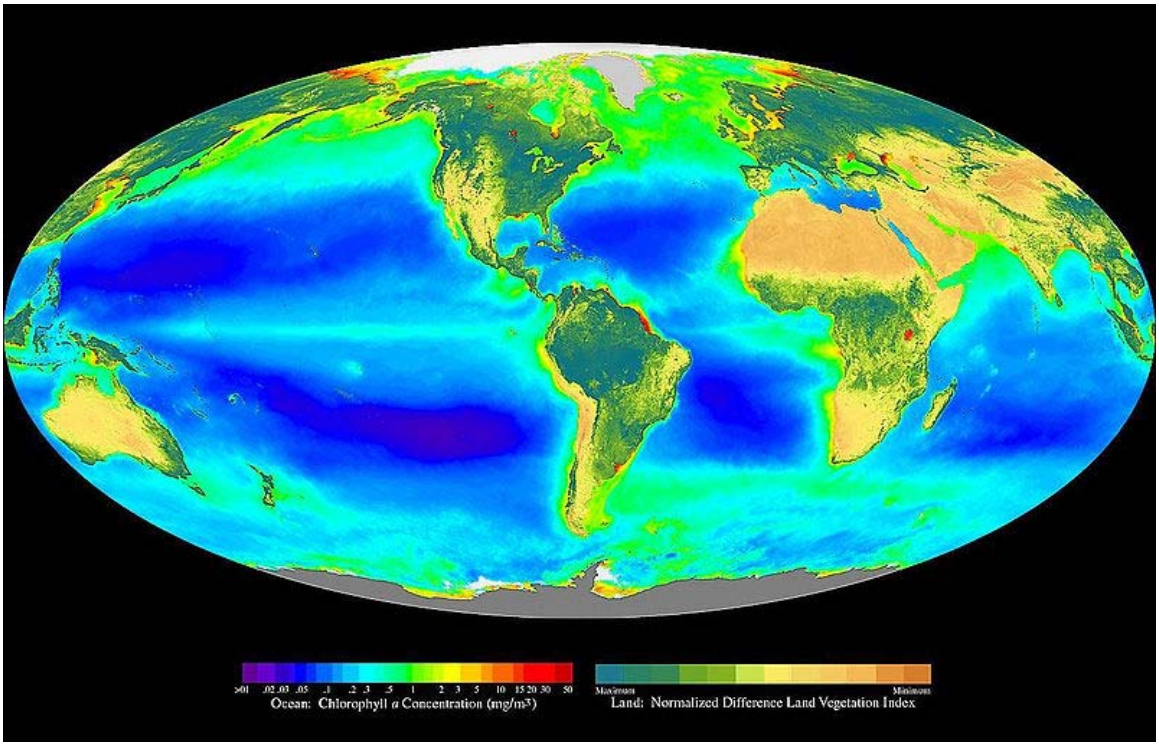
Complex plastids start by secondary endosymbiosis, when a eukaryote engulfs a red or green alga and retains the algal plastid, which is typically surrounded by more than two membranes. In some cases these plastids may be reduced in their metabolic and/or photosynthetic capacity. Algae with complex plastids derived by secondary endosymbiosis of a red alga include the heterokonts, haptophytes, cryptomonads, and most dinoflagellates (= rhodoplasts). Those that endosymbiosed a green alga include the euglenids and chlorarachniophytes (= chloroplasts). The Apicomplexa, a phylum of obligate parasitic protozoa including the causative agents of malaria (*Plasmodium* spp.), toxoplasmosis (*Toxoplasma gondii*), and many other human or animal diseases also

harbor a complex plastid (although this organelle has been lost in some apicomplexans, such as *Cryptosporidium parvum*, which causes cryptosporidiosis). The 'apicoplast' is no longer capable of photosynthesis, but is an essential organelle, and a promising target for antiparasitic drug development.

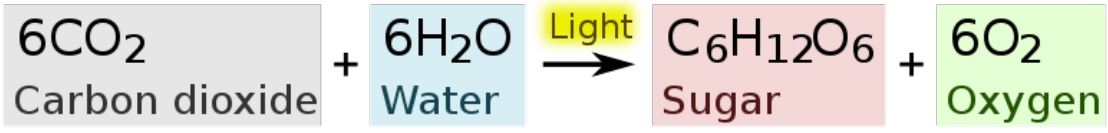
Some dinoflagellates and sea slugs, particularly of the genus Elysia, take up algae as food and keep the plastid of the digested alga to profit from the photosynthesis; after a while the plastids are also digested. These captured plastids are known as kleptoplastids.

Chapter 14

Photosynthesis



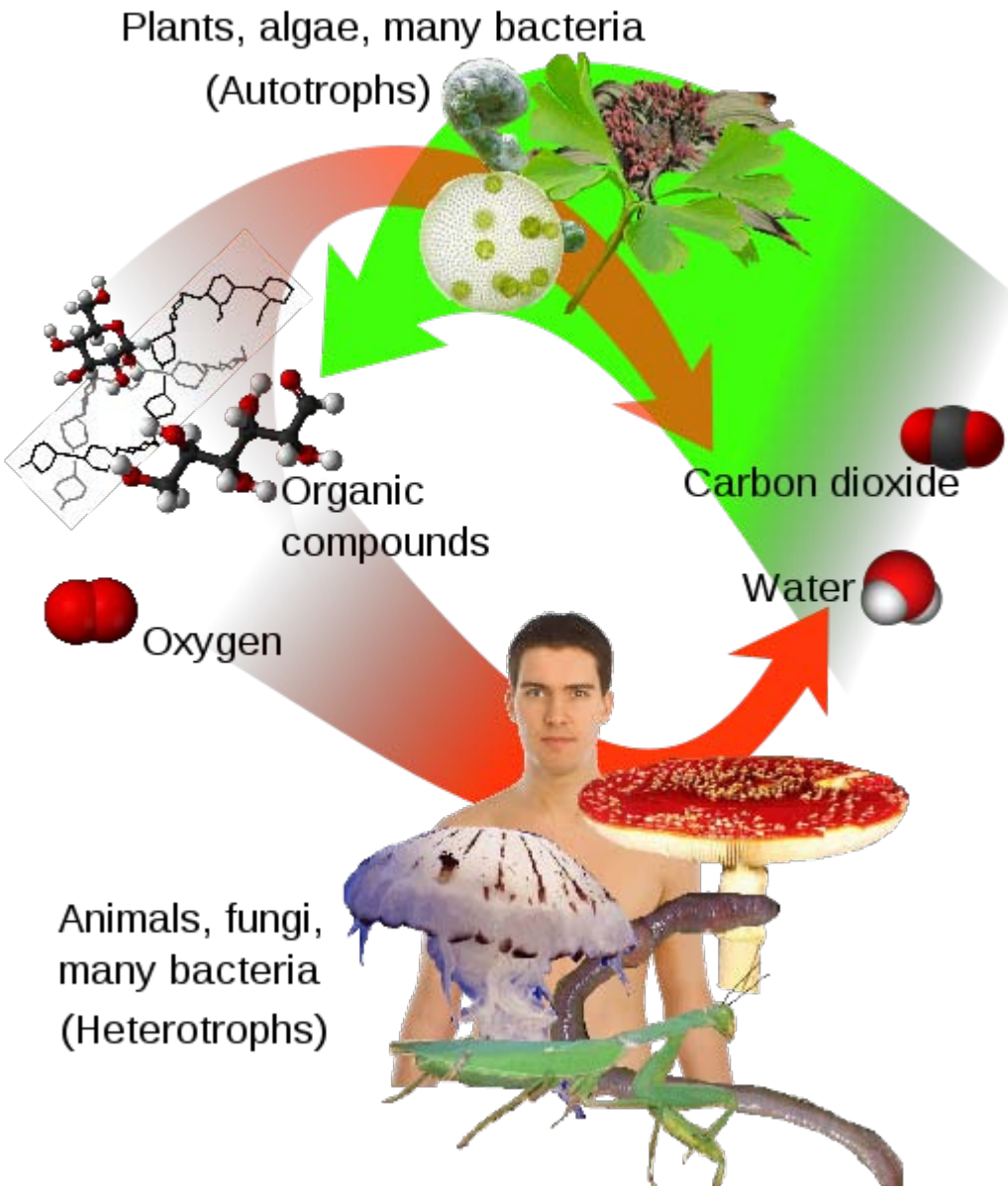
Composite image showing the global distribution of photosynthesis, including both oceanic phytoplankton and vegetation



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.

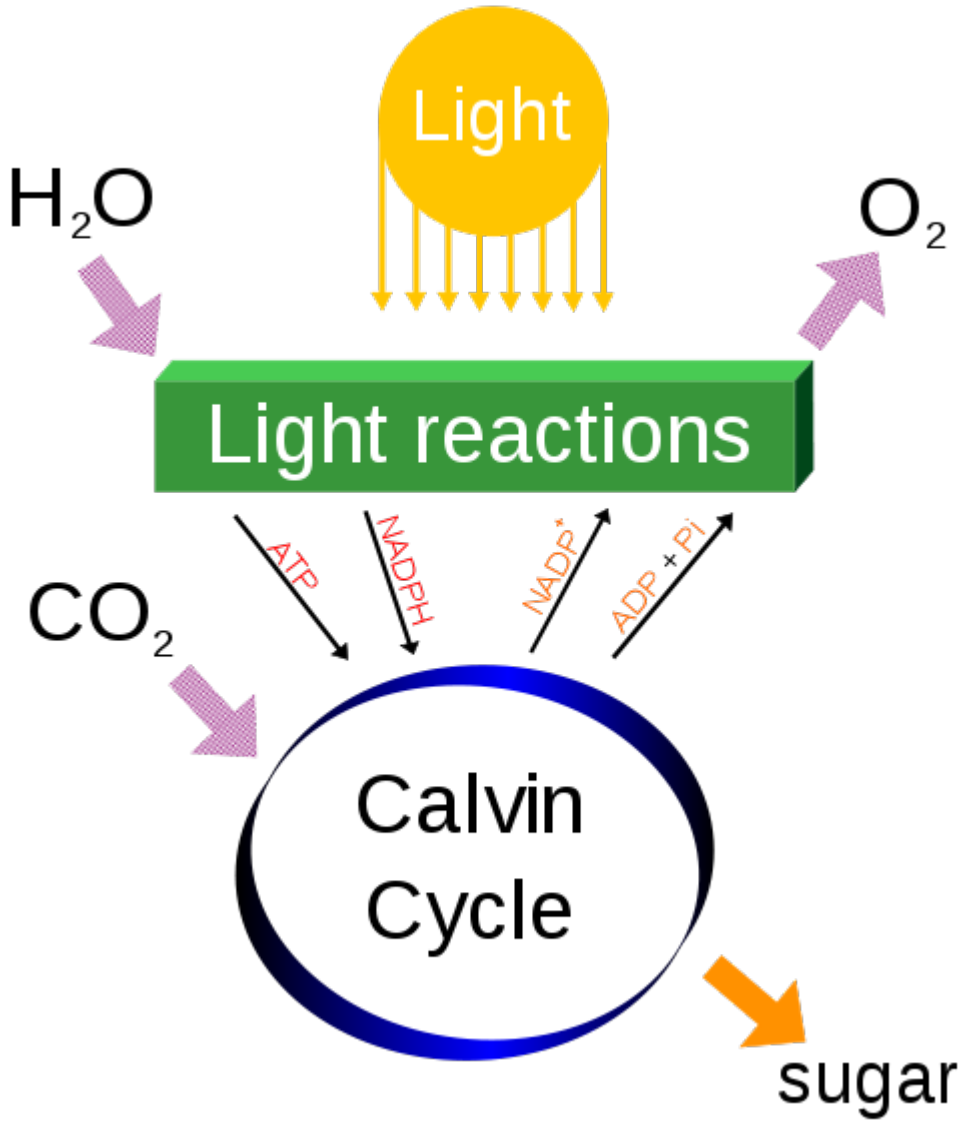


Overview of cycle between autotrophs and heterotrophs. Photosynthesis is the main means by which plants, algae and many bacteria produce organic compounds and oxygen from carbon dioxide and water (green arrow).

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:

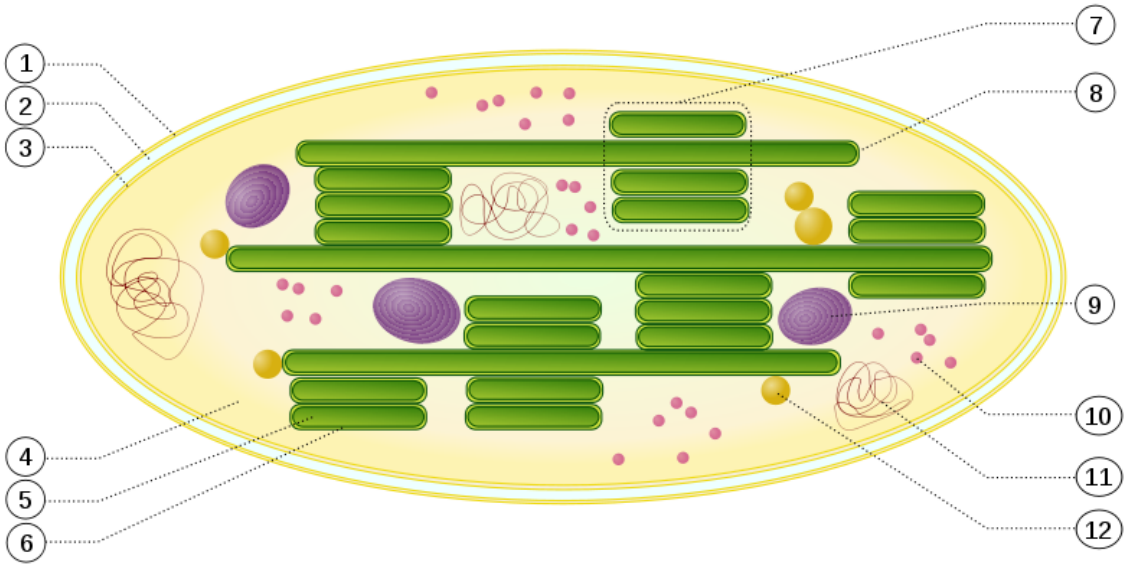
$$(AsO_3^{3-}) + CO_2 + \text{photons} \rightarrow CO + (AsO_4^{3-})$$

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,

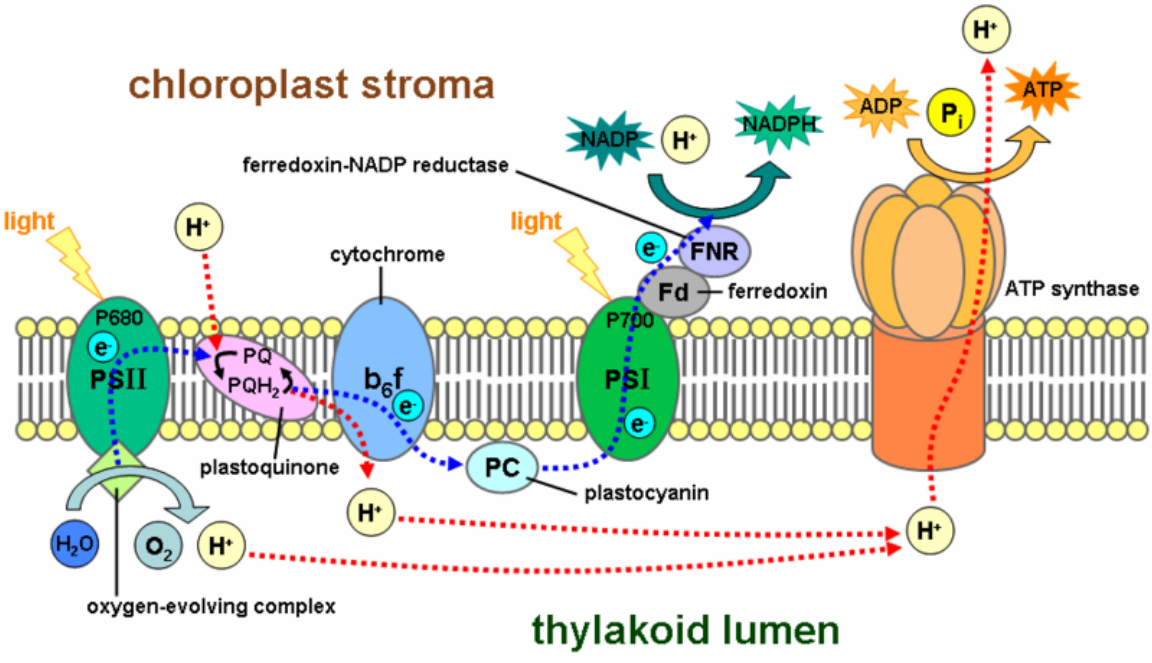
bounded by a membrane with a lumen or thylakoid space within it. The site of photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems.

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



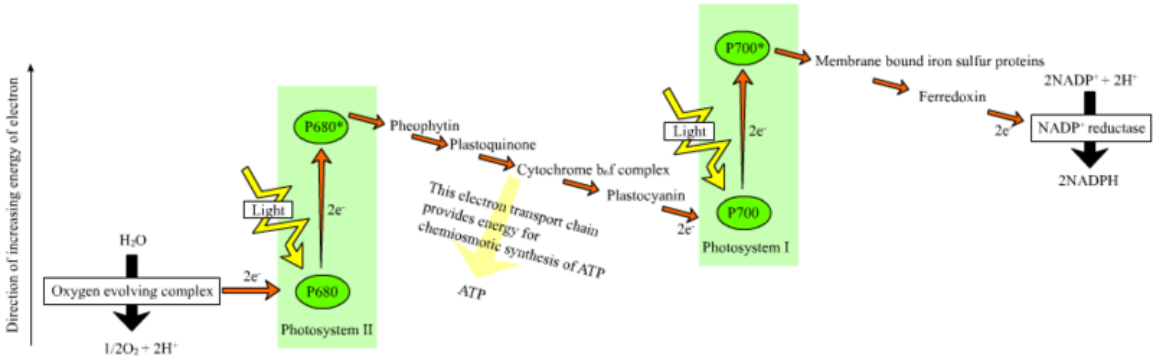
Light-dependent reactions of photosynthesis at the thylakoid membrane

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

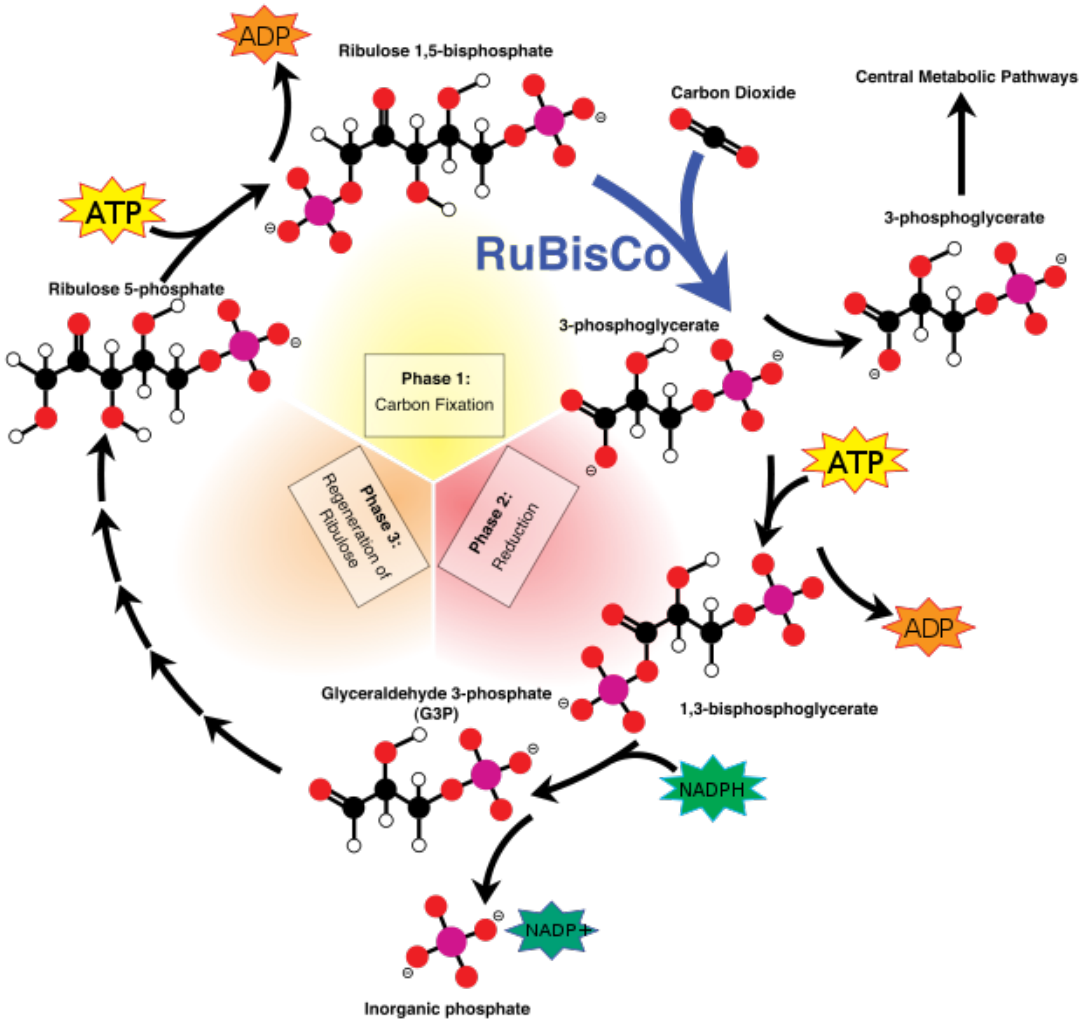
The NADPH is the main reducing agent in chloroplasts, providing a source of energetic electrons to other reactions. Its production leaves chlorophyll with a deficit of electrons (oxidized), which must be obtained from some other reducing agent. The excited electrons lost from chlorophyll in photosystem 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

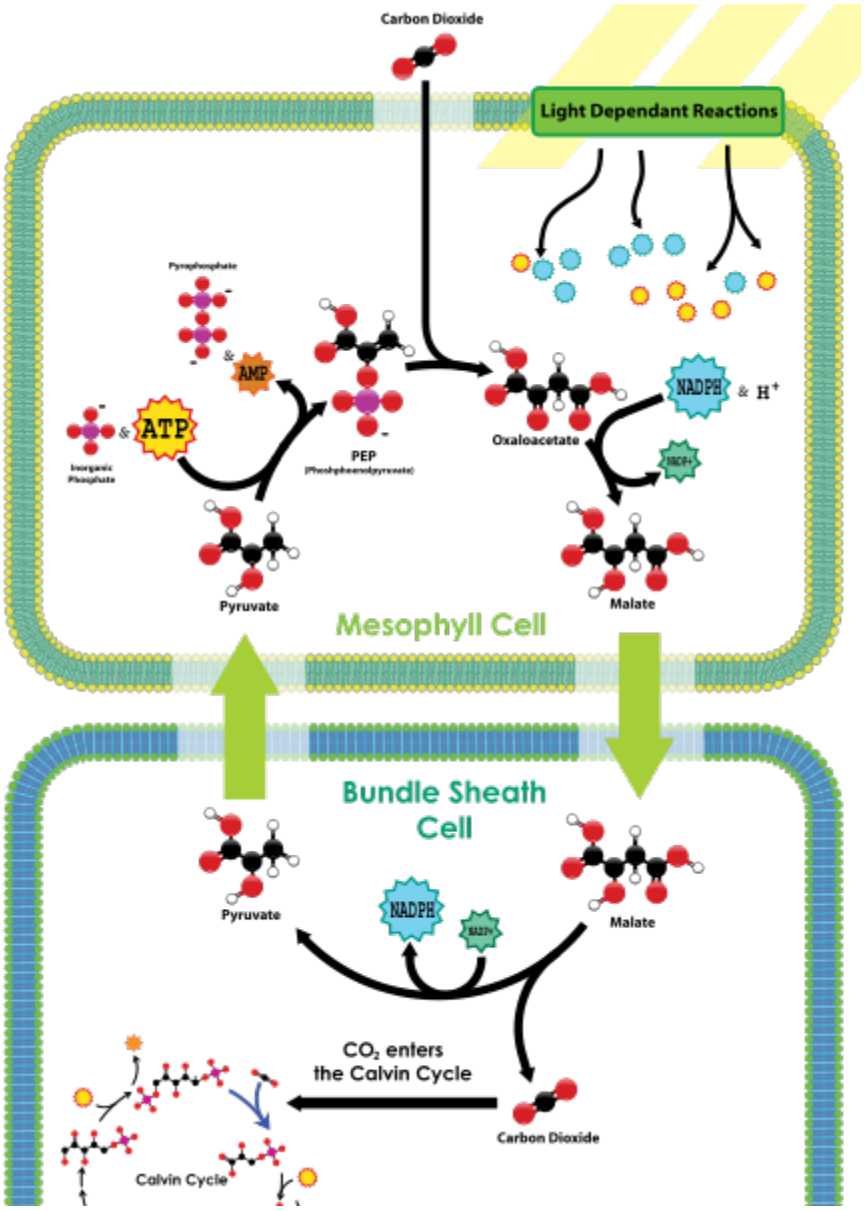
To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then variously used to form other organic compounds, such as the building material cellulose, as precursors for lipid 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, creating 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. Sixteen thousand 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

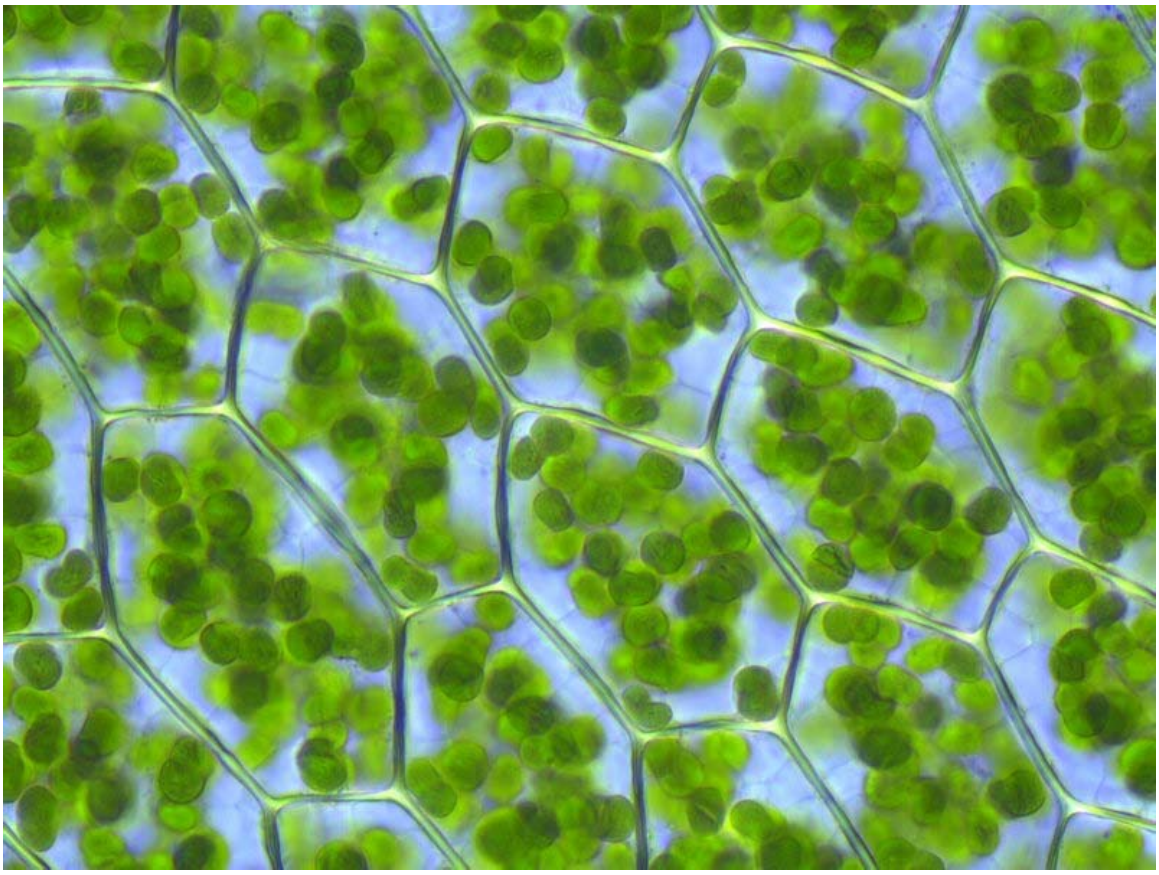
The overall process of photosynthesis takes place in four stages. The first, energy transfer in antenna chlorophyll takes place in the femtosecond (1 femtosecond (fs) = 10^{-15} s) to

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, which 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. It is presumed that this is due to the particularly simple body plans and large surface areas of these animals 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, it is speculated, 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 afterward, 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 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.

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:

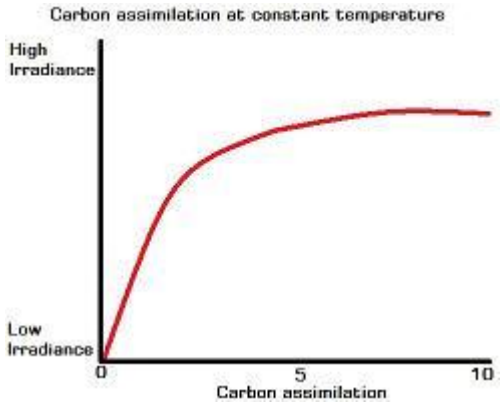
- Light irradiance and wavelength
- Carbon dioxide concentration
- Temperature.

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 seen only 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: First, from research it is known that, in general, photochemical reactions are not 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.