Plant Physiology and Biochemistry

Photosynthesis and transport of organic substances

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PHOTOSYNTHESIS

Photosynthesis and significance

All organisms use energy to carry out the functions of life. Some organisms obtain this energy directly from sunlight. Photosynthesis is the process by which plants, some bacteria, and some protistans use the energy from sunlight to produce sugar, which cellular respiration converts into ATP, the “fuel” used by all living things. The conversion of unusable sunlight energy into usable chemical energy is associated with the actions of the green pigment chlorophyll. The photosynthetic process uses water and release the oxygen.

The overall reaction of this process as:

\[ 6\text{H}_2\text{O} + 6\text{CO}_2 \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \]

Fig. 1

Historical aspects

Most autotrophs or producers use photosynthesis, to convert the energy of sunlight into chemical energy. Photosynthesis is the formation of carbohydrates from carbon dioxide and water, through the action of light energy on a light-sensitive pigment, such as chlorophyll, and usually resulting in the production of oxygen. Prior to this, the atmosphere was mainly composed of carbon dioxide, with other gases such as nitrogen, carbon monoxide, methane, hydrogen and sulphur gases present in smaller quantities. The earliest evidence for photosynthetic bacteria - suspected to be cyanobacteria (blue green algae) - is dated at sometime between 3.5 and 2.75 billion years ago. These first photosynthetic organisms would have been responsible for releasing oxygen into the atmosphere.
It probably took over 2 billion years, from the initial advent of photosynthesis for the oxygen concentration in the atmosphere to reach the level it is at today. As oxygen levels rose, some of the early anaerobic species probably became extinct, and others probably became restricted to habitats that remained free of oxygen. Some assumed a lifestyle permanently lodged inside aerobic cells. The anaerobic cells might, initially, have been incorporated into the aerobic cells after those aerobes had engulfed them as food. Alternatively, the anaerobes might have invaded the aerobic hosts and become parasites within them. Either way, a more intimate symbiotic relationship subsequently evolved between these aerobic and anaerobic cells. In these cases the survival of each cell was dependent on the function of the other cell.

Research on photosynthesis has been closely linked to knowledge of the growth cycles and physical structure of plants. In the 1640s, the work of both Johannes (Jan) Baptista van Helmont (1577 - 1644) and English clergyman and physiologist Stephen Hales indicated that plants require air and water to grow. In the 1700s, chemists began to identify the individual gases involved in the processes of combustion, respiration, and photosynthesis. Joseph Priestley (1733 - 1804) demonstrated that green plants can replenish stale, or oxygen-poor, air so that it is capable of supporting combustion and respiration. Dutch doctor and plant physiologist Jan Ingenhousz (1730 - 1799), inspired by Priestley's research, later learned that only the green parts of plants can revitalize stale air that is, take in carbon dioxide and release oxygen and that they do so only in the presence of sunlight. This was the first indication of light's role in the photosynthetic process. Ingenhousz also discovered that only the light of the Sun and not the heat it generates is necessary for photosynthesis.

In the nineteenth century, research on photosynthesis is centered on the chemical processes in which carbon is "fixed" in carbohydrates. In the late 1800s, German botanist Julius von Sachs (1832 - 1897) suggested that starch is a product of carbon dioxide. He also argued in 1865 that, in the presence of light, chlorophyll catalyzes photosynthetic reactions, and he discovered the chlorophyll-containing chloroplasts. In the 1880s, German physiologist Theodor Wilhelm Engelmann (1843-1909) showed that the light reactions, which capture solar energy and convert it into chemical energy, occur within the chloroplasts and respond only to the red and blue hues of natural light.

It was not until the twentieth century that scientists began to understand the complex biochemistry of photosynthesis. Richard Willstätter recognized that there were two major types of chlorophyll in land plants: blue-green, or "a" type, and yellow-green, or "b" type. Martin David Kamen, a Canadian-born American biochemist, used the isotope (forms of an element having the same atomic number but a different atomic weight due to a different number of neutrons) oxygen-18 to trace the chemical's role in the process. He confirmed that the oxygen created during photosynthesis comes only from the water molecules. Germ biochemist Otto Warburg found that, under suitable conditions, the efficiency of the photosynthetic process can approach 100%, meaning that nearly all of the Sun's energy is converted to chemical energy.

In 1940, the discovery of carbon-14, a radioactive isotope of carbon isolated by Kamen, allowed for more detailed studies of photosynthesis. Using carbon-14, Melvin Calvin was able to trace carbon's path through the entire photosynthetic process. During the 1950s and 1960s, he confirmed that the light reactions involving chlorophyll instantly capture the Sun's energy. Then he studied the subsequent dark reactions, so-called because they can take place without sunlight, find that carbohydrate molecules begin to form at this stage of the process. Working with green algal cells, Calvin interrupted the photosynthetic process at different stages and plunged the cells into an alcohol solution. Then, using the laboratory technique called paper chromatography; he analyzed the cells and the chemicals that had been produced, identifying at least ten intermediate products that had been created within a few seconds. This series of reactions is now called the Calvin Benson Cycle.
Photosynthetic pigments

A pigment is any substance that absorbs light. The colour of the pigment comes from the wavelengths of light reflected (in other words, those not absorbed). Chlorophyll, the green pigment common to all photosynthetic cells, absorbs all wavelengths of visible light except green, which it reflects to be detected by our eyes. Black pigments absorb all of the wavelengths that strike them. White pigments/lighter colors reflect all or almost all of the energy striking them. Pigments have their own characteristic absorption spectra, the absorption pattern of a given pigment.

Chlorophyll is a complex molecule. Several modifications of chlorophyll occur among plants and other photosynthetic organisms. All photosynthetic organisms (plants, certain protistans, prochlorobacteria, and cyanobacteria) have chlorophyll a.

**Fig.2. Structure of Chlorophyll a**

Accessory pigments absorb energy that chlorophyll a does not absorb. Accessory pigments include chlorophyll b (also c, d, and e in algae and protistans), xanthophylls, and carotenoids (such as beta-carotene). Chlorophyll a absorbs its energy from the Violet-Blue and Reddish orange-Red wavelengths, and little from the intermediate (Green-Yellow-Orange) wavelengths. Accessory pigments absorb light of shorter (i.e. higher energy) wavelengths than does chlorophyll a, so they increase the width of the spectrum available for photosynthesis. Energy transfer occurs in the sequence: carotenoids → chlorophyll b → chlorophyll a → reaction centre, with the whole antenna acting like a funnel, channelling the energy of absorbed quanta to the reaction centre. Different accessory pigments occur in other photosynthetic organisms; for example, red algae and cyanobacteria contain chlorophyll d, carotenoids and phycobiliproteins (which absorb green wavelengths).

In addition to pigments, antennae also contain structural proteins, which are different for photosystems I and II and play an important protective role. The terms light-harvesting complexes I and II (LHCI and LHClI) are often used to describe the pigment–protein complexes associated with PSI and PSII respectively. Photosynthetic electron transport and photophosphorylation occur on the membranes or lamellae (singular lamella) in chloroplasts, but the molecular components are not arranged randomly. This non-random arrangement is significant for control and coordination of the light reactions, so it is worth examining closely.
**Photoinhibition and photo-oxidation**

Excess light can be harmful and plants have evolved a wide variety of mechanisms that prevent or minimize this harm, with the minimum ‘cost’ in terms of energy and resources. A first line of defence operates at the level of leaf behaviour and structure and involves decreased light absorption. Some plants adjust the orientation of leaf blades so that they lie parallel to the Sun’s rays and thus minimize light interception.

The efficiency of the light reactions was estimated by measuring fluorescence characteristics which are proportional to quantum efficiency (the number of light quanta absorbed per molecule of O₂ evolved). Leaf folding (compared with not folding) results in: (1) greater efficiency of the light reactions, i.e. less photoinhibition, during sunflecks; and (2) no inhibition of photosynthesis after the sunfleck. Post-sunfleck inhibition in non-folding leaves results from damage, which takes time (and energy) to repair. Some plants from open habitats show similar leaf movements, but it is not a common response to excess light.

More common in high-light environments are longer-term adaptations such as the development of a thick waxy layer on the leaf surface. Such layers may perform several functions, including protection from insect or fungal attack and reduction of water loss, but they also reflect light very effectively. Experimental removal of surface wax from the succulent plant *Cotyledon orbiculata*, for example, increased light absorption by 50% and greatly increased photoinhibition in strong light.

The other lines of defence against excess light all involve molecular mechanisms that are universal among plants but are developed to different degrees in different habitats. Before describing these mechanisms, we need to examine more closely why light may cause damage. The basic cause is the formation of highly dangerous forms of oxygen, known as reactive oxygen species (ROS), which include the superoxide anion radical (essentially an oxygen molecule with an extra electron). ROS are all free radicals and are especially harmful to membranes.

During the photosynthetic light reactions, ROS may form either by energy transfer from excited chlorophyll molecules to O₂ (when reaction centers are too few to accept all the absorbed energy), or by transfer of electrons to O₂ from carriers such as ferredoxin (when there are too few suitable electron acceptors).

**Molecular protective mechanisms**

Molecular protective mechanisms against light-induced ROS operate at three levels:

1. Prevention of ROS formation by dissipating as heat the excess light energy absorbed by pigments. Heat dissipation is mediated by a pigment called zeaxanthin, a type of carotenoid pigment belonging to a group called the xanthophylls. As light levels increase, zeaxanthin is synthesized enzymically from another, precursor xanthophyll and accumulates in chloroplasts. The reverse occurs as light levels fall, i.e. conversion of zeaxanthin to its precursor, so that a xanthophyll cycle, finely tuned to prevailing light conditions, operates to protect leaves from light through thermal energy dissipation.
2. Rapid destruction of any ROS that form. The enzyme superoxide dismutase (SOD), for example, converts O$_2$ very efficiently to hydrogen peroxide, H$_2$O$_2$, which is then disposed of by other enzymes because its bleaching action is also potentially damaging.

3. If ROS start to build up, damage occurs first to PSII, culminating in the destruction of a particular protein, named D1, which acts as a weak link within the PSII complex. D1 has a very rapid turnover rate, with a half-life of about 21h, so it can be replaced within hours or days. So, by breaking the electron transport chain at one easily repaired link, damage to the rest of the photosynthetic machinery is minimized.
**Action spectra and enhancement effects**

The action spectrum of photosynthesis is the relative effectiveness of different wavelengths of light at generating electrons. If a pigment absorbs light energy, one of three things will occur. Energy is dissipated as heat. The energy may be emitted immediately as a longer wavelength, a phenomenon known as **fluorescence**.

Energy may trigger a chemical reaction, as in photosynthesis. Chlorophyll only triggers a chemical reaction when it is associated with proteins embedded in a membrane (as in a chloroplast) or the membrane infoldings found in photosynthetic prokaryotes such as cyanobacteria and prochlorobacteria.

![Absorption spectra of different plant pigments](image)

**Fig. 4. Absorption spectra of different plant pigments**

**Stages of Photosynthesis**

Photosynthesis is a two stage process. The first process is the Light Dependent Process (Light Reactions), requires the direct energy of light to make energy carrier molecules that are used in the second process. The Light Independent Process (or Dark Reactions) occurs when the products of the Light Reaction are used to form C-C covalent bonds of carbohydrates. The Dark Reactions can usually occur in the dark, if the energy carriers from the light process are present. Recent evidence suggests that a major enzyme of the Dark Reaction is indirectly stimulated by light, thus the term Dark Reaction is somewhat of a misnomer. The Light Reactions occur in the grana and the Dark Reactions take place in the stroma of the chloroplasts.

Chlorophyll is a complex molecule. Several modifications of chlorophyll occur among plants and other photosynthetic organisms. All photosynthetic organisms (plants, certain protists, prochlorobacteria, and cyanobacteria) have chlorophyll a. Accessory pigments absorb energy that chlorophyll a does not absorb. Accessory pigments include chlorophyll b (also c, d, and e in algae and protists), xanthophylls, and carotenoids (such as beta-
carotene). Chlorophyll a absorbs its energy from the Violet-Blue and Reddish orange-Red wavelengths, and little from the intermediate (Green-Yellow-Orange) wavelengths.

Carotenoids and chlorophyll b absorb some of the energy in the green wavelength. Why not so much in the orange and yellow wavelengths? Both chlorophylls also absorb in the orange-red end of the spectrum (with longer wavelengths and lower energy). The origins of photosynthetic organisms in the sea may account for this. Shorter wavelengths (with more energy) do not penetrate much below 5 meters deep in sea water. The ability to absorb some energy from the longer (hence more penetrating) wavelengths might have been an advantage to early photosynthetic algae that were not able to be in the upper (photic) zone of the sea all the time.

**The structure of the chloroplast and photosynthetic membranes**

The thylakoid is the structural unit of photosynthesis. Both photosynthetic prokaryotes and eukaryotes have these flattened sacs-vesicles containing photosynthetic chemicals. Only eukaryotes have chloroplasts with a surrounding membrane.

Thylakoids are stacked like pancakes in stacks known collectively as grana. The areas between grana are referred to as stroma. While the mitochondrion has two membrane systems, the chloroplast has three, forming three compartments.

![Fig. 5 Structure of chloroplast](image)

![Fig. 5 Structure of chloroplast](image)
**Light Reactions**

In the Light Dependent Processes (Light Reactions) light strikes chlorophyll a in such a way as to excite electrons to a higher energy state. In a series of reactions the energy is converted (along an electron transport process) into ATP and NADPH. Water is split in the process, releasing oxygen as a by-product of the reaction. The ATP and NADPH are used to make C-C bonds in the Light Independent Process (Dark Reactions).

In the Light Independent Process, carbon dioxide from the atmosphere (or water for aquatic/marine organisms) is captured and modified by the addition of Hydrogen to form carbohydrates (general formula of carbohydrates is \([CH_2O]_n\)). The incorporation of carbon dioxide into organic compounds is known as carbon fixation. The energy for this comes from the first phase of the photosynthetic process. Living systems cannot directly utilize light energy, but can, through a complicated series of reactions, convert it into C-C bond energy that can be released by glycolysis and other metabolic processes.

Photosystems are arrangements of chlorophyll and other pigments packed into thylakoids. Many Prokaryotes have only one photosystem, Photosystem II (so numbered because, while it was most likely the first to evolve, it was the second one discovered). Eukaryotes have Photosystem II plus Photosystem I. Photosystem I uses chlorophyll a, in the form referred to as P700. Photosystem II uses a form of chlorophyll a known as P680. Both "active" forms of chlorophyll a function in photosynthesis due to their association with proteins in the thylakoid membrane.

Photophosphorylation is the process of converting energy from a light-excited electron into the pyrophosphate bond of an ADP molecule. This occurs when the electrons from water are excited by the light in the presence of P680. The energy transfer is similar to the chemiosmotic electron transport occurring in the mitochondria. Light energy causes the removal of an electron from a molecule of P680 that is part of Photosystem II. The P680 requires an electron, which is taken from a water molecule, breaking the water into H\(^+\) ions and O\(^{-2}\) ions. These O\(^{-2}\) ions combine to form the diatomic O\(_2\) that is released. The electron is "boosted" to a higher energy state and attached to a primary electron acceptor, which begins a series of redox reactions, passing the electron through a series of electron carriers, eventually attaching it to a molecule in Photosystem I.

Light acts on a molecule of P700 in Photosystem I, causing an electron to be "boosted" to a still higher potential. The electron is attached to a different primary electron acceptor (that is a different molecule from the one associated with Photosystem II). The electron is passed again through a series of redox reactions, eventually being attached to NADP\(^+\) and H\(^-\) to form NADPH, an energy carrier needed in the Light Independent Reaction. The electron from Photosystem II replaces the excited electron in the P700 molecule. There is thus a continuous flow of electrons from water to NADPH. This energy is used in Carbon Fixation. Cyclic Electron Flow occurs in some eukaryotes and primitive photosynthetic bacteria. No NADPH is produced, only ATP. This occurs when cells may require additional ATP, or when there is no NADP\(^+\) to reduce to NADPH. In Photosystem II, the pumping to H\(^+\) ions into the thylakoid and the conversion of ADP + P into ATP is driven by electron gradients established in the thylakoid membrane.

Cyclic electron flow involves absorption of light by PSI and transfer of excited electrons via ferredoxin to plastoquinone, cytochrome b6f and then, via the mobile carrier plastocyanin, back to PSI. No NADP.2H is produced, but the coupled proton pumping can be used for ATP synthesis. This cyclic flow operates mainly in the stroma lamellae and provides a flexible way of generating ATP in situations where there is a need for ATP but relatively less demand for reducing power. At present, however, there is no completely satisfactory explanation for the wide separation of the two photosystems in chloroplast lamellae. The arrangement is strikingly different from that in mitochondria, where electron carriers are all packed closely together.
Noncyclic photophosphorylation which is also known as noncyclic electron flow occurs in the thylakoids at the same time as cyclic electron flow. Actually Photosystem I participates in both processes. In noncyclic electron flow however the excited electron is not cycled back to chlorophyll but instead is passed off through a series of redox reactions to oxidized NADP+ to form reduced NADPH (nicotinamide adenine dinucleotide phosphate), a high energy reduced enzyme that represents trapped solar energy.

![Localization of electron transport chain](image)

**Fig. 6 Localization of electron transport chain**

Halobacteria, which grow in extremely salty water, are facultative aerobes, they can grow when oxygen is absent. Purple pigments, known as retinal (a pigment also found in the human eye) act similar to chlorophyll. The complex of retinal and membrane proteins is known as bacteriorhodopsin, which generates electrons which establish a proton gradient that powers an ADP-ATP pump, generating ATP from sunlight without chlorophyll. This supports the theory that chemiosmotic processes are universal in their ability to generate ATP.
Dark Reactions

Carbon-Fixing Reactions are also known as the Dark Reactions (or Light Independent Reactions). Carbon dioxide enters single-celled and aquatic autotrophs through no specialized structures, diffusing into the cells. Land plants must guard against drying out (desiccation) and so have evolved specialized structures known as stomata to allow gas to enter and leave the leaf.

The Calvin Cycle occurs in the stroma of chloroplasts (where would it occur in a prokaryote?). Carbon dioxide is captured by the chemical ribulose biphosphate (RuBP). RuBP is a 5-C chemical. Six molecules of carbon dioxide enter the Calvin Cycle, eventually producing one molecule of glucose. The reactions in this process were worked out by Melvin Calvin and his co-workers.

Fig. 6 Localization of electron transport chain

The first stable product of the Calvin Cycle is phosphoglycerate (PGA), a 3-C chemical. Hence called C3 pathway. The energy from ATP and NADPH energy carriers generated by the photosystems is used to attach phosphates to (phosphorylate) the PGA. Eventually there are 12 molecules of glyceraldehyde phosphate (also known as phosphoglyceraldehyde or PGAL, a 3-C), two of which are removed from the cycle to make a glucose. The remaining PGAL molecules are converted by ATP energy to reform 6 RuBP molecules, and thus start the cycle again. Remember the complexity of life, each
reaction in this process, as in Kreb's Cycle, is catalyzed by a different reaction-specific enzyme.

Fig. 7. Overall reactions of the Calvin cycle
Adaptations to different light environments

The difference in light intensity, or ‘strength’, between a deeply shaded forest floor and midday tropical sun in the open is about 160-fold, i.e. from 15–2400 µmol m⁻² s⁻¹ of light quanta of wavelengths 400–700 nm (the photosynthetic photon flux density or PPFD), yet plants flourish over this whole range of light conditions. Some species are genetically adapted to permanent shade, others to full sun and yet others tolerate or adapt physiologically to a wide range of light.

**Shade plants** have a range of adaptations to their environment, which may be determined genetically or result from acclimation. For example, they have very low rates of respiration. Here, however, we consider the adaptations that allow them to harvest light very efficiently when it is available at low average intensity, is relatively enriched in longer wavelengths compared with sunlight and may show brief periods of high intensity during sunflecks.

The first type of adaptation is at the level of whole leaves. Compared with a leaf that developed in the open (sun leaf), a leaf that developed in shade is much thinner overall and, in particular, has a very shallow layer of palisade mesophyll and patchy spongy mesophyll with more air spaces. It takes energy and resources to construct and maintain thick leaves, so this minimal structure of shade leaves is an efficient way in which to harvest the meagre supply of light normally available.

Other types of adaptation occur at a biochemical level within chloroplasts. For example, shade leaves have more chlorophyll molecules in the antenna systems that collect and feed light energy to each reaction centre. In addition, there is much greater proportion of PSII relative to PSI, which is often reflected in the presence of wide grana containing large numbers of stacked thylakoids, giving a ratio of appressed to non-appressed lamellae up to five times greater than in sun leaves. PSII is located mainly on the appressed lamellae of grana and PSI on the non-appressed lamellae on the outside of grana or in the stroma. The increase in PSII in shade plants relates to the far-red enrichment of shade light. The reaction centre of PSII shows maximum light absorption at a slightly shorter wavelength (680 nm) compared with the reaction centre of PSI (700 nm) hence the names of the reaction centres, P680 and P700 respectively, where P stands for ‘pigment’. This difference in absorption properties means that in far-red-enriched light, PSI is relatively more excited (emitting energized electrons at a faster rate) than PSII. But the smooth operation of non-cyclic electron transport requires that the two photosystems are excited equally, hence the requirement in shady habitats for increased absorption by PSII, by either increasing the number of reaction centres or the size of the pigment funnels channelling energy to each PSII reaction centre.

Finally, there is the question of sunflecks which, for leaves in deep shade, may provide nearly half the daily light income, but which are potentially damaging for shade leaves. Damage may arise because sunflecks are not far-red-enriched and hence over-excitation of PSII can occur. Such over-excitation literally, funnelling more energy to PSII reaction centres than they can deal with or, alternatively, having too small a pool of electron acceptors to cope with the flow of excited electrons from reaction centres. This leads to a form of reversible inhibition of photosynthesis called photoinhibition. This phenomenon is widespread in plants and is not restricted to shade plants exposed to sunflecks. Other adaptations that are necessary for the efficient use of precious sunfleck light do not involve the light reactions per se but rather: an ability to increase rapidly the rate of carbon fixation, i.e. make use of the increased supply of NADP2H and ATP.

An ability to tolerate abrupt increases in leaf temperature (as much as 201°C during prolonged, bright sunflecks), which increase water loss and may cause wilting; there is evidence that plants growing in moderately shaded habitats where sunflecks are relatively prolonged or abundant have larger root systems, which facilitate water uptake compared with plants adapted to more deeply shaded sites. So adaptation of individual leaves or whole plants to variable light conditions (i.e. sunflecks) involves many aspects of their...
physiology. Not all sunflecks are intense enough to cause photoinhibition, however, and in shade leaves, redistribution of light energy from PSII to PSI must occur so that both photosystems are again equally excited. **Energy redistribution** is thought to occur frequently in shade leaves, as the spectral composition of incident light shifts at different times of day or with different atmospheric conditions. It is a short-term, fine tuning of the photosynthetic light reactions, which allows light to be used with maximum efficiency. PSII is now thought to exist as a huge dimeric complex with two reaction centres plus associated proteins and two pigment–protein funnels or light-harvesting complexes (LHCII).

**C4 PHOTOSYNTHESIS**

This pathway is discovered by Hatch, Slaek and Kortschak (1960-1966). Some plants have developed a preliminary step to the Calvin Cycle (which is also referred to as a C-3 pathway), this preamble step is known as C-4. While most C-fixation begins with RuBP, C-4 begins with a new molecule, phosphoenolpyruvate (PEP), a 3-C chemical that is converted into oxaloacetic acid (OAA, a 4-C chemical) when carbon dioxide is combined with PEP. The OAA is converted to malic acid and then or aspartic acid from the mesophyll cell into the bundle-sheath cell, where malic acid or aspartic acid are broken down into PEP plus carbon dioxide. The carbon dioxide then enters the Calvin Cycle, with PEP returning to the mesophyll cell. The resulting sugars are now adjacent to the leaf veins and can readily be transported throughout the plant.

The capture of carbon dioxide by PEP is mediated by the enzyme PEP Carboxylase, which has a stronger affinity for carbon dioxide than does RuBP Carboxylase. When carbon dioxide levels decline below the threshold for RuBP carboxylase, RuBP is catalyzed with oxygen instead of carbon dioxide. The product of that reaction forms glycolic acid, a chemical that is be broken down by photorespiration which evolves CO₂, producing neither NADH nor ATP, in effect dismantling the Calvin Cycle. C-4 plants, which often grow close together, have had to adjust to decreased levels of carbon dioxide by artificially raising the carbon dioxide concentration in certain cells to prevent photorespiration. C-4 plants evolved in the tropics and are adapted to higher temperatures than are the C-3 plants found at higher latitudes. Common C-4 plants include crabgrass, corn, and sugar cane. OAA and malic acid and aspartic acid also have functions in other processes, thus the chemicals would have been present in all plants, leading scientists to hypothesize that C-4 mechanisms evolved several times independently in response to a similar environmental condition, a type of evolution known as convergent evolution.

In C3 plants, the vascular tissue is more or less embedded in the spongy mesophyll cells. In C4 plants, however, the vascular tissue is surrounded by an additional ring of bundle sheath cells that are closely connected with the spongy mesophyll. This is referred to as Kranz anatomy, because “kranz” is German for wreath, and Germans were big into plant anatomy in the 1800's. It's important to note that all of the machinery for C3 dark reactions (RUBISCO, etc.) is concentrated in the bundle sheath cells. So what's the purpose of the mesophyll cells? Let's look at C4 photosynthesis as a 4-step process.
Fig. 8: Leaf Anatomy of a C3 Plant  Leaf Anatomy of a C4 Plant

Stages of CO₂ fixation

1. Fixation of CO₂ in mesophyll with a 3-C molecule of PEP to form a 4-C organic acid, such as OAA (the enzyme that does this is PEP carboxylase). OAA is unstable compound and is immediately gets converted into malate and or aspartate. Note that this is not the typical fixation of CO₂ by Rubisco, which binds CO₂ to the 5-C RuBp.

2. transport of the C4 acids (malate or aspartate) to the bundle sheath cells

3. decarboxylation of the C4 acids within the bundle sheath cells to generate CO₂ and pyruvate. CO₂ is then used in the Calvin cycle—just like regular C3 photosynthesis.

4. transport of the C3 acid (PEP) back to the mesophyll cells, where the whole process starts all over again.
Fig. 9. C4 Photosynthetic Pathway


**Fig. 9. C4 Photosynthetic Pathway**

*Why is this ecologically significant?*

The PEP carboxylase in the mesophyll cells has a high affinity for CO₂. By rapidly converting CO₂ into a 4-C molecule, this reduces the internal concentration of CO₂ and creates a steep gradient of high CO₂ outside the leaf and low CO₂ inside the mesophyll cell. Further, C4 acids synthesized translocate to bundle sheath cells and their decarboxylation creates high CO₂ concentration in the bundle sheath cells which increase rate of CO₂ fixation by Rubisco.

- The C4 plant's strategy is to make a large gradient and then lower the conductivity = increase leaf resistance by closing stomates. For C4 plants growth in ambient 360 ppm CO₂, the Ci is around 100 ppm, creating a gradient of 260 ppm.

- The C3 leaf, lacking the CO₂ accumulation "pump", has a weaker gradient and must have higher leaf conductivity to let more CO₂ into the leaf. For C3 plants growth in ambient 360 ppm CO₂, the Ci is around 250 ppm, creating a gradient of 110 ppm.

So in the end, both plants may photosynthesize at the same rate, but the C4 plant does it with much tighter stomates, preventing water loss. We say that this kind of photosynthesis has high **water use efficiency (carbon uptake/water loss)** and this is a beneficial strategy where water is limited (like grasslands and the deserts).

Physiologically C3 and C4 plants do not inherently different in the degree to which they withstand severe drought and the main difference in performance in response to aridity is greater water use efficacy of the C4 pathway. At operating internal CO₂ concentrations in normal air for non-stressed plants, C4 species have over twice the water use efficacy of C3 plants with equivalent photosynthesis rate. This is because C4 plants can match the CO₂ assimilation rate of a C3 plant with about half the stomatal conductance and hence half the rate of water loss. As a result, for given amount of soil water, C4 species are able to develop a larger canopy, grow more root mass and produce more seeds than their C3 competitors. The bundle sheath cells accumulate CO₂ at a much higher concentration than you would expect if they were in direct contact with the atmosphere. Higher levels of CO₂ means that Rubisco spends more of its time in fixing CO₂ rather than O₂ and
photorespiration is reduced. Remember that photorespiration increases dramatically in C3 plants as temperature is increased. This doesn't happen in C4 plants, so C4 plants are often more competitive in environments with higher temperature (because C3 plants lose a lot of C to photorespiration)

**Evolution of C4 pathway**

Until the last 50 million years ago, Earth's atmosphere was loaded with CO₂--levels between 5-10 times higher than today's atmosphere. From 540 MYA (Million Years Ago) to about 260 MYA the earth's plates were moving together to forming more-or-less one giant continent called "Pangea." Over the last 200 MY, the continental plates have been moving apart, separating the continents with wide oceans. Around 50 million years ago, the Indian subcontinent slammed into Asia as a result of plate techtonics, initiating the uplift of the Himalayan Mountains.

This large amount of exposed rock surface was subject to weathering by carbonic acid, produced when atmospheric H₂O and CO₂ combine to form H₂CO₃. The weathering process of silicate rocks can be represented generally as

\[
\text{CaSiO}_3 + \text{H}_2\text{CO}_3 \rightarrow \text{HCO}_3^- + \text{Ca}^{2+} + \text{SiO}_2
\]

(silicate)

These materials flow in rivers to the ocean where marine organisms use them to build shells:

\[
\text{Ca}^{2+} + \text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{H}_2\text{O}
\]

(Calcium carbonate shells)

Thus, this overall rock weathering process scrubs CO₂ out of the atmosphere and buries the C in the deep ocean. Over the last 50 million years, this weathering process has lowered the atmospheric CO₂ to levels between 180-400 ppm. Therefore, CO₂ in the last 5-7 million years has become a limiting resource for plants, and natural selection would favor innovative biochemical pathways that allow plants to concentrate CO₂.

Plants subject to lower CO₂ are subject to relatively higher rates of O₂ and photorespiration, leading to carbon loss. C-4 photosynthesis evades photorespiration by nearly saturating Rubisco with CO₂.

C4 plants represent only about 3% of the world's flora (C3 are 87%; CAM are 10%), suggesting that not many plants use the C4 path. C4 plants need an additional 2 ATP to regenerate PEP. As we might expect, this is an extra energy expense. However, think about what environments this added expense is truly costly. If the light reactions make ATP, then you might expect a couple of extra ATP would be easy to make in high light environments. This is not as simple for shaded plants or plants that grow in cloudy conditions or plants growing under temperate conditions where light is limiting factor. Rate of photosynthesis of the C4 plants is always more than the C3 plants under tropical and subtropical conditions where sufficient amounts of light is available.

You see it in plants that live all over the world, and it has evolved independently many times.
• it has evolved 45 separate times
• found in at least 19 families
• found only in advanced families
• within families, it's only in advanced genera, indicating it's a recent evolutionary step
• its absence from woody plants except Chenopodeaceae

There are three common variants of the pathway, indicating that the basic CO2 pump is fairly easy to evolve using different biochemical constituents for the 3 and 4-C organic acids.

**Carbon isotopes** are an incredibly cool way to estimate WUE (Water Use Efficiency) among the C3, C4, and CAM pathways.

Carbon occurs naturally in three forms, or isotopes, depending on the number of neutrons: 12C, 13C, and 14C

- 12C is by far the most abundant form (6 protons and 6 neutrons in the nucleus)
- 13C is stable isotope of carbon (1.1% of total carbon) with 6 protons and 7 neutrons in the nucleus
- 14C is radiocarbon that has 6 protons and 8 neutrons in the nucleus. It is unstable and one of the neutrons degrades into a proton and a negatron (e-, a nuclear electron). The negatron is emitted from the atom as Beta emission, and the resulting atom is 14N (7 protons and 7 neutrons).

Among the carbon isotopes, 14C is the heaviest, 13C next heaviest, and 12C the lightest form of C. Here's why this is useful:

- 13C is heavier than 12C so moves more slowly in all physical and chemical processes
- Diffusion into the leaf discriminates 4‰ (parts per thousand)—think of % as parts per hundred ‰ adds extra 0: parts per thousand
- Carbon fixation by RUBISCO discriminates 27‰. This is because Rubisco has a higher affinity for the lighter isotope, 12C.

Therefore, biochemical pathways of plants are a major factor determining which carbon isotopes are incorporated into plant matter. For example, the atmosphere has a 13C content of -8‰ relative to standard (Pee Dee belemnite—a limestone from North Carolina). In contrast, plants have lower 13C content because discriminate against 13C. C3 plants under well watered conditions have 13C = -30 to -35.

Therefore autotrophic organisms always have more negative delta 13C ratios, because they discriminate against 13C. So far as we know, only living autotrophic organisms can impart this isotopic fractionation. Thus, you can grab a chunk of organic matter, figure out the C isotopes and determine if an organism produced it. This is how here's where things get interesting for physiological ecology:

C3 plants under well watered conditions have 13C = -30 to -35 (atmos + diffusion) under drought stress stomates close

- physical diffusion becomes the major limiting step
- plants discriminate: 13C comes closer to the atmosphere -20 to -30
- the less negative the number the greater the drought stress
C4 plants: PEP carboxylase shows virtually no discrimination between 12C and 13C.

- It has an affinity so high it absorbs everything that diffuses into cell
- Therefore the 13C isotopic signal of C4 plants is $-8 + (-4) = -12$ to -15 (atmos + diffusion)

Recent studies indicated that many enzymes of the C3 and C4 pathways of photosynthesis are light dependent and hence the terminology Dark reactions of photosynthesis is not valid toady. Now it is called as C3 PCR (Photosynthetic Carbon Reduction) and C4 PCR cycle.

**CRASSULACEAN ACID METABOLISM (CAM)**

CAM photosynthesis is a second evolutionary strategy employing a "CO$_2$ pump" to accumulate CO$_2$. It is technically also a C4 pathway, because CO$_2$ is fixed first into a 4-C organic acid like oxaloacetate.

This path occurs in a wide variety of plant species, mainly in arid and tropical regions

- Cacti (barrel, saguaro, prickly pear, teddy bear cholla)
- Crassulaceae (Crassula aquatica, Sedum rosea)
- Bromeliads (pineapples, "air plants"—epiphytes such as Spanish moss)
- Orchids (Vanilla, Phalaenopsis, Dendrobium)
- Agave (Century plant, yucca)

*Ecological Significance*

The understanding of the potential value of CAM for plant carbon gain is based largely on studies of desert succulents and upper canopy epiphytes from tropical forests. When taken together, the phylogeny, biogeography, and physiology of CAM plants provide compelling evidence for the ecological significance of this photosynthetic pathway in sun-exposed, water limited habitats. Nocturnal CO$_2$ uptake coupled with stomatal closure during the day maximizes the ratio of carbon gain to water loss. High daytime partial pressures of CO$_2$ inside the leaf help to mitigate the photoinhibitory effects of high light common to these environments. The capacity to maintain a functioning photosynthetic apparatus by re-fixation of respired CO$_2$ when stomates are continuously closed during prolonged drought helps CAM plants to survive in exposed, xeric sites.

*Overall Process*

To make the C4 "CO$_2$ pump" happen, a plant needs spatial separation between the sites of initial CO$_2$ fixation (mesophyll) and decarboxylation of C4 acids and fixation of CO$_2$ by the C3 cycle in bundle sheath. The specialized Kranz anatomy allows this to happen.

CAM plants achieve the CO$_2$ accumulating mechanism, not by a spatial separation of CO$_2$ uptake and unloading, but, rather, a temporal separation of these two events. In CAM plants, C4 fixation occurs during the night when stomates are open. CO$_2$ is fixed by PEP carboxylase by combining with PEP to form oxaloacetate. This 4-C organic acid is easily transformed (reduced by NADPH) to malate and malate is stored in vacuoles.
Fig. 10. CAM (Crassulacean Acid Metabolism) Photosynthesis
1. This malate is then shuttled into the vacuole, where it is stored in high concentrations.

2. During daylight hours, the stomates are shut, preventing CO₂ uptake. At these hours, the malate is transported out of the vacuole, where it is decarboxylated to make CO₂ and pyruvate.

3. CO₂ released during day is fixed by the C3 photosynthesis (Calvin cycle)

The net result is that the plant cell has a lot of CO₂ in the mesophyll during daylight hours when it can be fixed by Rubisco in the Calvin cycle. The important, however, is that it loaded up all of the CO₂ without ever having to open the stomates during the daylight hours! At night, the relative humidity of the air is higher, and there is less water lost from the plant.

This is an extremely inefficient process for several reasons:

- requires large investment in organic intermediates
- ATP cost of PEP resynthesis
- plant growth rate is therefore very low
- Two types of C fixation--first by PEP carboxylase during night and the second by Rubisco during day.
- Malate accumulates in the dark as PEP carboxylase is binding a CO₂ with a PEP.
- The triose glucan 3 PGA is regenerated in the light as intermediate of the Calvin cycle and PEP is regenerated in the dark during glycolysis whereas malate is decarboxylated in the light.

**Interesting points about CAM photosynthesis:**

1. During the wet seasons, such as the late summer monsoons in the deserts many succulents switch to exclusively C3 photosynthesis. That is, the open their stomates during the day, and the CO₂ that enters the leaf is immediately fixed by Rubisco in the Calvin cycle.

2. CAM plants exhibit a very high WUE. There is often a tradeoff, however, between efficiency and rate of process.

3. **CAM idling** -- this is a phenomenon that happens with plants that shut their stomates for a long period of time.

- Stems of some desert succulents never open stomates during drought
- The CAM pathway serves only to recycle CO₂ released by respiration
- There is no net CO₂ gain.
- After rain, these plants can begin to gain carbon within 24 hours using the C3 pathway. This is a slow process, and plants require around 10 days to produce leaves and gain C.

Something similar to CAM idling occurs in some desert C3 species that keep their stomates shut for extended periods of time.
4. CAM plants have 13C of about -12 to -20

- when operating as CAM it is about -12
- when operating as C3 is about -25 (just like C3)
5. CAM Cycling

CAM cycling where stomata remain closed during the dark period but some nocturnal synthesis of organic acid fed by respiratory CO2 occurs, and where stomata are open during the light period with uptake of atmospheric CO2 and direct Calvin-cycle CO2 reduction (C3-photosynthesis) in addition to assimilation of CO2 remobilized from nocturnally stored organic acid.

Fig. 12. CAM Cycling
PHOTORESPIRATION

Photorespiration is a special type of respiration shown by green plants when they are exposed to light. The normal dark respiration (usual mitochondrial respiration) is independent of light and its rate is same in both light and dark. The photorespiration process is carried on only in the presence of light. The term photorespiration is referred to “the release of CO₂ in respiration in presence of light during photosynthesis”.

Importance of Photorespiration

a) Photorespiration is closely related to CO₂ compensation point.

b) It usually occurs only in those plants, which have comparatively high CO₂ compensation point and follow C3 photosynthesis (eg. Tomato, wheat, oats etc.)

c) It is insignificant or rather practically absent in C4 plants, which have very low CO₂ compensation point. (eg. maize, sugarcane, sorghum, pearl millet, amaranth etc.).

Sites of photorespiration

Photorespiration occurs only in chlorophyllous tissues of plants. The process of photorespiration occurs in three different organelles viz.,

1. Chloroplasts
2. Peroxisomes and
3. Mitochondria.

Steps of Photorespiration

Like usual mitochondrial respiration, the photorespiration is also an oxidative process where oxidation of RuBP occurs in chloroplast and hence is translocation of one molecule of 3 PGA (which enters in to Calvin cycle) and one molecule of phosphoglycerate which was 2C. This reaction is catalyzed by Rubisco i.e., RuBP oxygenase. Rubisco when it catalize RuBP + CO₂ reaction it is called RuBP carboxylase. Thus it has two activities because it can not distinguish between CO₂ and O₂. Various steps of photospiration are given in Fig. 13

A. Reactions in Chloroplast

O₂ competes with CO₂ for the enzyme RuBP carboxylase. This enzyme can not distinguish between CO₂ and O₂. When this enzyme reacts with O₂ instead of CO₂, it is called as RuBP oxygenase. In this case, one molecule of phosphoglyceric acid (PGA) and one molecule of phosphoglycolic acid are formed from RuBP. PGA then enters into the Calvin cycle. Phosphoglycolic acid is dephosphorylated to form glycolate, in the presence of the enzyme, phosphatase.

B. Reactions in Peroxisome

From chloroplasts, the glycolate migrates into peroxisome where it is oxidised to glyoxalate in the presence of glycolic acid oxidase. In this reaction, hydrogen peroxide (H₂O₂) is formed with the utilization of one molecule of O₂. The H₂O₂ is then removed by the enzyme catalase as follows:
Glyoxalate is converted into an amino acid, glycine by transamination reaction in the presence of L-Glutamate glyoxalate transaminase.

C. Reactions in Mitochondrion

Glycine (2C) formed in peroxisomes migrates into mitochondria. Now, two molecules of glycine react to form one molecule of another aminoacid, Serine (3C), liberating CO₂ (post-illumination burst of CO₂ i.e., photorespiration) and NH₃. This reaction is catalyzed by serine hydroxymethyl transferase. Serine thus formed is apparently recycled back into the pool of photosynthetic intermediates of Calvin cycle in chloroplasts. This is mediated by the formation of hydroxypyruvate and glyceric acid. On phosphorylation with ATP, glyceric acid is converted into PGA enters into Calvin cycle.

Therefore, starting from intermediates of Calvin cycle and with the synthesis of glycolate, serine is formed which is again converted into intermediates of Calvin cycle thus completing the glycolate cycle. The photorspiratory cycle is also called as C₂ cycle because the initial product of RuBP + CO₂ is phosphoglycerate which enters into phloem respiratory cycle and it has two carbons. Further glyoxalate and glycine are 2-C compounds.
Fig. 13. Reactions of the Photorespiratory (Oxidative Photosynthetic Carbon (C2)) Pathway
**Photorespiration – C4 plants:**

Theoretically photorespiration exists in the C4 plants because all enzymes of photorespiration present in the C4 plants. Further all intermediates of photorespiratory cycle are present in the C4 plants. However, evolved CO₂ by photorespiratory cycle is refixed either by PEPcase or by RuBPcase. Hence practically no evolution of CO₂. Hence practically photorespiration is absent in the C4 plant.

**Significance of Photorespiration in Crop-Productivity**

1. Often, presence of photorespiration is considered as a **wasteful** and **energy consuming** process in crop plants which ultimately leads to **reduction in final yield of crops**.

2. It is estimated that during C3 photosynthesis, up to 50% of the CO₂ fixed may have to pass through photorespiratory process (glycolate pathway) to form carbohydrates such as sucrose thereby resulting in considerable decrease of photosynthetic productivity. i.e., net rate of photosynthesis.

3. Unlike usual mitochondrial respiration, assimilatory powers (ATP or NADH₂) are not generated in photorespiration.

4. However, photorespiration is considered as metabolic adjunct to the Calvin cycle (i.e., it has been added to the **Calvin cycle**).

5. Because photorespiration process decreases photosynthetic efficiency of crop plants, scientists are working to increase the efficiency of C3 plants by decreasing photorespiration.

**Ways to reduce the effect of photorespiration would be:**

a) to increase the CO₂ concentration which avoids O₂ competition and

b) to develop varieties or strains of C3 plants, which have low photorespiration rate.

**Regulation of Photorespiration**

Negative effects of photorespiration on crop plants can be regulated and consequently, the photosynthetic productivity can be increased manifold. Possible measures would be:

1. By manipulating different atmospheric conditions i.e., by increasing atmospheric CO₂ etc.

2. Use of inhibitors of glycolic acid oxidase, such as hydroxysulphonates.

3. Through genetic manipulation also, the process of photorespiration can be regulated.

**Positive Effects of Photorespiration**

Recently, photorespiration process has been considered as a **protective** and **supportive mechanism**, which **reduces O₂ injury to chloroplasts**.

Free radicals of O₂ gas are very reactive which react with membrane components and destroy them. In the absence of photorespiration, concentration of such O₂ free radicals may reach very high and can attain a destructive level in the chloroplasts. Therefore, under such circumstances, efforts to reduce photorespiration may prove dangerous. Further in the
absence of CO₂, photorespiratory process maintain intermediates of Calvin cycle so that when CO2 is available Calvin cycle starts.

**Factors Affecting Photorespiration**

Following factors are known to influence the rate of photorespiration:

1. Comparative concentration of CO₂ and O₂.
2. Plant species (C3 or C4 plant)
3. Higher temperature.
4. Increase in CO₂ concentration.
5. Inhibitors of glycolic acid oxidase such as hydroxysulphonates inhibit the process of photorespiration.
THE CARBON CYCLE

Plants may be viewed as carbon sinks, removing carbon dioxide from the atmosphere and oceans by fixing it into organic chemicals and release O₂. Plants also produce some carbon dioxide by their respiration, but this is quickly used by photosynthesis. Plants also convert energy from light into chemical energy of C-C covalent bonds. Animals are carbon dioxide producers that derive their energy from carbohydrates and other chemicals produced by plants by the process of photosynthesis.

The balance between the plant carbon dioxide removal and animal carbon dioxide generation is equalized also by the formation of carbonates in the oceans. This removes excess carbon dioxide from the air and water (both of which are in equilibrium with regard to carbon dioxide). Fossil fuels, such as petroleum and coal, as well as more recent fuels such as peat and wood generate carbon dioxide when burned. Fossil fuels are formed ultimately by organic processes, and represent also a tremendous carbon sink.

Human activity has greatly increased the concentration of carbon dioxide in air. This increase has led to global warming, an increase in temperatures around the world, the Greenhouse Effect. The increase in carbon dioxide and other pollutants in the air has also led to acid rain, where water falls through polluted air and chemically combines with carbon dioxide, nitrous oxides, and sulfur oxides, producing rainfall with pH as low as 4. This results in fish kills and changes in soil pH which can alter the natural vegetation and uses of the land. The Global Warming problem can lead to melting of the ice caps in Greenland and Antarctica, raising sea-level as much as 120 meters. Changes in sea-level and temperature would affect climate changes, altering belts of grain production and rainfall patterns.
TRANSPORT OF ORGANIC SUBSTANCES

The plant is a complex of specialized organs. Proper functioning of these organs depends on balanced and integrated transfer of materials within these complex structures. Materials absorbed by the roots must be moved to the leaves for assimilation. Water and inorganic salts move up in the xylem while minerals are redistributed from the leaves through the phloem. There must be ways for the solutes from photosynthesis and other biochemical pathways to depart from the leaf and go both down to the root and up to flowers, fruits, and apical meristems to provide energy for respiration carried out in these non-photosynthetic areas. So leaves are the likely source of small organic molecules, and the rest of the plant organs are sinks for those molecules. The flow of these solutes then must be able to be both upward and downward in the plant. The solutes could be any of the subunits of the macromolecules; examples include sugars, amino acids, nucleotides, and fatty acids.

The flow of these organic molecules is called translocation and this process occurs mostly through the phloem. While phloem lies alongside the xylem in veins in leaves, vascular bundles in stems, and the vascular cylinder of roots, it is a completely different tissue conducting in different directions and by different mechanisms!

Mechanism of phloem transport was first suggested in 1926. Later it was confirmed by most plant physiologists during the late 1970s and the early 1985. Although, the transport model has been modified, the contemporary form is supported by a large and convincing body of evidence.

Early Techniques

One of the first experiments in the field of plant physiology was done by Italian anatomist and microscopist Marcello Malpighi in 1675. He girdled a tree by removing a strip of bark from around its trunk. Later the experiment was repeated by Stephen Hales in 1727. Plant physiologists tried to measure translocation directly by following the movement of marked materials in the transport system. Early investigators used dyes like fluorescein which moves readily in phloem cells and is still used as an effective tracer.

Modern Techniques

Although viruses and herbicides have also been used as markers, but by far the most important tracers are the radioactive nuclides. Radioactive phosphorus, sulfur, chlorine, calcium, strontium, rubidium, potassium, hydrogen (tritium), and carbon have been used as tracers. Heavy stable isotopes such as those of oxygen and nitrogen are also used. Tracers can be applied by the reverse-flap technique or by abrading the epidermis of a leaf so that the cuticle is removed, sometimes breaking open a few epidermal cells. When tracer solutions are applied, they readily penetrate into the leaf mesophyll cells, the leaf veins, or both. Another common approach is by exposing the leaf in a closed container to carbon dioxide labeled with carbon-14 or carbon-11. The labeled CO₂ is incorporated into products of assimilation and metabolism and in this form it is exported from the leaf in the translocation stream.

Both girdling experiments and techniques utilizing tracers have provided some specific conclusions. In the earliest studies it was apparent that the removal of the bark from the stem or trunk had no immediate effects on the growth of the plant or on transpiration from the leaves. Movement of water with tritium replacing the hydrogen took place through the wood in the transpiration stream even though the bark has been removed. Analysis of the xylem sap shows that it contains mostly dissolved minerals from the soil plus small amounts of various organic compounds, including sugars and amino acids. These findings clearly support the view that the upward movement of water with its dissolved minerals in the plant is through xylem tissues.
Malpighi and Hale girdle experiments clearly showed that assimilates from leaves, including the products of photosynthesis are necessary for the growth of plant parts that cannot photosynthesize and even for some parts that photosynthesize only at low levels, such as some stems and fruits. The detailed studies, especially with radioactive tracers, have shown that assimilates move through the sieve tubes in phloem tissue. Hence the second conclusion: Assimilates, including photosynthates, move primarily through sieve tubes in the phloem. This is phloem transport.

Girdle experiments on other parts of the plant besides the main stem clearly showed that assimilate transport in plant parts follow some sort of source and sink relationship. The leaves, with their photosynthetic capacity, (tubers in some cases) typically constitute the source and developing seedlings any growing, storing, or metabolizing tissue as sinks. Hence Assimilates move from source to sink.

**Mechanism of Phloem transport**

The model of phloem transport was first proposed by E. Munch in Germany in 1926. Much of the knowledge about translocation was learned in validating Munch’s model. Munch's pressure flow hypothesis is simple and can be built in the laboratory: The model is a simple and consists of two osmometers connected to each other with a tube. The osmometers can be immersed in the same solution or in different solutions, which may or may not be connected. The first osmometer contains a more concentrated than its surrounding solution; the second osmometer contains a less concentrated solution than that in the first osmometer, but either more or less concentrated than its surrounding medium. Water moves into the first osmometer by osmosis, and pressure builds up. Since the osmometers are connected, pressure is transferred from the first osmometer to the second. Soon the increasing pressure in the second osmometer causes it to have a more positive water potential than exists in its surrounding medium; as a result, water molecules diffuse out through the membrane, which retains the solute molecules.

The total result is osmotic movement of water into the first osmometer from its surrounding solution, bulk flow of solution through the tube into the second osmometer, and osmotic movement of water out of the second osmometer into its surrounding solution. If the walls of the second osmometer stretch, pressure are relieved even if no water moves out, and if the second osmometer is surrounded by a solution more concentrated than that inside it, water will diffuse into the surrounding solution even without a buildup of pressure.
Sieve elements of photosynthesizing leaf mesophyll cells are similar to the first osmometer, but the concentration of assimilates is kept high in these sieve cells by sugars that are produced photosynthetically in the nearby mesophyll cells. The concentration of assimilates in the other end of the phloem system, near the sink cells by incorporating them into protoplasm (growth), or storage as starch or fats. The connecting channel between source and sink is the phloem system with its sieve tubes; the surrounding dilute solutions are those of the apoplast, specifically those in cell walls and in the xylem.

Flow through the sieve tubes is passive and is due to pressure gradient caused by osmotic diffusion of water into the sieve tubes. There is no active pumping of solution by sieve cells along the route, although there is evidence that metabolism is required to maintain these cells in a condition that will prevent leakage.

Dutch botanist, Hugo de Vries (1885) was proposed an active transport of solutes due to cytoplasmic streaming. The cytoplasm streaming is common to many cells, and any solute that passes from one cell to another will be accelerated in its transport across the cell. This must be an important active transport mechanism in many plant tissues and it could be important in moving sugars from leaf mesophyll cells to phloem sieve tubes, but since the cytoplasm does not stream in mature sieve elements, cytoplasmic streaming cannot play role in phloem transport.
Phloem Anatomy

The sieve elements, which are the elongated living cells, usually without nuclei, in which transport actually takes place. They may be connected end to end with pore-filled sieve
plates as in case of angiosperms and are called **sieve elements**. However, the existence of sieve plates is not as clear in gymnosperms and lower vascular plants and sieve areas with smaller pores on lateral walls. Hence the units are called **sieve cells** instead of sieve elements. Second are the **companion cells** (in angiosperms) or albuminous cells (in gymnosperms), which are closely associated with the sieve elements or sieve cells and have relatively dense cytoplasm and distinct nuclei. There are usually many plasmodesmata in the walls between sieve elements and their companion cells, with the plasmodesmatal pores frequently being branched on the side of the companion cell. The exact function of the companion cells remains unknown. In leaves, they apparently absorb sugars and transfer them to sieve elements (phloem loading). Third are the **phloem parenchyma cells**, which are thin-walled cells that are similar to other parenchyma cells throughout the plant except that some are more elongated. They may act in storage as well as in lateral transport of solutes and water. Fourth are the thick walled **phloem fibers**, which sometimes are grouped in a bundle. They provide strength.

Understanding of the vascular anatomy of the minor veins in leaves is important in phloem transport. The large veins in a leaf branch into smaller veins and eventually into the minor leaf veins. Each minor vein may contain only one vessel representing xylem and one or two sieve tubes. The vessel is usually above the phloem tissue, and the sieve elements are typically smaller than and surrounded by companion cells. Intermingled with the companion cells are large phloem parenchyma cells, and vascular parenchyma may separate the phloem tissue from the xylem.

Companion cells and phloem parenchyma cells sometimes contain chloroplasts, and actively photosynthesizing mesophyll cells (usually consisting of palisade and spongy parenchyma tissues) are often in close contact with the minor vein. Indeed, typically no mesophyll cell in a leaf is separated from a minor vein by more than two or three other mesophyll cells.

Sometimes the companion cells have cell-wall ingrowths which highly increase the membrane surface area of the cell. These cells are called **transfer cells**. Transfer cells contribute significantly to the transfer of assimilates from the mesophyll cells to the sieve tubes. Transfer cells are not restricted to phloem but occur throughout the plant. They are found in xylem and phloem parenchyma of leaf nodes and in reproductive structures.

**Phloem development**

A sieve element and companion cell are formed from a single cambial cell. The sieve element expands rapidly and becomes highly vacuolated, with a thin layer of cytoplasm pressed against the cell wall. Minute bodies appear in this cytoplasm. They are generally ovoid in shape, some appearing rather amorphous, while others have a more fibrillar or stranded appearance called slime bodies consist of phloem protein. At about this time, the nucleus begins to degenerate and eventually it disappears completely in most sieve elements in most cases. The tonoplast (the membrane between the vacuole and the cytoplasm) also disappears at about this stage, but the plasmalemma remains intact. Cytoplasmic streaming has been observed in some developing sieve elements, but when the elements are mature, this activity ceases.

Simultaneously, the sieve plate is developed with small deposits of a special glucose polymer called callose, usually around plasmodesmata. The deposits increase in size until they assume the shape of the final pore. The plasmalemma extends through the pore and is thus continuous from cell to cell. Some sieve tubes remain functional for several years like in perennial monocots, such as palm trees.

Companion cells have dense cytoplasm, well-defined nucleus, small vacuoles and rich in mitochondria. Contrast to this, phloem parenchyma cells have large vacuoles and with few organelles. In sieve tubes, smooth endoplasmic reticulum occurs as an almost continuous network along the inner surface. Mitochondria apparently remain unmodified throughout the differentiation of the sieve tube. Plastids, sometimes contains starch, protein, or both, occur in young as mature sieve elements. Microtubules are abundant in
the cytoplasm of young sieve tubes but disappear at later stages. Microfilament bundles are frequently observed in differentiating sieve elements.

**The Rates of Phloem Transport**

Classical experiments conducted by Alden S. Crafts and O. Lorenz (1944) on pumpkins that material moved into each fruit through its peduncle at an average rate of 0.61 g h⁻¹. Crafts and Lorenz estimated that the average cross section of phloem tissue in the peduncles was 18.6 mm²; of this, about 20 percent consisted of sieve tubes (3.72 mm²). Thus, material was moving through sieve tubes with a mass transfer rate of 0.61g h⁻¹/3.72 mm² = 0.164 g mm⁻²h⁻¹. The mass transfer rate is the quantity of material passing through a given cross section of sieve tubes per unit of time. The velocity of movement, which is a measure of the linear distance transversed by an assimilate molecule per unit of time. More meaningful data in this direction are now obtained radioactive tracer studies.

**Transported Solute**

To determining what solutes are contained in phloem sap is simply to cut the phloem and let the sap run it, forming droplets that can then be collected and analyzed. There are problems with this technique as bleeding is often rapidly stopped by P-protein and other particulate matter in the phloem. The other approach is by using miniature hypodermic needle that we could insert into a single sieve tube, carefully extracting some of the contents without a sudden pressure release. In a similar way, phloem sap also obtained from feeding aphids.

Ninety percent of more of the material translocated in phloem consists of carbohydrates. There are species for which this is not necessarily true, with phloem sap containing as much as 45 percent nitrogen compounds; but sugars make up the great bulk of translocated solutes in the phloem sap of most species. Furthermore, virtually all the sugars transported in the phloem are nonreducing sugars. There are a great many plants, perhaps the majority, in which sucrose is nearly the only sugar that is transported. Other sugars, if they occur at all, are present only in trace amounts. (The reducing sugars glucose and fructose, often found along with sucrose in fruits, are sometimes found in phloem exudate, but it has been shown that they are breakdown products of sucrose and are not themselves translocated.) The major, if not the sole nonreducing carbohydrates that are transported in higher plants, belong to the raffinose series of sugars: sucrose, raffinose, stachyose, and verbascose or the sugar alcohols: mannitol, sorbitol, galactitol, and myoinositol.

Experiments done by H. Ziegler (1975) on many plants strongly support the statements made above; namely, that sucrose is by far the most common transported sugar, although raffinose and stachyose (sometimes verbascose) also appear. Myoinositol also appears in trace to very small amounts in many species.

**Phloem Loading**

Experiments by Brunhild Roeckl (1949) on osmotic potentials of photosynthetic cells and sieve sap of *Robinia pseudoacacia* (black locust) and later many studies using radioactive tracers, revealed that the mesophyll cells of trees have an osmotic potential of about -1.3 to -1.8 MPa, whereas sieve elements in leaves have an osmotic potential of about -2.0 to -3.0 MPa.
Herbaceous plants frequently have somewhat less negative osmotic potentials in the mesophyll cells. Since most of the osmotic potential is caused by the presence of sugars in both kinds of cells, it is clear that the sugar concentration is approximately 1.5 to 3 times as high in the sieve elements as in the surrounding mesophyll cells. The process in which sugars are raised to high concentrations in phloem lose to a source such as photosynthesizing leaf is called **phloem loading**.

**Pathway of transport**

Experimental evidence (14CO₂ assimilation into carbohydrates in the mesophyll cells) showed that sucrose movement to the minor veins occurs through the symplast (from cell to cell through plasmodesmata). Contrasted to the many plasmodesmal connections between adjacent mesophyll cells, plasmodesmata are rare or absent between mesophyll and adjacent companion cells and sieve elements. This is true for the majority of species that been examined (e.g., broadbean, maize, sugar beets). In any case, there is considerable evidence that sugar is transported actively out of mesophyll cells into the apoplast (cell walls outside of the protoplast) of the minor veins; that is secreted into the apoplast. Evidence of active loading into companion cells and then sieve elements came from the studies using, p-chloromercuribenzene sulfonic acid (PCMBS). This strongly suggests that sucrose produced in photosynthesis must enter the apoplast before it is loaded into the phloem. There is some evidence that the companion cells play the most important role in absorbing sucrose from the apoplast. Active loading of sucrose into the companion cells produces a very negative osmotic potential in those cells. This leads to an osmotic entrance of water, which then passes in bulk flow across the plasmodesmatal connections between the companion cells and sieve elements, carrying the sucrose along with it. Indeed, it is this high concentration of sucrose produced by loading in companion cells and then sieve elements and the consequent osmotic uptake of water that produces the high pressure and mass flow in sieve tubes.

**Role of metabolism in transport**

Although Munch's pressure-flow model does not immediately suggest that metabolic energy might be required along the pathway to maintain flow, although some metabolism might be required to maintain the phloem tissues in a condition suitable for transport and to prevent leakage of sugars through the plasma membranes of sieve elements. Early studies seemed to suggest that any inhibition of metabolism (e.g., by low temperatures or respiration inhibitors) along the pathway did inhibit transport. This requirement for metabolism was often cited by those who presented alternative theories to pressure flow. Indeed, it was suggested that metabolic energy was required along the pathway to move solutes across the sieve plates (Hence these alternative theories were often referred to as active theories as contrasted to the passive pressure-flow mechanism.)
Development of loading capacity

Young leaves normally act as sinks rather than sources. This is true even after they develop some photosynthetic capability. At a certain time, however, they begin to export carbohydrates through the phloem, although import of carbohydrate may continue for a while through different vascular strands. The development of phloem loading capacity by the minor-vein companion cells could account for this switch from import to export. Once sucrose begins to be actively loaded into the companion cells and then the sieve elements, water will enter by osmosis and flow will begin out of the minor veins: The leaf will become a source instead of a sink.

Phloem Unloading

Removal of sucrose and other solutes from the sieve elements at the sink end of the system has been much less studied than phloem loading. There is, nevertheless, accumulating evidence that an active unloading process plays an important role. Indeed, the degree of unloading could determine the sinks into which most translocation occurs. Unloading of solutes at the sink end of the transport system would be another highly appropriate modification of Munch's original hypothesis.

Unloading would insure that turgor pressures at the sink end of the system remained low, since low sugar concentrations would allow water to move out osmotically in response to the pressure transmitted' from the source. Furthermore, the solute unloaded at the sink could then be actively absorbed into developing fruit or other cells where concentrations could reach values as high or higher than occurred in the sieve tubes at the source.

Suggested Readings: