Handbook of Photosynthesis & Photobiology

Shira Shafer
Karrie Crittenden

First Edition, 2012

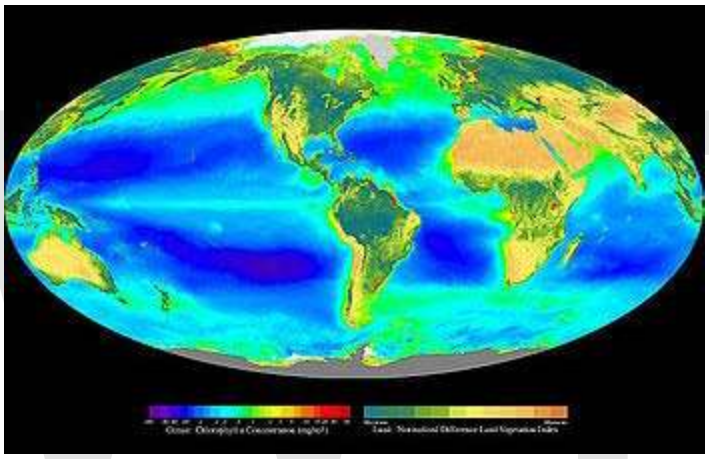
ISBN 978-81-323-0715-0

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

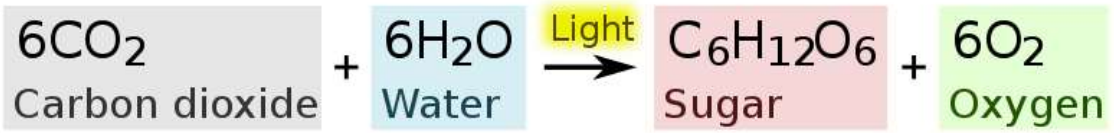
Table of Contents

Chapter- 1

Photosynthesis



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

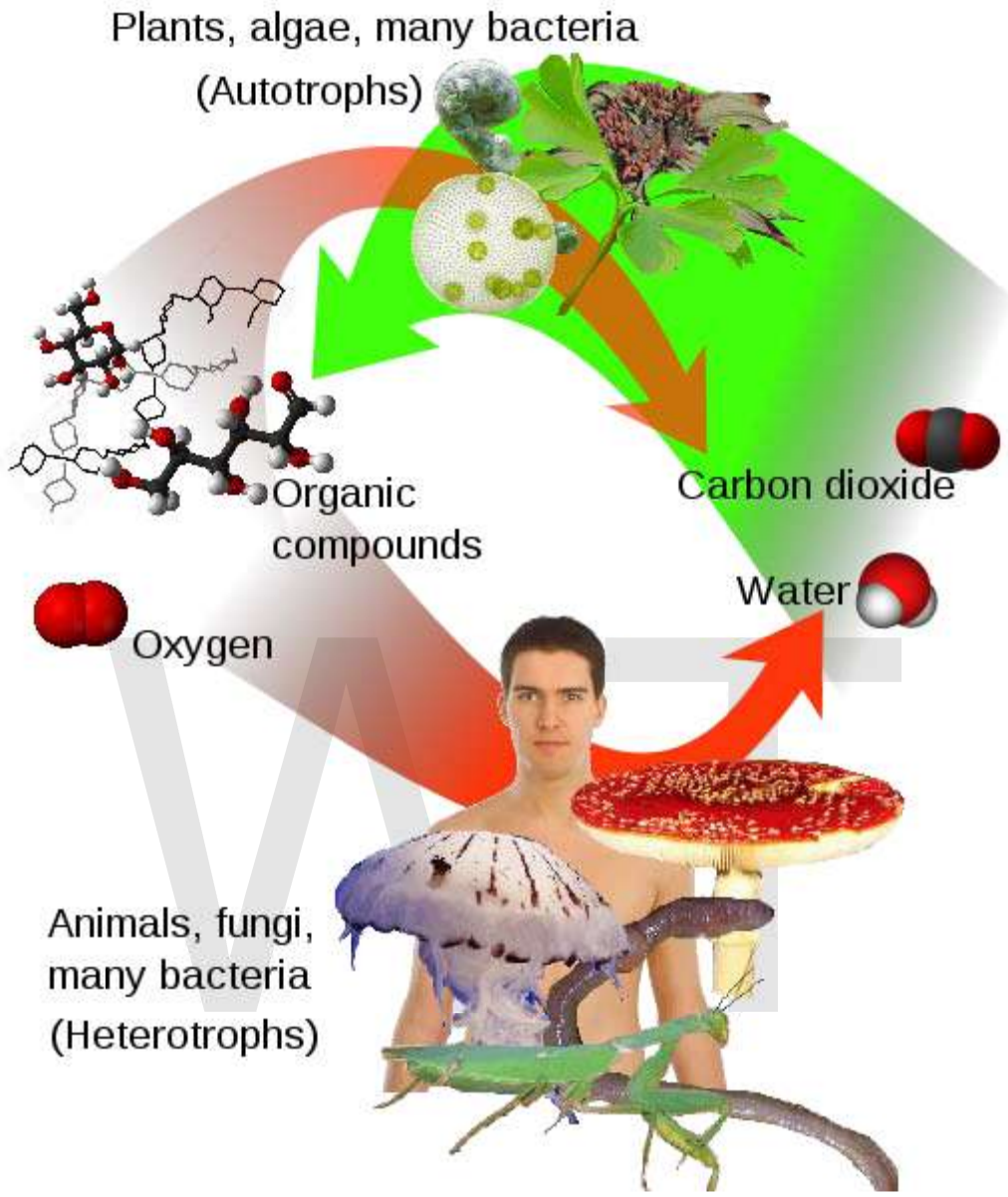


Overall equation for the type of photosynthesis that occurs in plants

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.

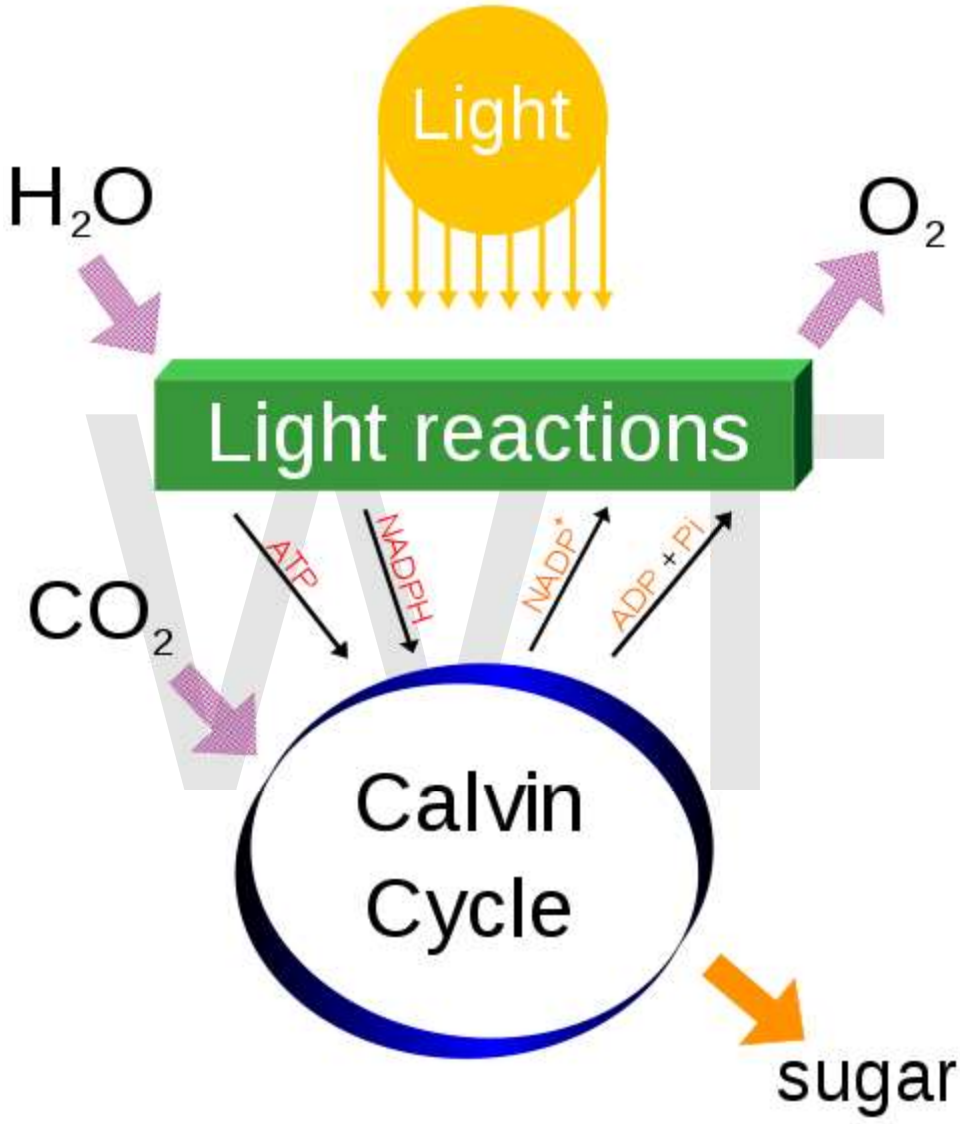


Overview of cycle between autotrophs and heterotrophs. Photosynthesis is the main means by which plants, algae and many bacteria produce organic compounds and oxygen from carbon dioxide and water (green arrow).

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 and 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:

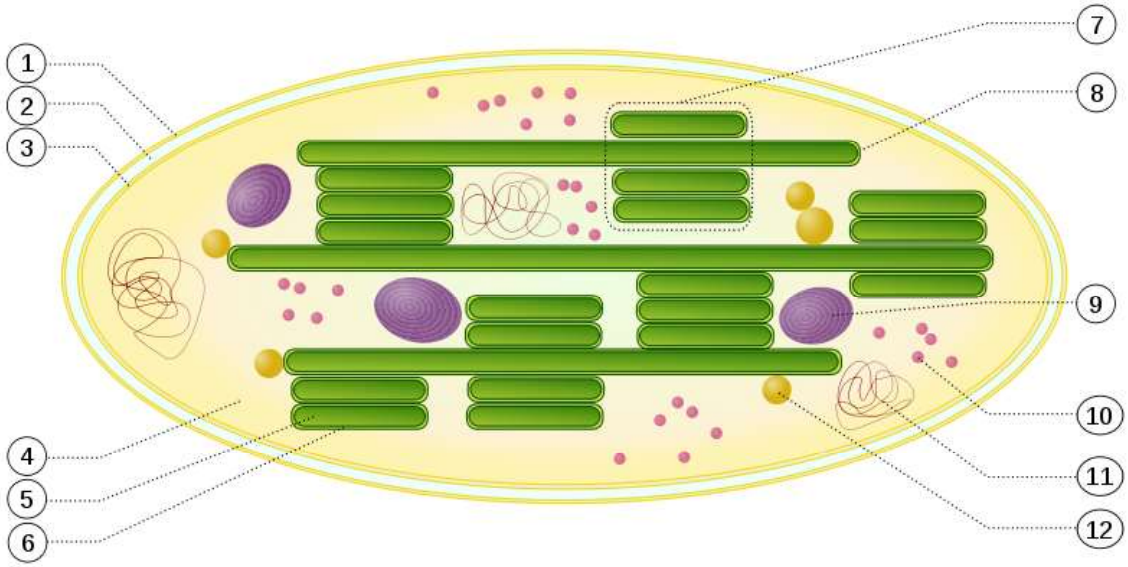
$$(AsO_3^{3-}) + CO_2 + \text{photons} \rightarrow CO + (AsO_4^{3-})$$

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.

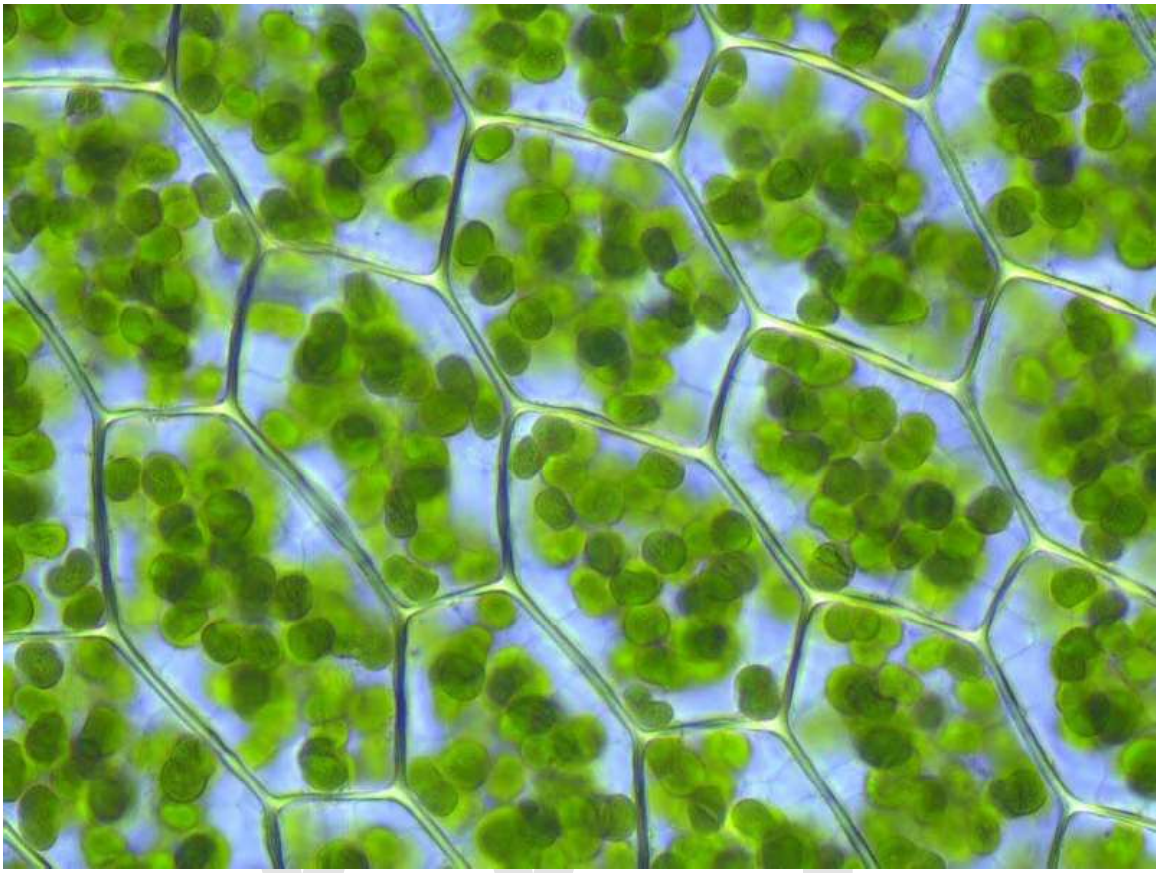
These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a light-harvesting complex.

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.

Order and kinetics

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

Evolution



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

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

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

The main source of oxygen in the atmosphere is oxygenic photosynthesis, and its first appearance is sometimes referred to as the oxygen catastrophe. Geological evidence suggests that oxygenic photosynthesis, such as that in cyanobacteria, became important during the Paleoproterozoic era around 2 billion years ago. Modern photosynthesis in plants and most photosynthetic prokaryotes is oxygenic. Oxygenic photosynthesis uses

water as an electron donor, which is oxidized to molecular oxygen (O_2) in the photosynthetic reaction center.

Symbiosis and the origin of chloroplasts

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

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

Cyanobacteria and the evolution of photosynthesis

The biochemical capacity to use water as the source for electrons in photosynthesis evolved once, in a common ancestor of extant cyanobacteria. The geological record indicates that this transforming event took place early in Earth's history, at least 2450–2320 million years ago (Ma), and possibly much earlier. Available evidence from geobiological studies of Archean (>2500 Ma) sedimentary rocks indicates that life existed 3500 Ma, but the question of when oxygenic photosynthesis evolved is still unanswered. A clear paleontological window on cyanobacterial evolution opened about 2000 Ma, revealing an already-diverse biota of blue-greens. Cyanobacteria remained principal primary producers throughout the Proterozoic Eon (2500–543 Ma), in part because the redox structure of the oceans favored photoautotrophs capable of nitrogen fixation. Green algae joined blue-greens as major primary producers on continental shelves near the end of the Proterozoic, but only with the Mesozoic (251–65 Ma) radiations of dinoflagellates, coccolithophorids, and diatoms did primary production in marine shelf waters take modern form. Cyanobacteria remain critical to marine ecosystems as primary producers in oceanic gyres, as agents of biological nitrogen fixation, and, in modified form, as the plastids of marine algae.

Discovery

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

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

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

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

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

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

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

Further experiments to prove that the oxygen developed during the photosynthesis of green plants came from water, were performed by Robert Hill in 1937 and 1939. He showed that isolated chloroplasts give off oxygen in the presence of unnatural reducing

agents like iron oxalate, ferricyanide or benzoquinone after exposure to light. The Hill reaction is as follows:

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

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

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

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

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

Factors

The leaf is the primary site of photosynthesis in plants.

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

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

Light intensity (irradiance), wavelength and temperature

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

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

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

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

Carbon dioxide levels and photorespiration

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

RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

1. One product of oxygenase activity is phosphoglycolate (2 carbon) instead of 3-phosphoglycerate (3 carbon). Phosphoglycolate cannot be metabolized by the Calvin-Benson cycle and represents carbon lost from the cycle. A high oxygenase activity, therefore, drains the sugars that are required to recycle ribulose 5-bisphosphate and for the continuation of the Calvin-Benson cycle.
2. Phosphoglycolate is quickly metabolized to glycolate that is toxic to a plant at a high concentration; it inhibits photosynthesis.
3. Salvaging glycolate is an energetically expensive process that uses the glycolate pathway and only 75% of the carbon is returned to the Calvin-Benson cycle as 3-phosphoglycerate. The reactions also produce ammonia (NH_3) which is able to diffuse out of the plant leading to a loss of nitrogen.

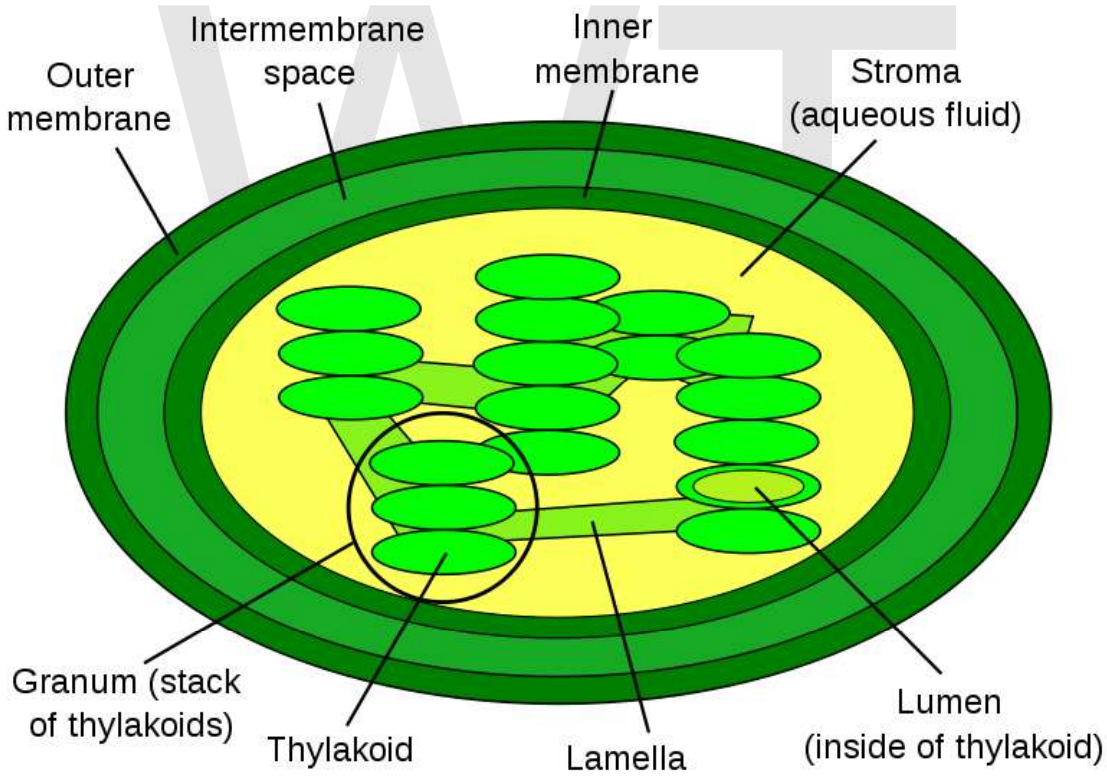
   A highly simplified summary is:
   2 glycolate + ATP → 3-phosphoglycerate + carbon dioxide + ADP +NH_3

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

Chapter- 2

Chloroplast and Thylakoid

Chloroplast

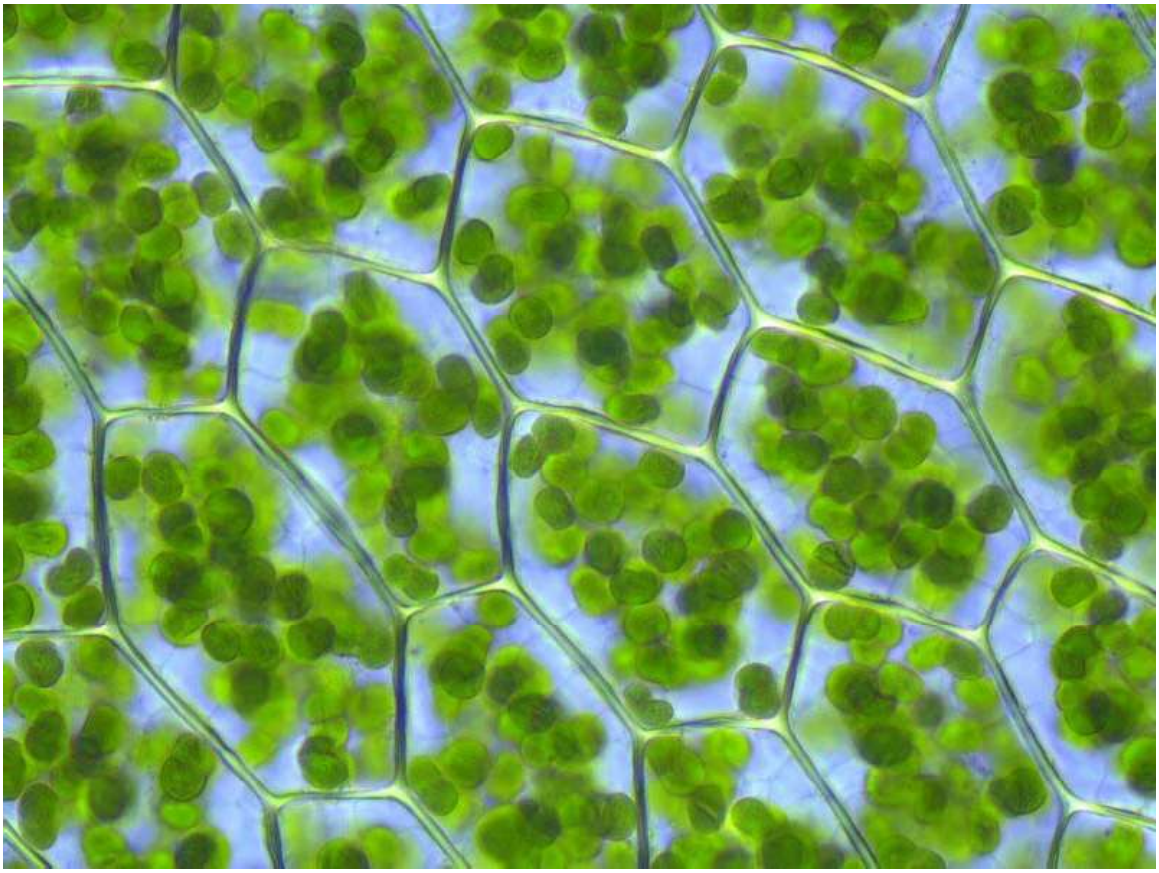


The simplified internal structure of a chloroplast

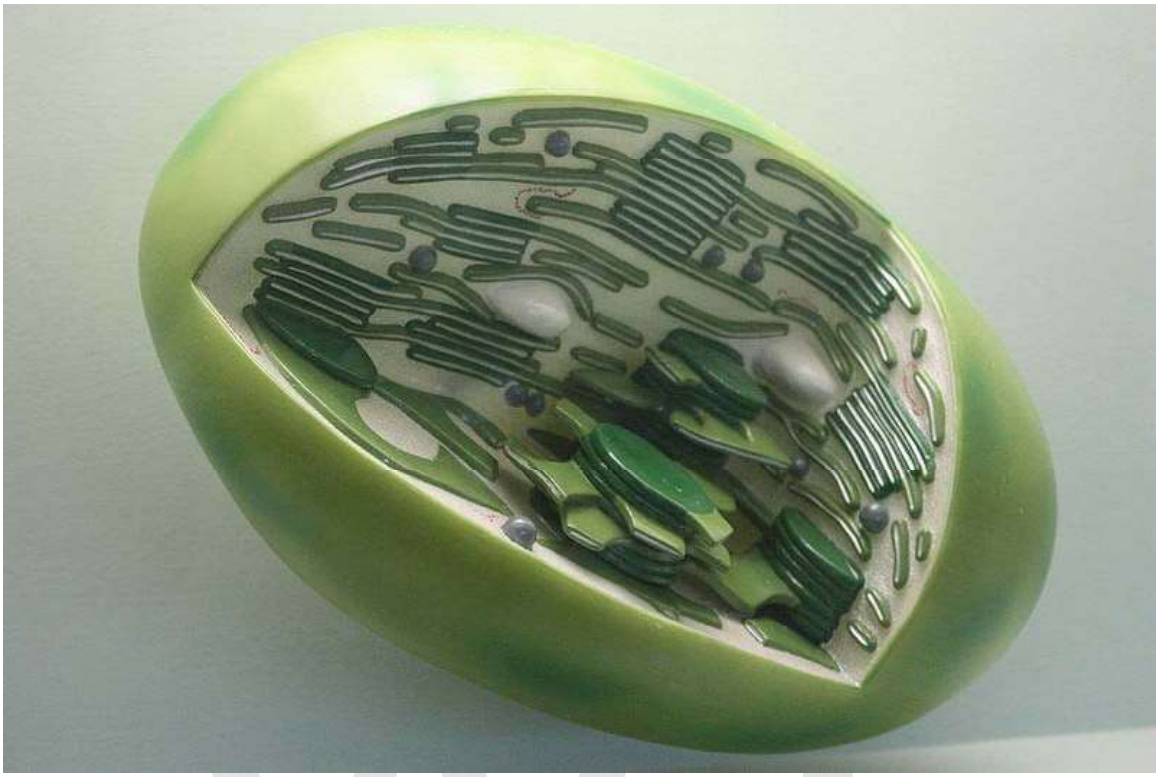
Chloroplasts are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy to conserve free energy in the form of ATP and reduce NADP to NADPH through a complex set of processes called photosynthesis.

The word chloroplast is derived from the Greek words *chloros*, which means green, and *plast*, which means form or entity. Chloroplasts are members of a class of organelles known as plastids.

Evolutionary origin



Chloroplasts visible in the cells of *Plagiomnium affine* — Many-fruited Thyme-moss



A model chloroplast

Chloroplasts are one of the many different types of organelles in the plant cell. In general, they are considered to have originated from cyanobacteria through endosymbiosis. This was first suggested by Mereschkowsky in 1905 after an observation by Schimper in 1883 that chloroplasts closely resemble cyanobacteria. All chloroplasts are thought to derive directly or indirectly from a single endosymbiotic event (in the Archaeplastida), except for *Paulinella chromatophora*, which has recently acquired a photosynthetic cyanobacterial endosymbiont which is not closely related to chloroplasts of other eukaryotes. In that they derive from an endosymbiotic event, chloroplasts are similar to mitochondria, but chloroplasts are found only in plants and protista. The chloroplast is surrounded by a double-layered composite membrane with an intermembrane space; further, it has reticulations, or many infoldings, filling the inner spaces. The chloroplast has its own DNA, which codes for redox proteins involved in electron transport in photosynthesis; this is termed the plastome.

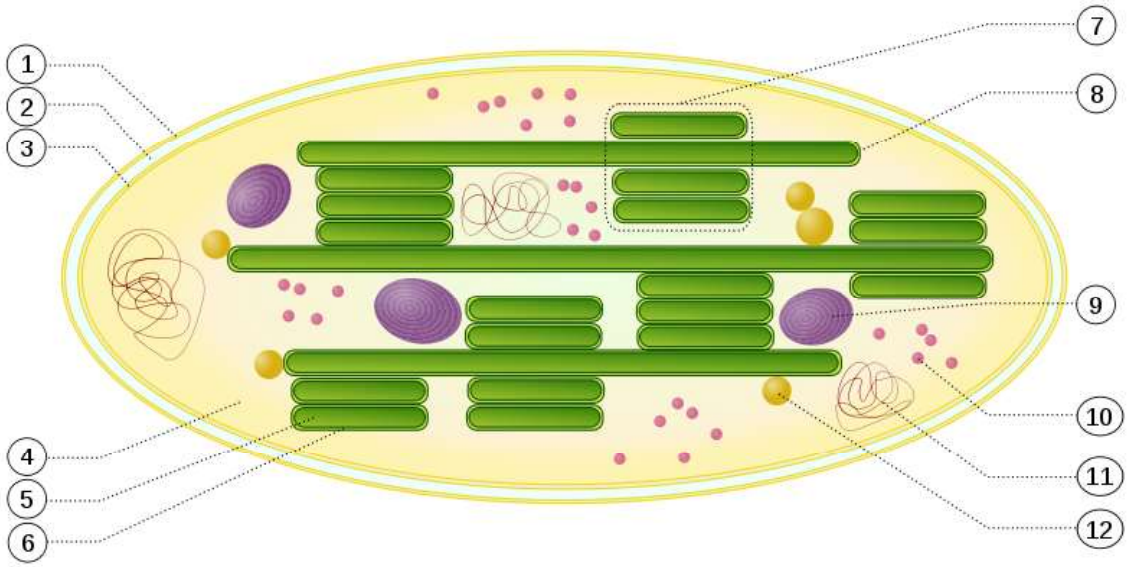
In green plants, chloroplasts are surrounded by two lipid-bilayer membranes. They are believed to correspond to the outer and inner membranes of the ancestral cyanobacterium. Chloroplasts have their own genome, which is considerably reduced compared to that of free-living cyanobacteria, but the parts that are still present show clear similarities with the cyanobacterial genome. Plastids may contain 60-100 genes whereas cyanobacteria often contain more than 1500 genes. Many of the missing genes are encoded in the nuclear genome of the host. The transfer of nuclear information has been estimated in tobacco plants at one gene for every 16000 pollen grains.

In some algae (such as the heterokonts and other protists such as Euglenozoa and Cercozoa), chloroplasts seem to have evolved through a secondary event of endosymbiosis, in which a eukaryotic cell engulfed a second eukaryotic cell containing chloroplasts, forming chloroplasts with three or four membrane layers. In some cases, such secondary endosymbionts may have themselves been engulfed by still other eukaryotes, thus forming tertiary endosymbionts. In the alga *Chlorella*, there is only one chloroplast, which is bell-shaped.

In some groups of mixotrophic protists such as the dinoflagellates, chloroplasts are separated from a captured alga or diatom and used temporarily. These klepto chloroplasts may only have a lifetime of a few days and are then replaced..

Structure

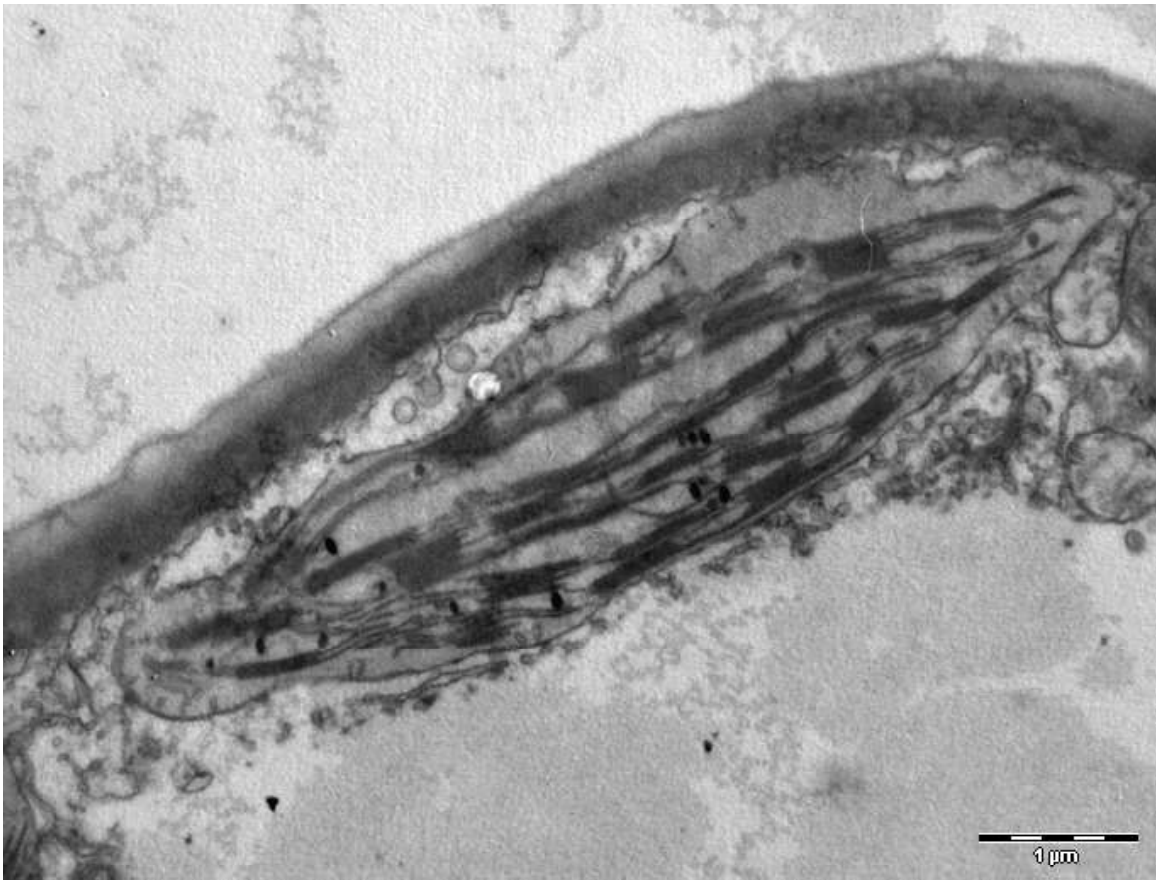
Chloroplasts are observable as flat discs usually 2 to 10 micrometers in diameter and 1 micrometer thick. In land plants, they are, in general, 5 μm in diameter and 2.3 μm thick. They are 200-400 nm (nano-meters). The chloroplast is contained by an envelope that consists of an inner and an outer phospholipid membrane. Between these two layers is the intermembrane space. A typical parenchyma cell contains about 10 to 100 chloroplasts.



Chloroplast ultrastructure:
1. outer membrane
2. intermembrane space
3. inner membrane (1+2+3: envelope)
4. stroma (aqueous fluid)
5. thylakoid lumen (inside of thylakoid)
6. thylakoid membrane
7. granum (stack of thylakoids)
8. thylakoid (lamella)
9. starch

10. ribosome
11. plastidial DNA
12. plastoglobule (drop of lipids)

The material within the chloroplast is called the stroma, corresponding to the cytosol of the original bacterium, and contains one or more molecules of small circular DNA. It also contains ribosomes; however most of its proteins are encoded by genes contained in the host cell nucleus, with the protein products transported to the chloroplast.



TEM image of a chloroplast

Within the stroma are stacks of thylakoids, the sub-organelles, which are the site of photosynthesis. The thylakoids are arranged in stacks called grana (singular: granum). A thylakoid has a flattened disk shape. Inside it is an empty area called the thylakoid space or lumen. Photosynthesis takes place on the thylakoid membrane; as in mitochondrial oxidative phosphorylation, it involves the coupling of cross-membrane fluxes with biosynthesis via the dissipation of a proton electrochemical gradient.

In the electron microscope, thylakoid membranes appear as alternating light-and-dark bands, each 0.01 µm thick. Embedded in the thylakoid membrane are antenna complexes, each of which consists of the light-absorbing pigments, including chlorophyll and carotenoids, as well as proteins that bind the pigments. This complex both increases the

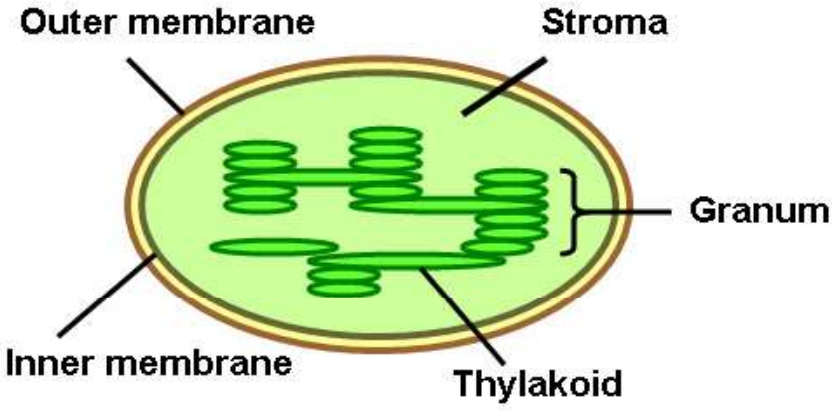
surface area for light capture, and allows capture of photons with a wider range of wavelengths. The energy of the incident photons is absorbed by the pigments and funneled to the reaction centre of this complex through resonance energy transfer. Two chlorophyll molecules are then ionised, producing an excited electron, which then passes onto the photochemical reaction centre.

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

Transplastomic plants

Recently, chloroplasts have caught attention by developers of genetically modified plants. In most flowering plants, chloroplasts are not inherited from the male parent, although in plants such as pines, chloroplasts are inherited from males. Where chloroplasts are inherited only from the female, transgenes in these plastids cannot be disseminated by pollen. This makes plastid transformation a valuable tool for the creation and cultivation of genetically modified plants that are biologically contained, thus posing significantly lower environmental risks. This biological containment strategy is therefore suitable for establishing the coexistence of conventional and organic agriculture. While the reliability of this mechanism has not yet been studied for all relevant crop species, recent results in tobacco plants are promising, showing a failed containment rate of transplastomic plants at 3 in 1,000,000.

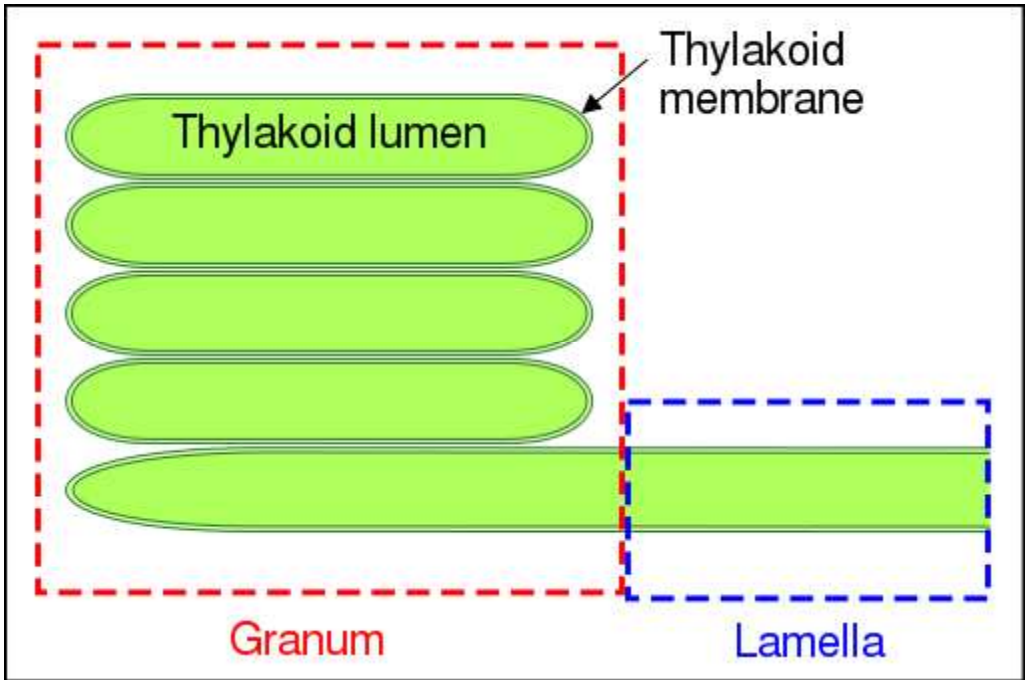
Thylakoid



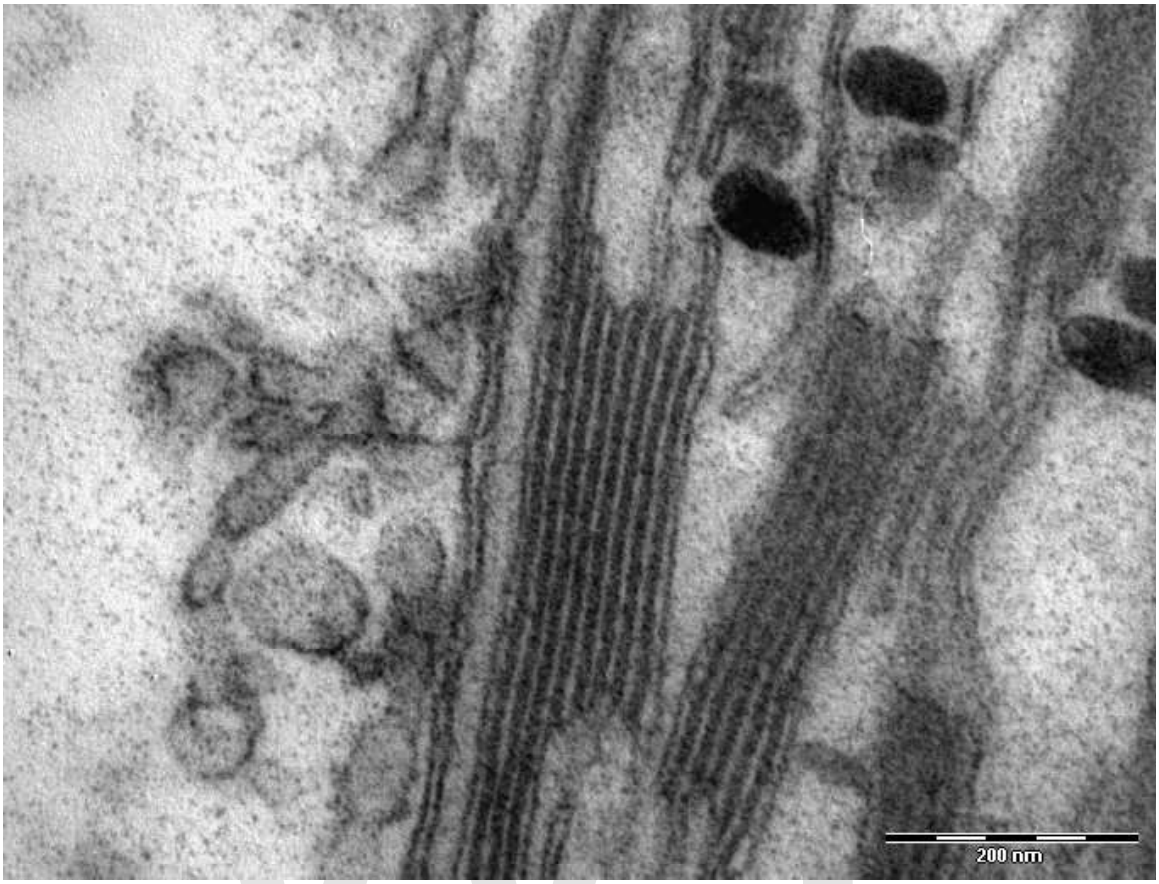
Thylakoids (green) inside a chloroplast

A **thylakoid** is a membrane-bound compartment inside chloroplasts and cyanobacteria. They are the site of the light-dependent reactions of photosynthesis. The word "thylakoid" is derived from the Greek *thylakos*, meaning "sac". Thylakoids consist of a **thylakoid membrane** surrounding a **thylakoid lumen**. Chloroplast thylakoids frequently form stacks of disks referred to as "grana" (singular: **granum**). Grana are connected by **intergrana** or **stroma** thylakoids, which join granum stacks together as a single functional compartment.

Thylakoid structure



Thylakoid structures



TEM image of grana

Thylakoids are membrane-bound structures embedded into the chloroplast stroma.

Membrane

The **thylakoid membrane** is the site of the light-dependent reactions of photosynthesis with the photosynthetic pigments embedded directly in the membrane. It is an alternating pattern of dark and light bands measuring each 1 nanometre. The thylakoid lipid bilayer shares characteristic features with prokaryotic membranes and the inner chloroplast membrane. For example, acidic lipids can be found in thylakoid membranes, cyanobacteria and other photosynthetic bacteria and are involved in the functional integrity of the photosystems. The thylakoid membranes of higher plants are composed primarily of phospholipids and galactolipids that are asymmetrically arranged along and across the membranes. The lipids for the thylakoid membranes are synthesized in a complex pathway involving exchange of lipid precursors between the endoplasmic reticulum and inner membrane of the plastid envelope and transported from the inner membrane to the thylakoids via vesicles.

Lumen

The **thylakoid lumen** is the compartment bounded by the thylakoid membrane. It plays a vital role for photophosphorylation during photosynthesis. During the light-dependent reaction, protons are pumped across the thylakoid membrane into the lumen making it acidic down to pH 4.

Granum

A **granum** (plural **grana**) is a stack of thylakoid discs. Chloroplasts can have from 10 to 100 grana. Grana are connected by stroma thylakoids, also called intergrana thylakoids or **lamellae**. Grana thylakoids and stroma thylakoids can be distinguished by their different protein composition. Grana contribute to chloroplasts' large surface area to volume ratio. Different interpretations of electron tomography imaging of thylakoid membranes has resulted in two models for grana structure. Both posit that lamellae intersect grana stacks in parallel sheets, though whether these sheets intersect in planes perpendicular to the grana stack axis, or are arranged in a right-handed helix is debated.

Thylakoid formation

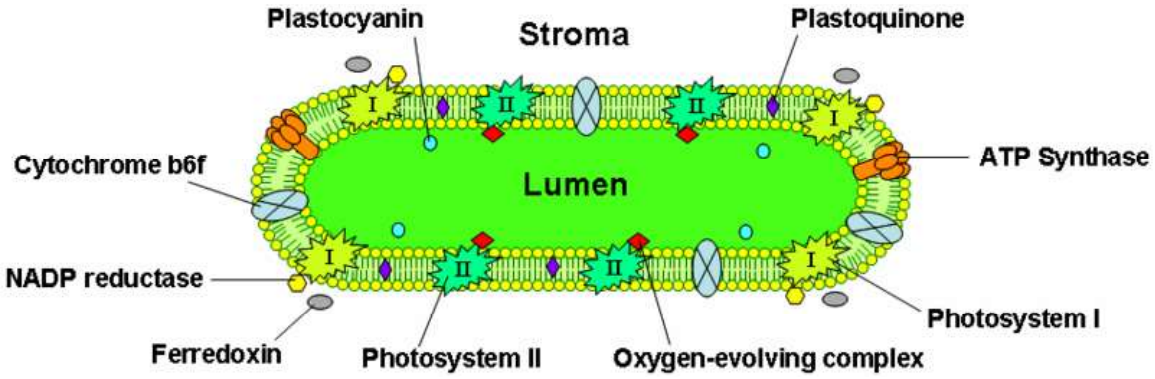
Chloroplasts develop from proplastids when seedlings emerge from the ground. Thylakoid formation requires light. In the plant embryo and in the absence of light, proplastids develop into etioplasts that contain semicrystalline membrane structures called prolamellar bodies. When exposed to light, these prolamellar bodies develop into thylakoids. This does not happen in seedlings grown in the dark, which undergo etiolation. An underexposure to light can cause the thylakoids to fail. This causes the chloroplasts to fail resulting in the death of the plant.

Thylakoid formation requires the action of *vesicle-inducing protein in plastids 1* (VIPP1). Plants cannot survive without this protein, and reduced VIPP1 levels lead to slower growth and paler plants with reduced ability to photosynthesize. VIPP1 appears to be required for basic thylakoid membrane formation, but not for the assembly of protein complexes of the thylakoid membrane. It is conserved in all organisms containing thylakoids, including cyanobacteria, green algae, such as Chlamydomonas, and higher plants, such as *Arabidopsis thaliana*.

Thylakoid isolation and fractionation

Thylakoids can be purified from plant cells using a combination of differential and gradient centrifugation. Disruption of isolated thylakoids, for example by mechanical shearing, releases the lumenal fraction. Peripheral and integral membrane fractions can be extracted from the remaining membrane fraction. Treatment with sodium carbonate (Na_2CO_3) detaches peripheral membrane proteins, whereas treatment with detergents and organic solvents solubilizes integral membrane proteins.

Thylakoid proteins



Thylakoid disc with embedded and associated proteins

Thylakoids contain many integral and peripheral membrane proteins, as well as lumenal proteins. Recent proteomics studies of thylakoid fractions have provided further details on the protein composition of the thylakoids. These data have been summarized in several plastid protein databases that are available online.

According to these studies, the thylakoid proteome consists of at least 335 different proteins. Out of these, 89 are in the lumen, 116 are integral membrane proteins, 62 are peripheral proteins on the stroma side, and 68 peripheral proteins on the lumenal side. Additional low-abundance lumenal proteins can be predicted through computational methods. Of the thylakoid proteins with known functions, 42% are involved in photosynthesis. The next largest functional groups include proteins involved in protein targeting, processing and folding with 11%, oxidative stress response (9%) and translation (8%).

Integral membrane proteins

Thylakoid membranes contain integral membrane proteins which play an important role in light harvesting and the light-dependent reactions of photosynthesis. There are four major protein complexes in the thylakoid membrane:

- Photosystems I and II
- Cytochrome b6f complex
- ATP synthase

Photosystem II is located mostly in the grana thylakoids, whereas photosystem I and ATP synthase are mostly located in the stroma thylakoids and the outer layers of grana. The cytochrome b6f complex is distributed evenly throughout thylakoid membranes. Due to the separate location of the two photosystems in the thylakoid membrane system, mobile electron carriers are required to shuttle electrons between them. These carriers are plastoquinone and plastocyanin. Plastoquinone shuttles electrons from photosystem II to

the cytochrome b6f complex, whereas plastocyanin carries electrons from the cytochrome b6f complex to photosystem I.

Together, these proteins make use of light energy to drive electron transport chains that generate a chemiosmotic potential across the thylakoid membrane and NADPH, a product of the terminal redox reaction. The ATP synthase uses the chemiosmotic potential to make ATP during photophosphorylation.

Photosystems

Photosystems are protein complexes involved in photosynthesis. They are found in the thylakoid membranes of plants, algae and cyanobacteria (in plants and algae these are located in the chloroplasts), or in the cytoplasmic membrane of photosynthetic bacteria. A photosystem (or Reaction Center) is an enzyme which uses light to reduce molecules. The membrane protein complex is made of several subunits and contains numerous cofactors. In the photosynthetic membranes, reaction centers provide the driving force for the bioenergetic electron and proton transfer chain. When light is absorbed by a reaction center (either directly or passed by neighbouring pigment-antennae), a series of oxido-reduction reactions is initiated, leading to the reduction of a terminal acceptor. Two families of photosystems exist: type I reaction centers (like photosystem I (P700) in chloroplasts and in green-sulphur bacteria) and type II reaction centers (like photosystem II (P680) in chloroplasts and in non-sulphur purple bacteria). Each photosystem can be identified by the wavelength of light to which it is most reactive (700 and 680 nanometers, respectively for PSI and PSII in chloroplasts), and the type of terminal electron acceptor. Type I photosystems use ferredoxin-like iron-sulfur cluster proteins as terminal electron acceptors, while type II photosystems ultimately shuttle electrons to a quinone terminal electron acceptor. One has to note that both reaction center types are present in chloroplasts and cyanobacteria, working together to form a unique photosynthetic chain able to extract electrons from water, creating oxygen as a byproduct.

Structure

A reaction center comprises several (>10 or >11) protein subunits, providing a scaffold for a series of cofactors. The latter can be pigments (like chlorophyll, pheophytin, carotenoids), quinones or iron-sulfur clusters.

Relationship between Photosystems I and II

For oxygenic photosynthesis, both photosystems I and II are required. Oxygenic photosynthesis can be performed by plants and cyanobacteria which are believed to be the progenitors of the photosystem-containing chloroplasts of eukaryotes. Photosynthetic bacteria which cannot produce oxygen have a single photosystem called BRC, bacterial reaction center.

Historically photosystem I was named "I" since it was discovered before photosystem II, but this does not represent the order of the electron flow.

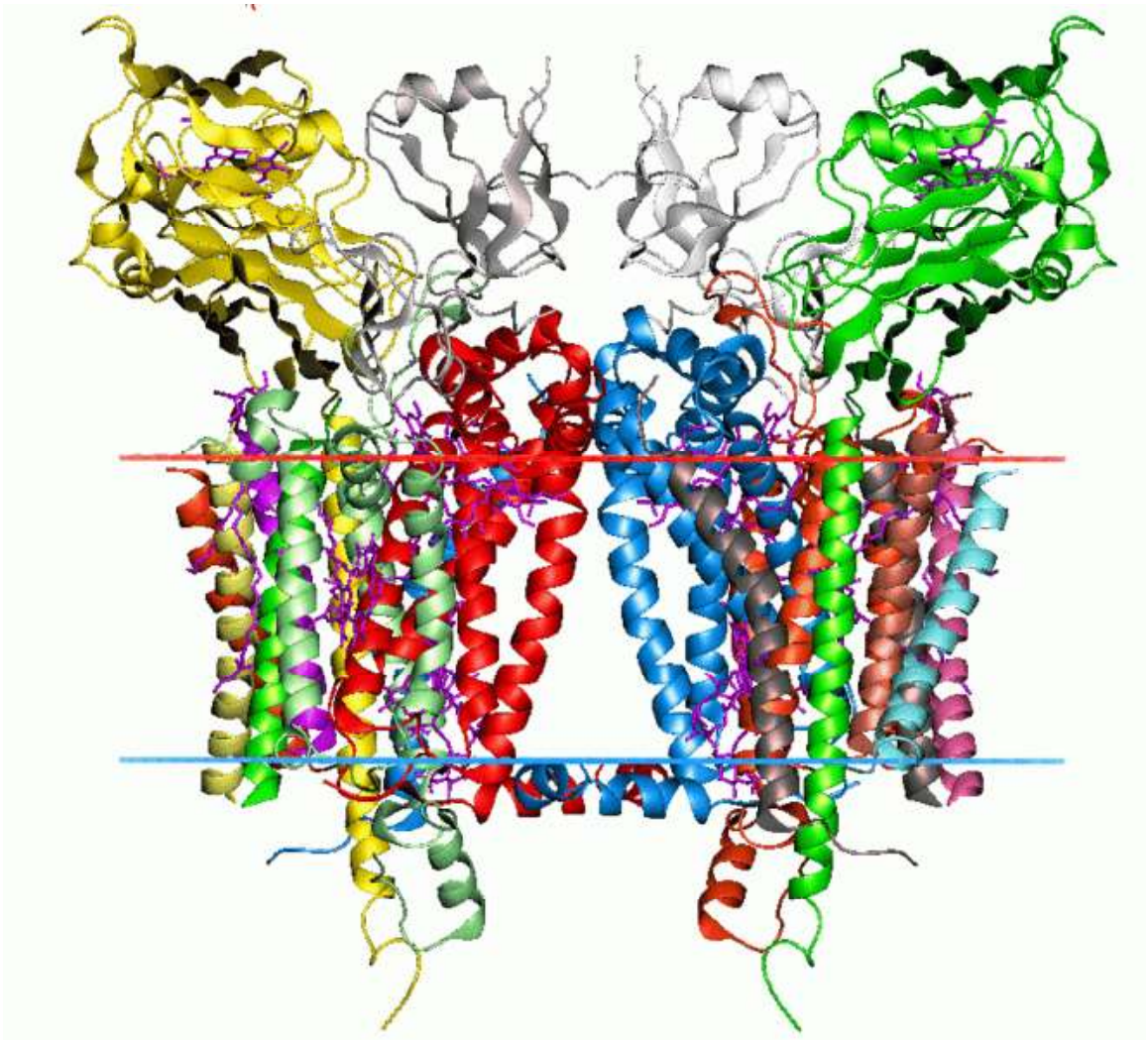
When photosystem II absorbs light, electrons in the reaction-center chlorophyll are excited to a higher energy level and are trapped by the primary electron acceptors. To replenish the deficit of electrons, electrons are extracted from water by a cluster of four Manganese ions in photosystem II and supplied to the chlorophyll via a redox-active tyrosine.

Photoexcited electrons travel through the cytochrome b6f complex to photosystem I via an electron transport chain set in the thylakoid membrane. This energy fall is harnessed, (the whole process termed chemiosmosis), to transport hydrogen (H^+) through the membrane, to the lumen, to provide a proton-motive force to generate ATP. The protons are transported by the plastoquinone. If electrons only pass through once, the process is termed noncyclic photophosphorylation.

When the electron reaches photosystem I, it fills the electron deficit of the reaction-center chlorophyll of photosystem I. The deficit is due to photo-excitation of electrons which are again trapped in an electron acceptor molecule, this time that of photosystem I.

ATP is generated when the ATP synthetase transports the protons present in the lumen to the stroma, through the membrane. The electrons may either continue to go through cyclic electron transport around PS I, or pass, via ferredoxin, to the enzyme $NADP^+$ reductase. Electrons and hydrogen ions are added to $NADP^+$ to form NADPH. This reducing agent is transported to the Calvin cycle to react with glycerate 3-phosphate, along with ATP to form glyceraldehyde 3-phosphate, the basic building block from which plants can make a variety of substances.

Cytochrome b6f complex



Cytochrome b6f complex (1q90). Hydrocarbon boundaries of the lipid bilayer are shown by red and blue dots.

The **cytochrome b_6f complex** (plastoquinol—plastocyanin reductase; EC 1.10.99.1) of chloroplasts and cyanobacteria transfers electrons between the two reaction center complexes of oxygenic photosynthetic membranes, photosystem I and photosystem II, and participates in formation of the transmembrane electrochemical proton gradient by also transferring protons from the stromal to the internal lumen compartment. It is minimally composed of four subunits: **cytochrome b_6**, carrying a low- and a high-potential heme groups (b_L and b_H); **cytochrome *f*** with one covalently bound heme *c*; **Rieske iron-sulfur protein** (ISP) containing a single [Fe_2S_2] cluster; and **subunit IV** (17 kDa protein). In its structure and functions, the cytochrome b_6f complex bears extensive

analogy to the cytochrome bc_1 complex of mitochondria and photosynthetic purple bacteria. However, there are important differences between the two complexes:

- The single-polypeptide cytochrome b in the cytochrome bc_1 complex corresponds to cytochrome b_6 and subunit IV in the cytochrome b_6f complex
- Cytochrome *f* and cytochrome c_1 are not homologous
- The cytochrome b_6f complex contains additional chromophores, chlorophyll *a*, β-carotene and atypical heme c_i (heme *x*), the latter being linked by a single thioether bond to cytochrome b_6

The cytochrome b_6f complex is responsible for "non-cyclic" **(1)** and "cyclic" **(2)** electron transfer between two mobile redox carriers, plastoquinol (QH_2) and plastocyanin:

$$H_2O \rightarrow \text{photosystem II} \rightarrow QH_2 \rightarrow \text{Cyt } b_6f \rightarrow \text{plastocyanin} \rightarrow \text{photosystem I} \rightarrow NADPH \quad \textbf{(1)}$$

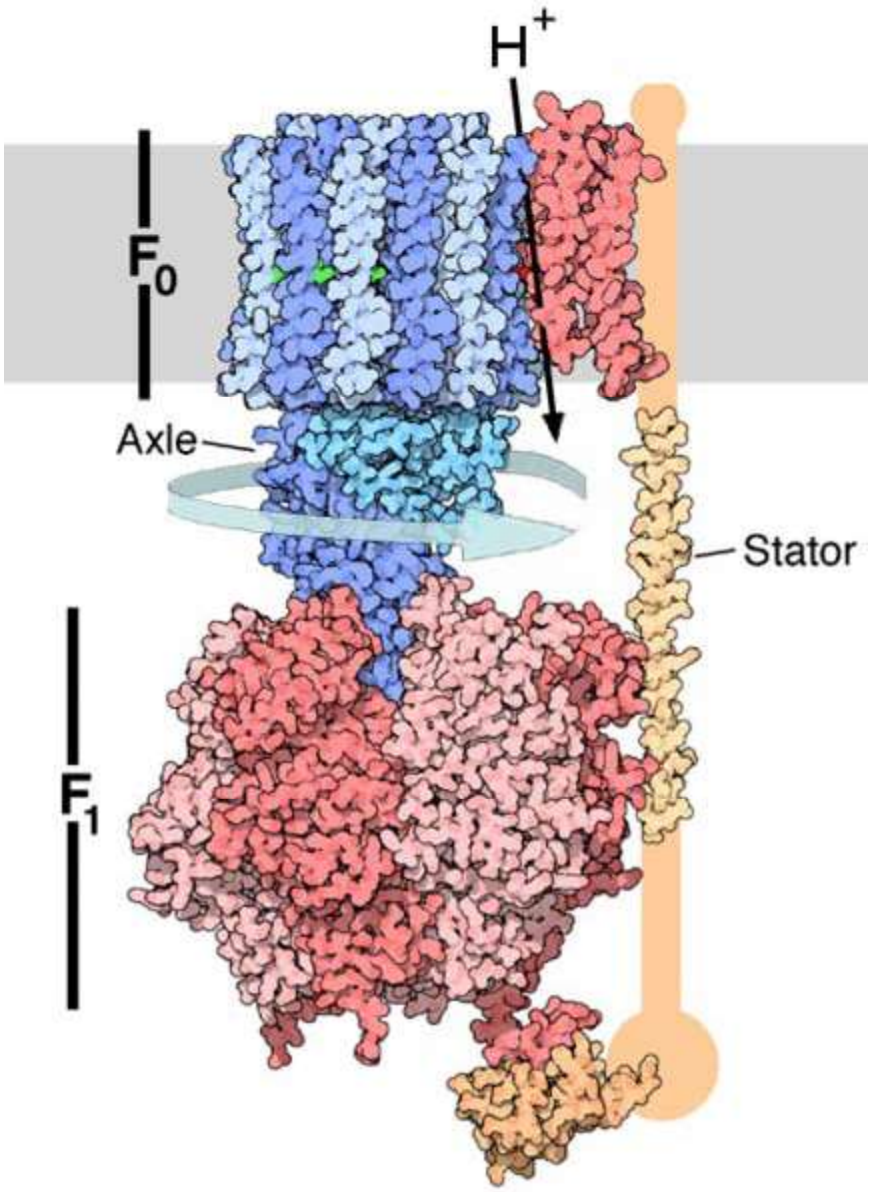
$$QH_2 \rightarrow \text{Cyt } b_6f \rightarrow \text{plastocyanin} \rightarrow \text{photosystem I} \rightarrow Q \quad \textbf{(2)}$$

Electron transfer is coupled with the translocation of protons across the membrane, thus generating proton-motive force in the form of an electrochemical proton potential which can drive ATP synthesis.

The crystal structure of cytochrome b_6f complexes from *Chlamydomonas reinhardtii* and *Mastigocladus laminosus* have been determined.

The cytochrome b6f complex is part of the thylakoid electron transport chain and couples electron transfer to the pumping of protons into the thylakoid lumen. Energetically, it is situated between the two photosystems and transfers electrons from photosystem II-plastoquinone to plastocyanin-photosystem I.

ATP synthase



Structure of ATP synthase, the F_O proton channel and rotating stalk are shown in blue, the F_1 synthase domain in red and the membrane in grey.

ATP synthase (EC 3.6.3.14) is a general term for an enzyme that can synthesize adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate by using a form of energy. This energy is often in the form of protons moving down an electrochemical gradient, such as from the lumen into the stroma of chloroplasts or from the inter-membrane space into the matrix in mitochondria. The overall reaction sequence is:

$ADP + P_i \rightarrow ATP$

These enzymes are of crucial importance in almost all organisms, because ATP is the common "energy currency" of cells.

The antibiotic oligomycin inhibits the F_O unit of ATP synthase.

Structure and nomenclature

In mitochondria, the F_1F_O ATP synthase has a long history of scientific study.

- the F_O portion is within the membrane.
- The F_1 portion of the ATP synthase is above the membrane, inside the matrix of the mitochondria.

The nomenclature of the enzyme suffers from a long history. The F_1 fraction derives its name from the term "Fraction 1" and F_O (written as a subscript "O", not "zero") derives its name from being the oligomycin binding fraction.

Taking as an example the nomenclature of subunits in the bovine enzyme, many subunits have alphabet names:

- Greek letters: alpha, beta, gamma, delta, epsilon
- Roman letters: a, b, c, d, e, f, g, h

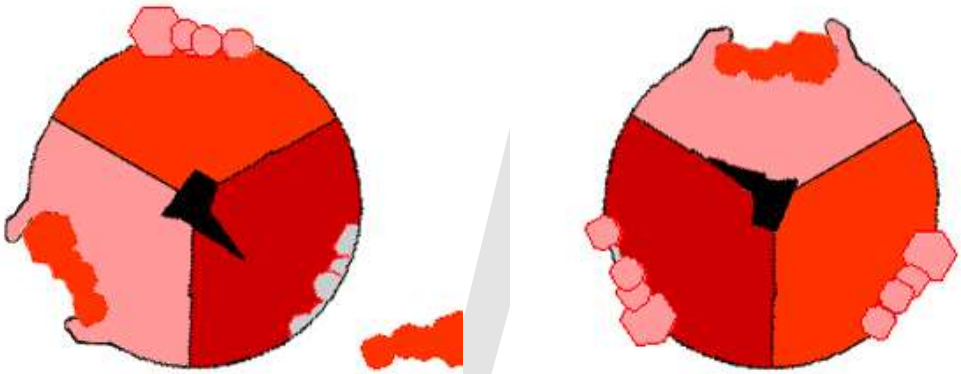
Others have more complex names:

- F_6 (from "Fraction 6")
- OSCP (the oligomycin sensitivity conferral protein), *ATP5O*
- A6L (named for the gene that codes for it in the mitochondrial genome)
- IF1 (inhibitory factor 1), *ATPIF1*

The F_1 particle is large and can be seen in the transmission electron microscope by negative staining. These are particles of 9 nm diameter that pepper the inner mitochondrial membrane. They were originally called elementary particles and were thought to contain the entire respiratory apparatus of the mitochondrion, but through a long series of experiments, Ephraim Racker and his colleagues (who first isolated the F_1 particle in 1961) were able to show that this particle is correlated with ATPase activity in uncoupled mitochondria and with the ATPase activity in submitochondrial particles created by exposing mitochondria to ultrasound. This ATPase activity was further associated with the creation of ATP by a long series of experiments in many laboratories.

Binding change mechanism

In the 1960s through the 1970s, Paul Boyer developed the binding change, or flip-flop, mechanism, which postulated that ATP synthesis is coupled with a conformational change in the ATP synthase generated by rotation of the gamma subunit. The research group of John E. Walker, then at the MRC Laboratory of Molecular Biology in Cambridge but now at the MRC Mitochondrial Biology Unit (also in Cambridge) crystallized the F_1 catalytic-domain of ATP synthase. The structure, at the time the largest asymmetric protein structure known, indicated that Boyer's rotary-catalysis model was essentially correct. For elucidating this Boyer and Walker shared half of the 1997 Nobel Prize in Chemistry. Jens Christian Skou received the other half of the Chemistry prize that year "for the first discovery of an ion-transporting enzyme, Na+, K+ -ATPase"



Mechanism of ATP synthase. ATP is shown in red, ADP and phosphate in pink and the rotating γ subunit in black.

The crystal structure of the F_1 showed alternating alpha and beta subunits (3 of each), arranged like segments of an orange around an asymmetrical gamma subunit. According to the current model of ATP synthesis (known as the alternating catalytic model), the proton-motive force across the inner mitochondrial membrane, generated by the electron transport chain, drives the passage of protons through the membrane via the F_O region of ATP synthase. A portion of the F_O (the ring of c-subunits) rotates as the protons pass through the membrane. The c-ring is tightly attached to the asymmetric central stalk (consisting primarily of the gamma subunit) which rotates within the $alpha_3beta_3$ of F_1 causing the 3 catalytic nucleotide binding sites to go through a series of conformational changes that leads to ATP synthesis. The major F_1 subunits are prevented from rotating in sympathy with the central stalk rotor by a peripheral stalk that joins the $alpha_3beta_3$ to the non-rotating portion of F_O. The structure of the intact ATP synthase is currently known at low-resolution from electron cryo-microscopy (cryo-EM) studies of the complex. The cryo-EM model of ATP synthase suggests that the peripheral stalk is a flexible structure that wraps around the complex as it joins F_1 to F_O. Under the right conditions, the enzyme reaction can also be carried out in reverse, with ATP hydrolysis driving proton pumping across the membrane.

The binding change mechanism involves the active site of a β subunit cycling between three states. In the "open" state, ADP and phosphate enter the active site, in the diagram to the right this is shown in red. The protein then closes up around the molecules and binds them loosely - the "loose" state (shown in orange). The enzyme then undergoes another change in shape and forces these molecules together, with the active site in the resulting "tight" state (shown in pink) binding the newly-produced ATP molecule with very high affinity. Finally, the active site cycles back to the open state, releasing ATP and binding more ADP and phosphate, ready for the next cycle of ATP production.

Physiological role

Like other enzymes, the activity of F_1F_O ATP synthase is reversible. Large enough quantities of ATP cause it to create a transmembrane proton gradient, this is used by fermenting bacteria which do not have an electron transport chain, and hydrolyze ATP to make a proton gradient, which they use for flagella and transport of nutrients into the cell.

In respiring bacteria under physiological conditions, ATP synthase generally runs in the opposite direction, creating ATP while using the protonmotive force created by the electron transport chain as a source of energy. The overall process of creating energy in this fashion is termed oxidative phosphorylation. The same process takes place in the mitochondria, where ATP synthase is located in the inner mitochondrial membrane (so that F_1-part sticks into mitochondrial matrix, where ATP synthesis takes place).

ATP synthase in different organisms

Plant ATP synthase

In plants ATP synthase is also present in chloroplasts (CF_1F_O-ATP synthase). The enzyme is integrated into thylakoid membrane; the CF_1-part sticks into stroma, where dark reactions of photosynthesis (Also called the light-independent reactions or the Calvin cycle) and ATP synthesis take place. The overall structure and the catalytic mechanism of the chloroplast ATP synthase are almost the same as those of the mitochondrial enzyme. However, in chloroplasts the proton motive force is generated not by respiratory electron transport chain, but by primary photosynthetic proteins.

Bovine ATP synthase

The ATP synthase isolated from bovine heart mitochondria (Bos taurus) is, biochemically and structurally, the best characterized ATP synthase. Beef heart is used as a source for the enyzme because of the high concentration of mitochondria in cardiac muscle.

E. coli ATP synthase

E. coli ATP synthase is the simplest known form of ATP synthase, with 8 different subunit types.

Yeast ATP synthase

Yeast ATP synthase is one of the best-studied eukaryotic ATP synthases and five F_1, eight F_O subunits and seven associated proteins have been identified. Most of these proteins have homologues in other eukaryotes.

Human ATP synthase

The following is a list of humans genes that encode components of ATP synthases:

- ATP5A1, ATP5AL1
- ATP5B, ATP5BL1
- ATP5C2, ATP5D, ATP5E, ATP5F1, ATP5G1, ATP5G2, ATP5G3, ATP5H, ATP5HP1, ATP5I, ATP5J, ATP5J2, ATP5L, ATP5L2, ATP5O, ATP5S
- ATP6, ATP6AP1, ATP6AP2
- ATPSBL1, ATPSBL2
- MT-ATP6, MT-ATP8

Evolution of ATP synthase

The evolution of ATP synthase is thought to be an example of modular evolution, where two subunits with their own functions have become associated and gained new functionality. This coupling must have occurred early in the evolution of life as evidenced by essentially the same structure and processes of ATP synthase enzymes conserved in all kingdoms of life. The F-ATP synthase shows large amounts of similarity both functionally and mechanically to the V-ATPase. However whilst the F-ATP synthase generates ATP by utilising a proton gradient the V-ATPase is responsible for generating a proton gradient at the expense of ATP, generating pH values as low as 1. The F_1 particle also shows significant similarity to hexameric DNA helicases and the F_O particle shows some similarity to H^+ powered flagellar motor complexes. The $\alpha_3\beta_3$ hexamer of the F_1 particle shows significant structural similarity to hexameric DNA helicases; both form a ring with 3 fold rotational symmetry with a central pore. Both also have roles dependent on the relative rotation of a macromolecule within the pore; the DNA helicases use the helical shape of DNA to drive their motion along the DNA molecule and to detect supercoiling whilst the $\alpha_3\beta_3$ hexamer uses the conformational changes due rotation of the γ subunit to drive an enzymatic reaction.

The H^+ motor of the F_O particle shows great functional similarity to the H^+ motors seen in flagellar motors. Both feature a ring of many small alpha helical proteins which rotate relative to nearby stationary proteins using a H^+ potential gradient as an energy source. This is, however, a fairly tenuous link - the overall structure of flagellar motors is far more complex than the F_O particle and the ring of rotating proteins is far larger, with around 30 compared to the 10, 11 or 14 known in the F_O complex.

The modular evolution theory for the origin of ATP synthase suggests that two subunits with independent function, a DNA helicase with ATPase activity and a H^+ motor, were

able to bind, and the rotation of the motor drive the ATPase activity of the helicase in reverse. This would then evolve to become more efficient, and eventually develop into the complex ATP synthases seen today. Alternatively the DNA helicase/H^+ motor complex may have had H^+ pump activity, the ATPase activity of the helicase driving the H^+ motor in reverse. This could later evolve to carry out the reverse reaction and act as an ATP synthase.

Thylakoid lumen proteins

The electron transport protein plastocyanin is present in the lumen and shuttles electrons from the cytochrome b6f protein complex to photosystem I. While plastoquinones are lipid-soluble and therefore move within the thylakoid membrane, plastocyanin moves through the thylakoid lumen.

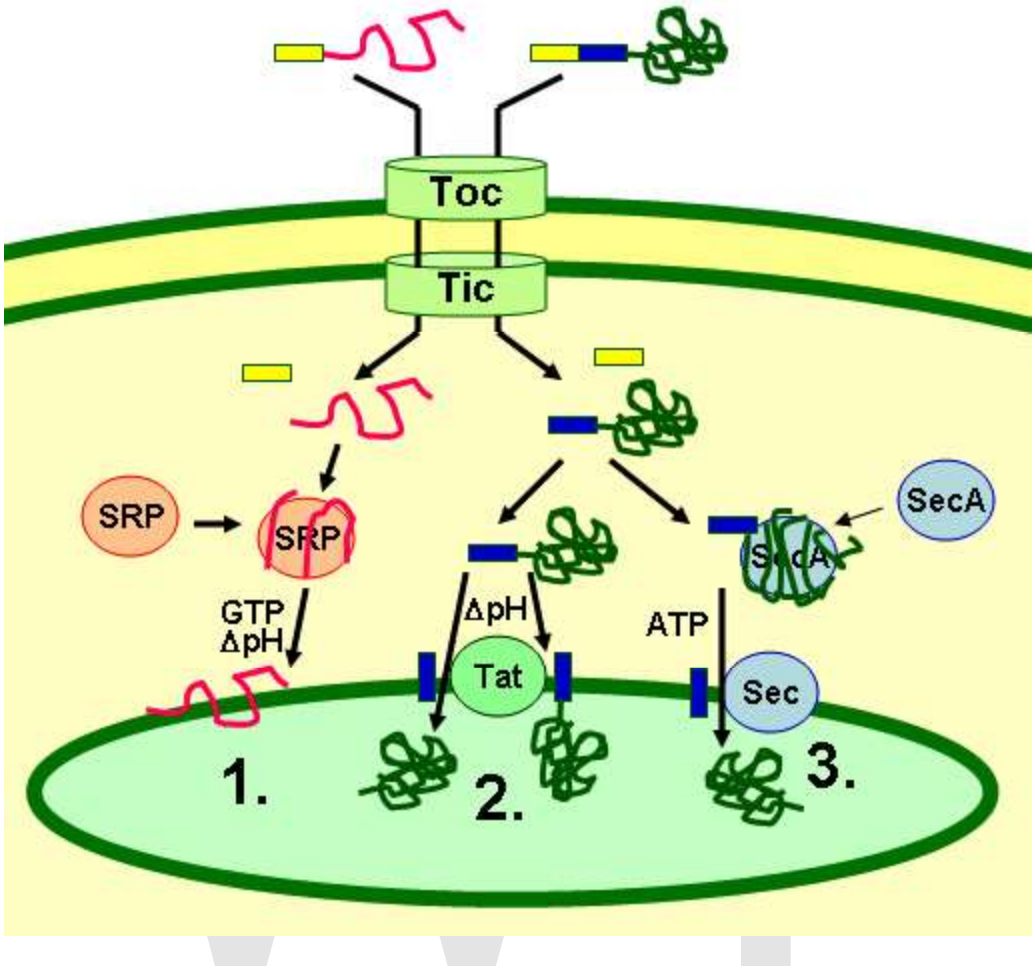
The lumen of the thylakoids is also the site of water oxidation by the oxygen evolving complex associated with the lumenal side of photosystem II.

Lumenal proteins can be predicted computationally based on their targeting signals. In Arabidopsis, out of the predicted lumenal proteins possessing the Tat signal, the largest groups with known functions are 19% involved in protein processing (proteolysis and folding), 18% in photosynthesis, 11% in metabolism, and 7% redox carriers and defense.

Thylakoid protein expression

Chloroplasts have their own genome, which encodes a number of thylakoid proteins. However, during the course of plastid evolution from their cyanobacterial endosymbiotic ancestors, extensive gene transfer from the chloroplast genome to the cell nucleus took place. This results in the four major thylakoid protein complexes being encoded in part by the chloroplast genome and in part by the nuclear genome. Plants have developed several mechanisms to co-regulate the expression of the different subunits encoded in the two different organelles to assure the proper stoichiometry and assembly of these protein complexes. For example, transcription of nuclear genes encoding parts of the photosynthetic apparatus is regulated by light. Biogenesis, stability and turnover of thylakoid protein complexes is regulated by phosphorylation via redox-sensitive kinases in the thylakoid membranes. The translation rate of chloroplast-encoded proteins is controlled by the presence or absence of assembly partners (control by epistasy of synthesis). This mechanism involves negative feedback through binding of excess protein to the 5' untranslated region of the chloroplast mRNA. Chloroplasts also need to balance the ratios of photosystem I and II for the electron transfer chain. The redox state of the electron carrier plastoquinone in the thylakoid membrane directly affects the transcription of chloroplast genes encoding proteins of the reaction centers of the photosystems, thus counteracting imbalances in the electron transfer chain.

Protein targeting to the thylakoids

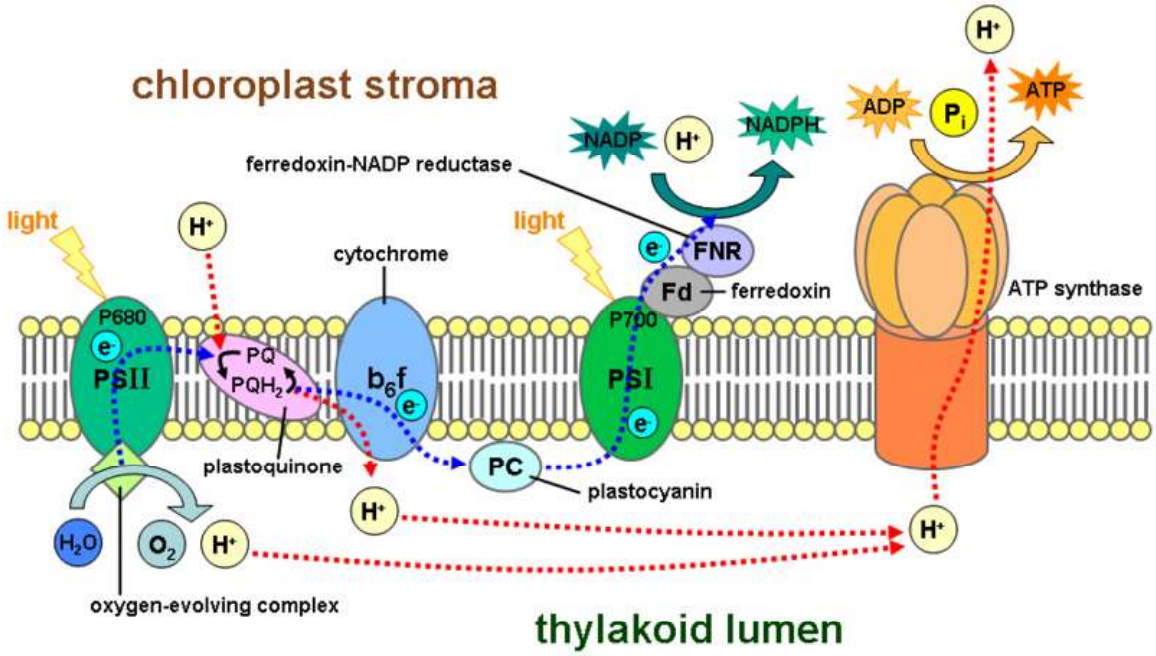


Schematic representation of thylakoid protein targeting pathways.

Thylakoid proteins are targeted to their destination via signal peptides and prokaryotic-type secretory pathways inside the chloroplast. Most thylakoid proteins encoded by a plant's nuclear genome need two targeting signals for proper localization: An N-terminal chloroplast targeting peptide (shown in yellow in the figure), followed by a thylakoid targeting peptide (shown in blue). Proteins are imported through the translocon of outer and inner membrane (Toc and Tic) complexes. After entering the chloroplast, the first targeting peptide is cleaved off by a protease processing imported proteins. This unmasks the second targeting signal and the protein is exported from the stroma into the thylakoid in a second targeting step. This second step requires the action of protein translocation components of the thylakoids and is energy-dependent. Proteins are inserted into the membrane via the SRP-dependent pathway (1), the Tat-dependent pathway (2), or spontaneously via their transmembrane domains (not shown in figure). Lumenal proteins are exported across the thylakoid membrane into the lumen by either the Tat-dependent pathway (2) or the Sec-dependent pathway (3) and released by cleavage from the thylakoid targeting signal. The different pathways utilize different signals and energy sources. The Sec (secretory) pathway requires ATP as energy source and consists of SecA, which binds to the imported protein, and a Sec membrane complex to shuttle the

protein across. Proteins with a twin arginine motif in their thylakoid signal peptide are shuttled through the Tat (twin arginine translocation) pathway, which requires a membrane-bound Tat complex and the pH gradient as an energy source. Some other proteins are inserted into the membrane via the SRP (signal recognition particle) pathway. The chloroplast SRP can interact with its target proteins either post-translationally or co-translationally, thus transporting imported proteins as well as those that are translated inside the chloroplast. The SRP pathway requires GTP and the pH gradient as energy sources. Some transmembrane proteins may also spontaneously insert into the membrane from the stromal side without energy requirement.

Thylakoid function



Light-dependent reactions of photosynthesis at the thylakoid membrane

The thylakoids are the site of the light-dependent reactions of photosynthesis. These include light-driven water oxidation and oxygen evolution, the pumping of protons across the thylakoid membranes coupled with the electron transport chain of the photosystems and cytochrome b6f complex, and ATP synthesis by the ATP synthase utilizing the generated proton gradient.

Water photolysis

The first step in photosynthesis is the light-driven oxidation (splitting) of water to provide the electrons for the photosynthetic electron transport chains as well as protons for the establishment of a proton gradient. The water-splitting reaction occurs on the lumenal side of the thylakoid membrane and is driven by the light energy captured by the photosystems. It is interesting to note that this oxidation of water conveniently produces

the waste product O_2 that is vital for cellular respiration. The molecular oxygen formed by the reaction is released into the atmosphere.

Electron transport chains

Two different variations of electron transport are used during photosynthesis:

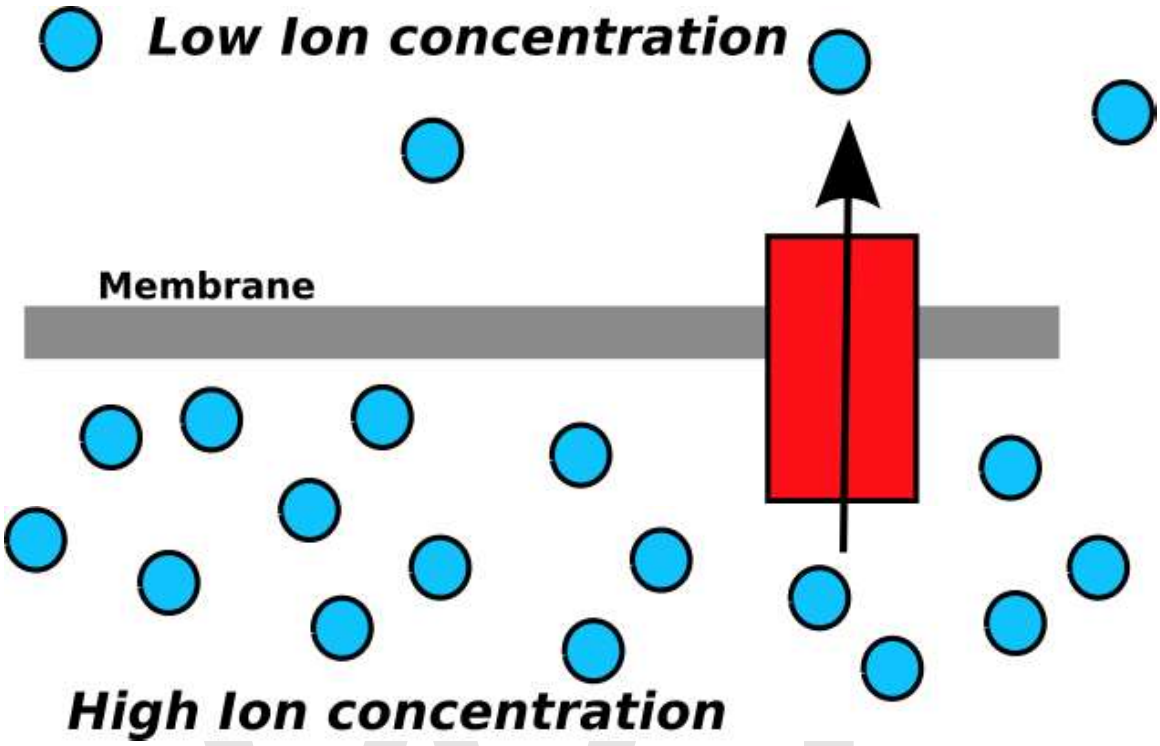
- **Noncyclic electron transport** or **Non-cyclic photophosphorylation** produces NADPH + H^+ and ATP.
- **Cyclic electron transport** or **Cyclic photophosphorylation** produces only ATP.

The noncyclic variety involves the participation of both photosystems, while the cyclic electron flow is dependent on only photosystem I.

- **Photosystem I** uses light energy to reduce $NADP^+$ to NADPH + H^+, and is active in both noncyclic and cyclic electron transport. In cyclic mode, the energized electron is passed down a chain that ultimately returns it (in its base state) to the chlorophyll that energized it.
- **Photosystem II** uses light energy to oxidize water molecules, producing electrons (e^-), protons (H^+), and molecular oxygen (O_2), and is only active in noncyclic transport. Electrons in this system are not conserved, but are rather continually entering from oxidized $2H_2O$ (O_2 + 4 H^+ + 4 e^-) and exiting with $NADP^+$ when it is finally reduced to NADPH.

Chemiosmosis

Chemiosmosis is the movement of ions across a selectively-permeable membrane, down their electrochemical gradient. More specifically, it relates to the generation of ATP by the movement of hydrogen ions across a membrane during cellular respiration.



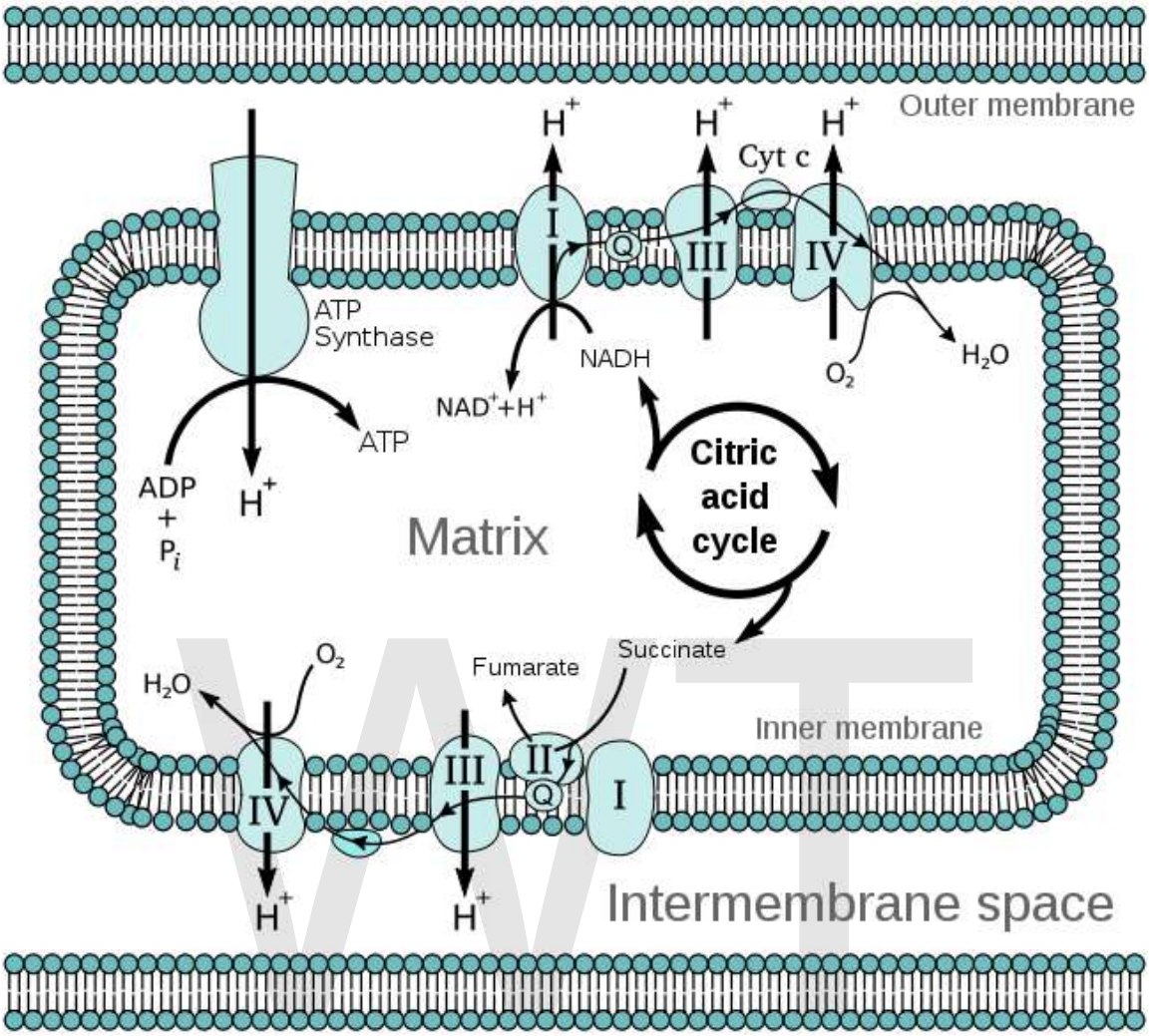
An Ion gradient has potential energy and can be used to power chemical reactions when the ions pass through a channel (red).

Hydrogen ions (protons) will diffuse from an area of high proton concentration to an area of lower proton concentration. Peter Mitchell proposed that an electrochemical concentration gradient of protons across a membrane could be harnessed to make ATP. He linked this process to osmosis, the diffusion of water across a membrane, which is why it is called *chemiosmosis*.

ATP synthase is the enzyme that makes ATP by chemiosmosis. It allows protons to pass through the membrane using the kinetic energy to phosphorylate ADP making ATP. The generation of ATP by chemiosmosis occurs in chloroplasts and mitochondria as well as in some bacteria.

The Chemiosmotic Theory

Peter D. Mitchell proposed the **chemiosmotic hypothesis** in 1961. The theory suggests essentially that most ATP synthesis in respiring cells come from the electrochemical gradient across the inner membranes of mitochondria by using the energy of NADH and $FADH_2$ formed from the breaking down of energy rich molecules such as glucose.



Chemiosmosis in a mitochondrion.

Molecules such as glucose are metabolized to produce acetyl CoA as an energy-rich intermediate. The oxidation of acetyl CoA in the mitochondrial matrix is coupled to the reduction of a carrier molecule such as NAD and FAD. The carriers pass electrons to the electron transport chain (ETC) in the inner mitochondrial membrane, which in turn pass them to other proteins in the ETC. The energy available in the electrons is used to pump protons from the matrix across the inner mitochondrial membrane, storing energy in the form of a transmembrane electrochemical gradient. The protons move back across the inner membrane through the enzyme ATP synthase. The flow of protons back into the matrix of the mitochondrion via ATP synthase provides enough energy for ADP to combine with inorganic phosphate to form ATP. The electrons and protons at the last pump in the ETC are taken up by oxygen to form water.

This was a radical proposal at the time, and was not well accepted. The prevailing view was that the energy of electron transfer was stored as a stable high potential intermediate, a chemically more conservative concept.

The problem with the older paradigm is that no high energy intermediate was ever found, and the evidence for proton pumping by the complexes of the electron transfer chain grew too great to be ignored. Eventually the weight of evidence began to favor the chemiosmotic hypothesis, and in 1978, Peter Mitchell was awarded the Nobel Prize in Chemistry.

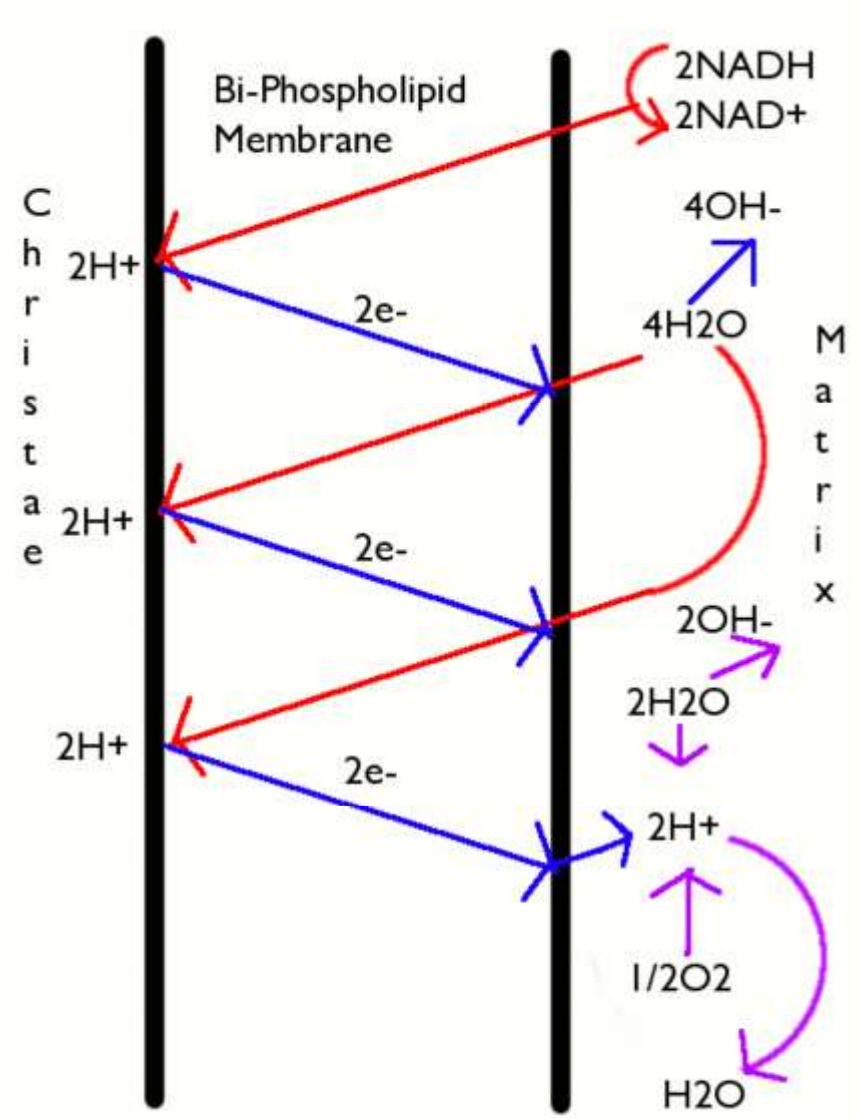
Chemiosmotic coupling is important for ATP production in chloroplasts and many bacteria.

The proton-motive force

In all mitochondria, chloroplasts and in many bacteria, chemiosmosis involves the **proton-motive force** (PMF) in some step. This can be described as the storing of energy as a combination of proton and voltage gradients across a membrane. The chemical potential energy refers to the difference in concentration of the protons on each side of the membrane and the electrical potential energy as a consequence of the charge separation across the membrane (when the protons move without a counter-ion, such as a proton).

In most cases the proton motive force is generated by an electron transport chain which acts as a proton pump, using the energy in electrons from an electron carrier to pump protons (hydrogen ions) out across the membrane, creating a separation of charge across the membrane. In mitochondria, free energy released from electrons by the electron transport chain is used to move protons from the mitochondrial matrix to the intermembrane space of the mitochondrion. Moving the protons out of the mitochondrion creates a lower concentration of positively charged protons inside it, resulting in a slight negative on the inside of the membrane: The electrical potential gradient is about -200 mV, inside negative. This charge difference and the proton concentration difference create a combined electrochemical gradient across the membrane. This electrochemical gradient for protons is both a concentration and charge difference and is often called the proton motive force (PMF). In mitochondria, the PMF is almost entirely made up of the electrical component but in chloroplasts the PMF is made up mostly of the pH gradient. In either case, the PMF needs to be about 50 kJ/mol for the ADP synthase to be able to make ATP.

In mitochondria



A diagram of chemiosmotic phosphorylation

Chemiosmotic phosphorylation is the third pathway that produces ATP from inorganic phosphate and an ADP molecule. This process is part of oxidative phosphorylation.

The complete breakdown of glucose in the presence of oxygen is called cellular respiration. The last steps of this process occur in mitochondria. The reduced molecules NADH and $FADH_2$ are generated by the Krebs cycle and glycolysis. These molecules pass electrons to an electron transport chain, which uses the energy released to create a proton gradient across the inner mitochondrial membrane. ATP synthase then uses the

energy stored in this gradient to make ATP. This process is called oxidative phosphorylation because oxygen is the final electron acceptor and the energy released by reducing oxygen to water is used to phosphorylate ADP and generate ATP.

In plants

The light reactions of photosynthesis generate energy by chemiosmosis. Light energy (photons) are received by the antenna complex of Photosystem 2, which excites a pair of electrons to a higher energy level. These electrons travel down an electron transport chain, causing H+ to diffuse across the thylakoid membrane into the inter-thylakoid space. These H+ are then transported down their concentration gradient through an enzyme called ATP-synthase, creating ATP by phosphorylation of ADP to ATP. The electrons from the initial light reaction reach Photosystem 1, then are raised to a higher energy level by light energy and then received by an electron receptor and reduce NADP+ to NADPH+H. The electrons from Photosystem 2 get replaced by the splitting of water, called "photolysis." Two water molecules must be split in order to gain 2 electrons (as well as O2, the oxygen eudicots require for survival).

In prokaryotes

Bacteria and archaea also can use chemiosmosis to generate ATP. Cyanobacteria, green sulfur bacteria, and purple bacteria create energy by a process called photophosphorylation. These bacteria use the energy of light to create a proton gradient using a photosynthetic electron transport chain. Non-photosynthetic bacteria such as *E. coli* also contain ATP synthase.

In fact, mitochondria and chloroplasts are believed to have been formed when early eukaryotic cells ingested bacteria that could create energy using chemiosmosis. This is called the endosymbiotic theory.

A major function of the thylakoid membrane and its integral photosystems is the establishment of chemiosmotic potential. The carriers in the electron transport chain use some of the electron's energy to actively transport protons from the stroma to the lumen. During photosynthesis, the lumen becomes acidic, as low as pH 4, compared to pH 8 in the stroma. This represents a 10,000 fold concentration gradient for protons across the thylakoid membrane.

Source of proton gradient

The protons in the lumen come from three primary sources.

- Photolysis by photosystem II oxidises water to oxygen, protons and electrons in the lumen.
- The transfer of electrons from photosystem II to plastoquinone during non-cyclic electron transport consumes two protons from the stroma. These are released in

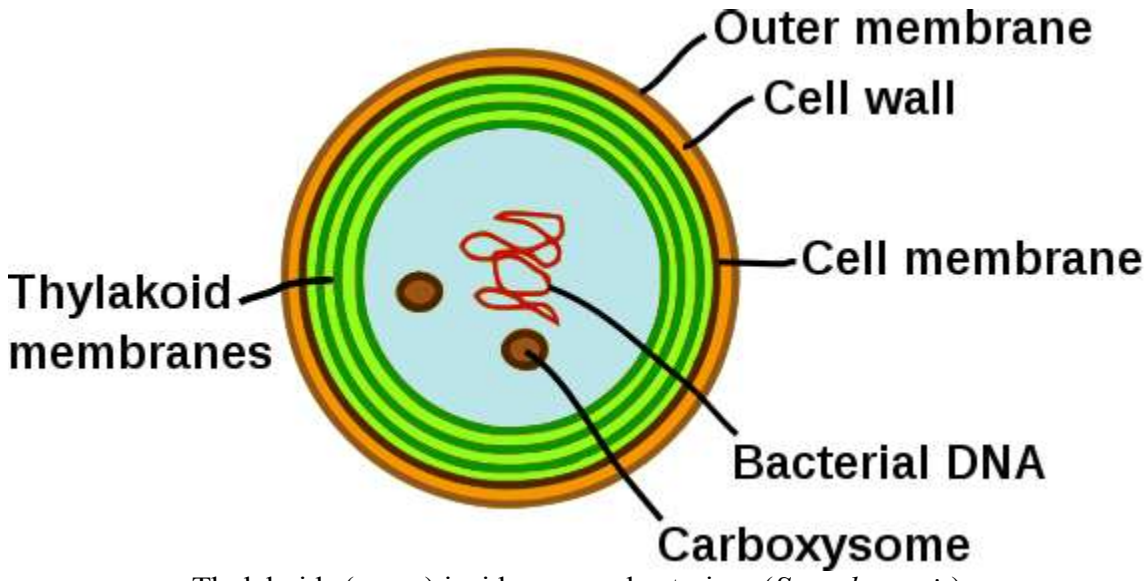
the lumen when the reduced plastoquinol is oxidized by the cytochrome b6f protein complex on the lumen side of the thylakoid membrane. From the plastoquinone pool, electrons pass through the cytochrome b6f complex. This integral membrane assembly resembles cytochrome bc1.
- The reduction of plastoquinone by ferredoxin during cyclic electron transport also transfers two protons from the stroma to the lumen.

The proton gradient is also caused by the consumption of protons in the stroma to make NADPH from NADP+ at the NADP reductase.

ATP generation

The molecular mechanism of ATP generation in chloroplasts is similar to that in mitochondria and takes the required energy from the proton motive force (PMF). However, chloroplasts rely more on the chemical potential of the PMF to generate the potential energy required for ATP synthesis. The PMF is the sum of a proton chemical potential (given by the proton concentration gradient) and a transmembrane electrical potential (given by charge separation across the membrane). Compared to the inner membranes of mitochondria, which have a significantly higher membrane potential due to charge separation, thylakoid membranes lack a charge gradient. To compensate for this, the 10,000 fold proton concentration gradient across the thylakoid membrane is much higher compared to a 10 fold gradient across the inner membrane of mitochondria. The resulting chemiosmotic potential between the lumen and stroma is high enough to drive ATP synthesis using the ATP synthase. As the protons travel back down the gradient through channels in ATP synthase, ADP + P_i is combined into ATP. In this manner, the light-dependent reactions are coupled to the synthesis of ATP via the proton gradient.

Thylakoid Membranes in Cyanobacteria



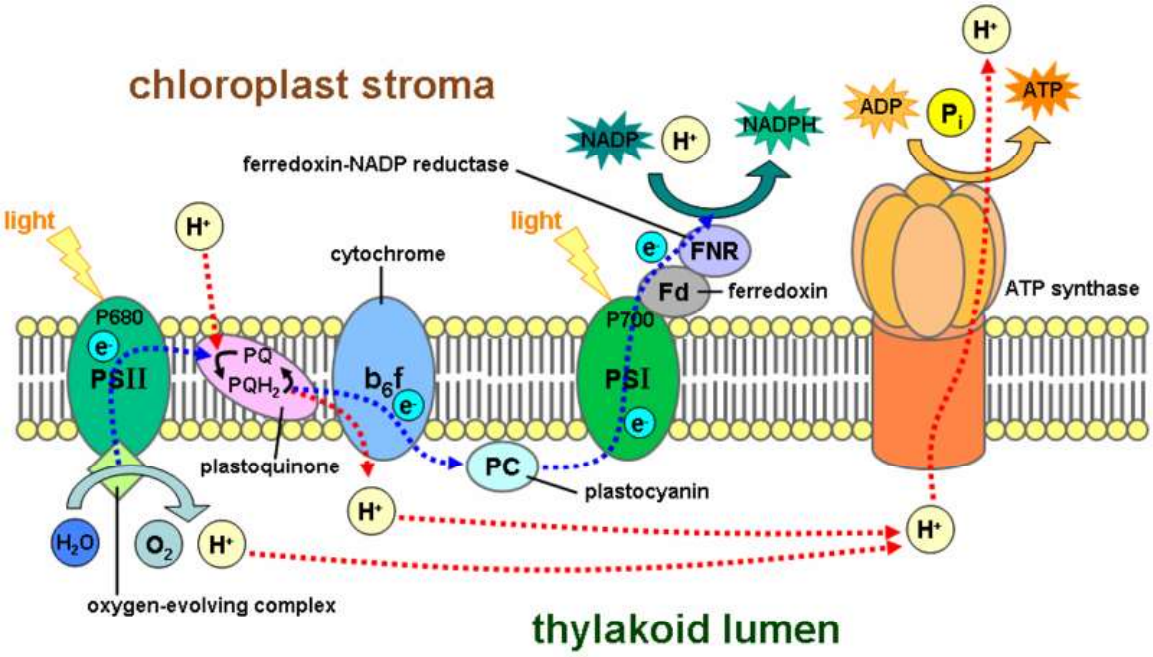
Thylakoids (green) inside a cyanobacterium (*Synechocystis*)

Cyanobacteria are photosynthetic prokaryotes with highly differentiated membrane systems. Cyanobacteria have an internal system of thylakoid membranes where the fully functional electron transfer chains of photosynthesis and respiration reside. The presence of different membrane systems lends these cells a unique complexity among bacteria. Cyanobacteria must be able to reorganize the membranes, synthesize new membrane lipids, and properly target proteins to the correct membrane system. The outer membrane, plasma membrane, and thylakoid membranes each have specialized roles in the cyanobacterial cell. Understanding the organization, functionality, protein composition and dynamics of the membrane systems remains a great challenge in cyanobacterial cell biology.

Chapter- 3

Light Reactions

Light-dependent reactions



Light-dependent reactions of photosynthesis at the thylakoid membrane

The **light-dependent reactions**, or **light reactions**, are the first stage of photosynthesis, the process by which plants capture and store energy from sunlight. In this process, light energy is converted into chemical energy, in the form of the energy-carrying molecules ATP and NADPH. In the *light-independent reactions*(also called dark reactions, by convention, as they are driven by products of light, ATP and NADPH-it should not be misunderstood that they occur in dark or they are independent of the need of light), the formed NADPH and ATP drive the reduction of CO_2 to more useful organic compounds, such as glucose.

The light-dependent reactions take place on the thylakoid membrane inside a chloroplast. The inside of the thylakoid membrane is called the lumen, and outside the thylakoid membrane is the stroma, where the light-independent reactions take place. The thylakoid membrane contains some integral membrane protein complexes that catalyze the light reactions. There are four major protein complexes in the thylakoid membrane: Photosystem I (PSI), Photosystem II (PSII), Cytochrome b6f complex and ATP synthase. These four complexes work together to ultimately create the products ATP and NADPH.

The two photosystems absorb light energy through proteins containing pigments, such as chlorophyll. The light-dependent reactions begin in photosystem II. When a chlorophyll *a* molecule within the reaction center of PSII absorbs a photon, an electron in this molecule attains a higher energy level. Because this state of an electron is very unstable, the electron is transferred from one to another molecule creating a chain of redox reactions, called an electron transport chain (ETC). The electron flow goes from PSII to cytochrome b6f to PSI. In PSI the electron gets the energy from another photon. The final electron acceptor is NADP. In *oxygenic photosynthesis*, the first electron donor is water, creating oxygen as a waste product. In *anoxygenic photosynthesis* various electron donors are used.

Cytochrome b6f and ATP synthase work together to create ATP. This process is called photophosphorylation, which occurs in two different ways. In *non-cyclic photophosphorylation*, cytochrome b6f uses the energy of electrons from PSII to pump protons from the stroma to the lumen. The proton gradient across the thylakoid membrane creates a proton-motive force, used by ATP synthase to form ATP. In *cyclic photophosphorylation*, cytochrome b6f uses the energy of electrons from not only PSII but also PSI to create more ATP and to stop the production of NADPH. Cyclic phosphorylation is important to create ATP and maintain NADPH in the right proportion for the light-independent reactions.

The net-reaction of all light-dependent reactions in oxygenic photosynthesis is:
$2H_2O + 2NADP^+ + 3ADP + 3P_i \rightarrow O_2 + 2NADPH + 3ATP$

Light to chemical energy

The two photosystems are protein complexes that absorb photons and are able to use this energy to create an electron transport chain. Photosystem I and II are very similar in structure and function. They use special proteins, called light-harvesting complexes, to absorb the photons with very high effectiveness. If a special pigment molecule in a photosynthetic reaction center absorbs a photon, an electron in this pigment attains the excited state and then is transferred to another molecule in the reaction center. This reaction, called *photoinduced charge separation*, is the start of the electron flow and is unique because it transforms light energy into chemical forms.

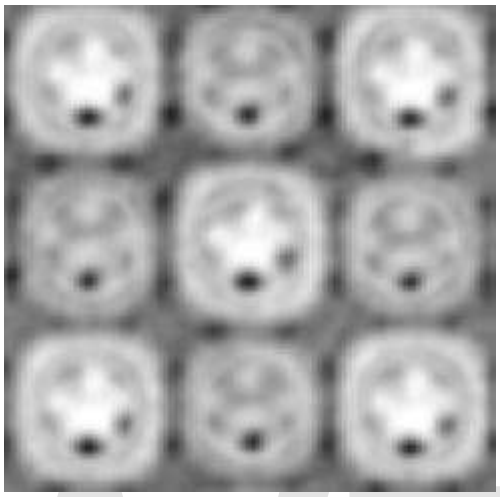
The light-harvesting system

A common misconception is that photosynthesis relies only on chlorophyll pigments. The truth is that photosynthesis would be rather inefficient using only chlorophyll molecules. Chlorophyll molecules absorb light only at specific wavelengths (see image). A large gap is present in the middle of the visible regions between approximately 450 and 650 nm. This gap corresponds to the peak of the solar spectrum, so failure to collect this light would constitute a considerable lost opportunity. That's why photosynthesis organisms have developed a light-harvesting system, which bundles different pigments to create a much wider absorption spectrum.

The light harvesting-system is composed of numerous light-harvesting complexes that completely surround the reaction center where the photoinduced charge separation takes place. Chlorophyll, carotenes and xanthophylls are arranged in such light-harvesting complexes or LHC-proteins. These pigments are referred to as accessory pigments and funnel the energy to a special pigment in the reaction center of PSI or PSII.

If a pigment molecule absorbs a photon, an electron in the molecule becomes excited. For most compounds that absorb light, the electron simply returns to the ground state and the absorbed energy is converted into heat and/or fluorescence. But in a LHC-protein the pigments are so arranged that the excitation energy can be transferred from one molecule to a nearby molecule. The rate of this process, called resonance energy transfer, depends strongly on the distance between the energy donor and energy acceptor molecules. For reasons of conservation of energy, energy transfer must be from a donor in the excited state to an acceptor of equal or lower energy. If the energy of a photon becomes lower, the wavelength also becomes longer. The pigments in an LHC-protein are so arranged that pigments are very close to each other and a pigment is near another pigment that absorbs photons with a longer wavelength. As a consequence, the pigment in the reaction center has to absorb photons with the longest wavelength and can not transfer this energy to another pigment. The function of LHC-proteins is to create a constant supply of excitation-energy to the reaction center pigment. Every reaction center has a couple of LHC-proteins.

Photosynthetic reaction centre



Electron micrograph of 2D crystals of the LH1-Reaction center photosynthetic unit.

A **photosynthetic reaction center** is a complex set of proteins, pigments and other co-factors where the primary energy conversion reactions of photosynthesis take place. Molecular excitations, either originating directly from sunlight or transferred as excitation energy via light-harvesting antenna systems, give rise to electron transfer reactions along a series of protein-bound co-factors. These co-factors are light-absorbing molecules (also named chromophores or pigments) such as chlorophyll and pheophytin, as well as quinones. The energy of the photon is used to promote an electron to a higher molecular energy level of a pigment. The free energy created is then used to reduce a chain of nearby electron acceptors, which have subsequently lowered redox-potentials. These electron transfer steps are the initial phase of a series of energy conversion reactions, ultimately resulting in the production of chemical energy during photosynthesis.

Transforming light energy into charge separation

Reaction centers are present in all green plants, algae, and many bacteria. Although these species are separated by billions of years of evolution, the reaction centers are homologous for all photosynthetic species. In contrast, a large variety in light-harvesting complexes exist between the photosynthetic species. Green plants and algae have two different types of reaction centers that are part of larger supercomplexes known as photosystem I and photosystem II. The structures of these supercomplexes are large, involving multiple light-harvesting complexes. The reaction center found in *Rhodopseudomonas* bacteria is currently best understood, since it was the first reaction center of known structure and has fewer polypeptide chains than the examples in green plants.

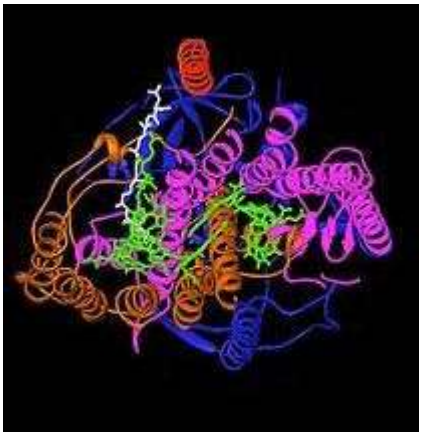
A reaction center is laid out in such a way that it captures the energy of a photon using pigment molecules and turns it into a usable form. Once the light energy has been absorbed directly by the pigment molecules, or passed to them by resonance transfer from a surrounding light-harvesting complex, they release two electrons into an electron transport chain.

Light is made up of small bundles of energy called photons. If a photon with the right amount of energy hits an electron, it will raise the electron to a higher energy level. Electrons are most stable at their lowest energy level, what is also called its ground state. In this state, the electron is in the orbit that has the least amount of energy. Electrons in higher energy levels can return to ground state in a manner analogous to a ball falling down a staircase. In doing so, the electrons release energy. This is the process that is exploited by a photosynthetic reaction center.

When an electron rises to a higher energy level, increase in the reduction potential of the molecule in which the electron resides occurs. This means that the molecule has a greater tendency to donate electrons, the key to the conversion of light energy to chemical energy. In green plants, the electron transport chain that follows has many electron acceptors including pheophytin, quinone, plastoquinone, cytochrome bf, and ferredoxin, which result in the reduced molecule NADPH. The passage of the electron through the electron transport chain also results in the pumping of protons (hydrogen ions) from the chloroplast's stroma into the lumen, resulting in a proton gradient across the thylakoid membrane that can be used to synthesise ATP using ATP synthase. Both the ATP and NADPH are used in the Calvin cycle to fix carbon dioxide into triose sugars.

Bacteria

Structure



Bacterial photosynthetic reaction centre.

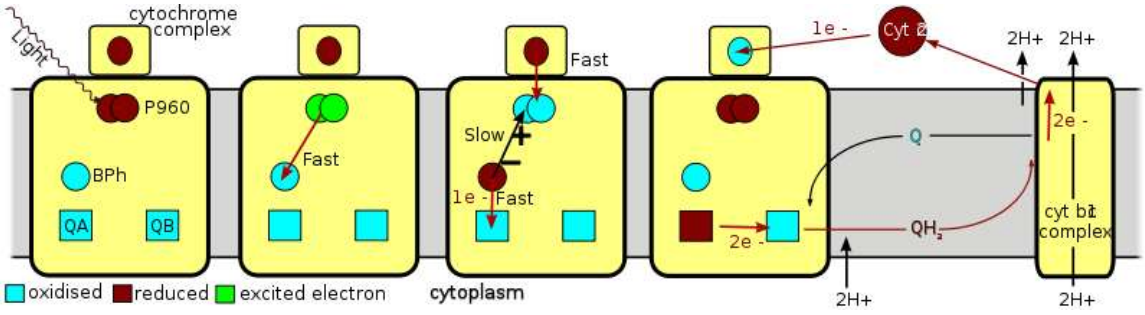
The bacterial photosynthetic reaction center has been an important model to understand the structure and chemistry of the biological process of capturing light energy. In the 1960s, Roderick Clayton was the first to purify the reaction center complex from purple

bacteria. However, the first crystal structure was determined in 1982 by Hartmut Michel, Johann Deisenhofer and Robert Huber for which they shared the Nobel Prize in 1988. This was also significant, since it was the first structure for any membrane protein complex.

Four different subunits were found to be important for the function of the photosynthetic reaction center. The L and M subunits, shown in blue and purple in the image of the structure, both span the plasma membrane. They are structurally similar to one another, both having 5 transmembrane polypeptide helices. Four bacteriochlorophyll b (BChl-b) molecules, two bacteriophaeophytin b molecules (BPh) molecules, two quinones (Q_A and Q_B), and a ferrous ion are associated with the L and M subunits. The H subunit, shown in gold, lies on the cytoplasmic side of the plasma membrane. A cytochrome subunit, here not shown, contains four c-type hemes and is located on the periplasmic surface (outer) of the membrane. The latter sub-unit is not a general structural motif in photosynthetic bacteria. The L and M subunits bind the functional and light-interacting cofactors, shown here in green.

Reaction centers from different bacterial species may contain slightly altered bacterio-chlorophyll and bacterio-pheophytin chromophores as functional co-factors. These alterations cause shifts in the color of light that can be absorbed, thus creating specific niches for photosynthesis. The reaction center contains two pigments that serve to collect and transfer the energy from photon absorption: BChl and Bph. BChl roughly resembles the chlorophyll molecule found in green plants, but, due to minor structural differences, its peak absorption wavelength is shifted into the infrared, with wavelengths as long as 1000 nm. Bph has the same structure as BChl, but the central magnesium ion is replaced by two protons. This alteration causes both an absorbance maximum shift and a lowered redox-potential.

Mechanism



The light reaction

The process starts when light is absorbed by two BChl molecules that lie near the periplasmic side of the membrane. This pair of chlorophyll molecules, often called the "special pair", absorbs photons between 870 nm and 960 nm, depending on the species and, thus, is called P870 (for the species rhodobacter sphaeroides) or P960 (for rhodopseudomonas viridis), with *P* standing for "pigment"). Once P absorbs a photon, it ejects an electron, which is transferred through another molecule of Bchl to the BPh in

the L subunit. This initial charge separation yields a positive charge on P and a negative charge on the BPh. This process takes place in 10 picoseconds (10^{-11} seconds).

The charges on the specialpair $^{+}$ and the BPh^{-} could undergo charge recombination in this state. This would waste the high-energy electron and convert the absorbed light energy in to heat. Several factors of the reaction center structure serve to prevent this. First, the transfer of an electron from BPh^{-} to $P960^{+}$ is relatively slow compared to two other redox reactions in the reaction center. The faster reactions involve the transfer of an electron from BPh^{-} (BPh^{-} is oxidised to BPh) to the electron acceptor quinone (Q_A), and the transfer of an electron to $P960^{+}$ ($P960^{+}$ is reduced to P960) from a heme in the cytochrome subunit above the reaction center.

The high-energy electron that resides on the tightly bound quinone molecule Q_A is transferred to an exchangeable quinone molecule Q_B. This molecule is loosely associated with the protein and is fairly easy to detach. Two of the high-energy electrons are required to fully reduce Q_B to QH_2, taking up two protons from the cytoplasm in the process. The reduced quinone QH_2 diffuses through the membrane to another protein complex (cytochrome bc_1-complex) where it is oxidised. In the process the reducing power of the QH_2 is used to pump protons across the membrane to the periplasmic space. The electrons from the cytochrome bc_1-complex are then transferred through a soluble cytochrome c intermediate, called cytochrome c_2, in the periplasm to the cytochrome subunit. Thus, the flow of electrons in this system is cyclical.

Green plants

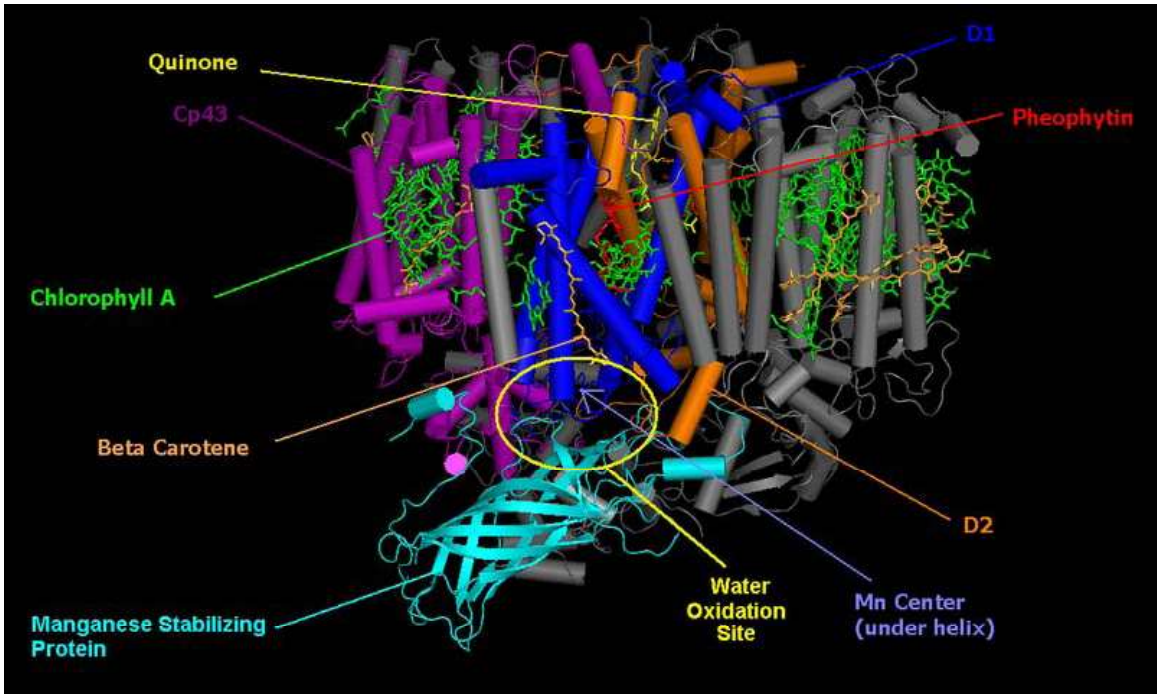
Oxygenic photosynthesis

In 1772, the chemist Joseph Priestley carried out a series of experiments relating to the gases involved in respiration and combustion. In his first experiment, he lit a candle and placed it under an upturned jar. After a short period of time, the candle burned out. He carried out a similar experiment with a mouse in the confined space of the burning candle. He found that the mouse died a short time after the candle had been extinguished. However, he could revivify the foul air by placing green plants in the area and exposing them to light. Priestley's observations were some of the first experiments that demonstrated the activity of a photosynthetic reaction center.

In 1779, Jan Ingenhousz carried out more than 500 experiments spread out over 4 months in an attempt to understand what was really going on. He wrote up his discoveries in a book entitled *Experiments upon Vegetables*. Ingenhousz took green plants and immersed them in water inside a transparent tank. He observed many bubbles rising from the surface of the leaves whenever the plants were exposed to light. Ingenhousz collected the gas that was given off by the plants and performed several different tests in attempt to determine what the gas was. The test that finally revealed the identity of the gas was placing a smouldering taper into the gas sample and having it relight. This test proved it was oxygen, or, as Joseph Priestley had called it, 'de-phlogisticated air'.

In 1932, Professor Robert Emerson and an undergraduate student, William Arnold, used a repetitive flash technique to precisely measure small quantities of oxygen evolved by chlorophyll in the algae *Chlorella*. Their experiment proved the existence of a photosynthetic unit. Gaffron and Wohl later interpreted the experiment and realized that the light absorbed by the photosynthetic unit was transferred. This reaction occurs at the reaction centre of photosystem II and takes place in cyanobacteria, algae and green plants.

Photosystem II



Cyanobacteria photosystem II, Monomer, PDB 2AXT.

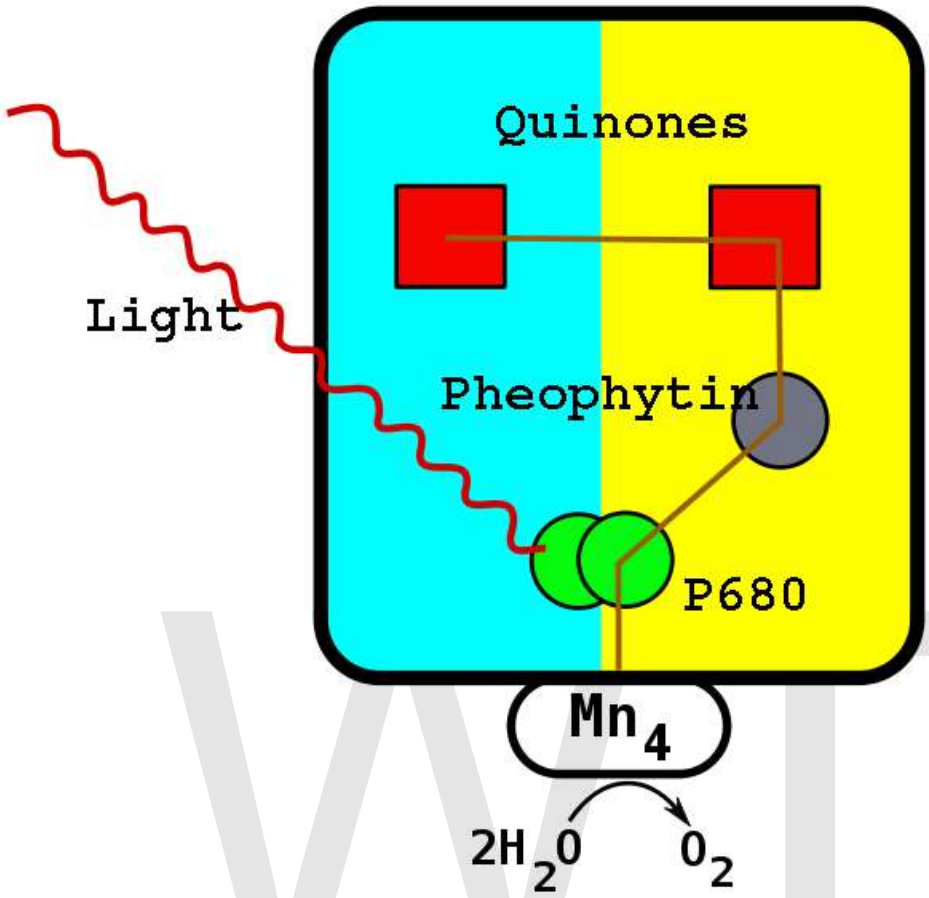
Photosystem II is the photosystem that generates the two electrons that will eventually reduce $NADP^+$ in Ferredoxin-NADP-reduktase. Photosystem II is present on the thylakoid membranes inside chloroplasts, the site of photosynthesis in green plants. The structure of Photosystem II is remarkably similar to the bacterial reaction center, and it is theorized that they share a common ancestor.

The core of photosystem II consists of two subunits referred to as D1 and D2. These two subunits are similar to the L and M subunits present in the bacterial reaction center. Photosystem II differs from the bacterial reaction center in that it has many additional subunits that bind additional chlorophylls to increase efficiency. The overall reaction catalyzed by photosystem II is:

$$2Q + 2H_2O \xrightarrow{light} O_2 + 2QH_2$$

Q represents plastoquinone, the oxidized form of Q. QH_2 represents plastoquinol, the reduced form of Q. This process of reducing quinone is comparable to that which takes place in the bacterial reaction center. Photosystem II obtains electrons by oxidizing water in a process called photolysis. Molecular oxygen is a byproduct of this process, and it is this reaction that supplies the atmosphere with oxygen. The fact that the oxygen from green plants originated from water was first deduced by the Canadian-born American biochemist Martin David Kamen. He used a natural, stable isotope of oxygen, O_{18} to trace the path of the oxygen, from water to gaseous molecular oxygen. This reaction is catalyzed by a reactive center in photosystem II containing four manganese ions.

The reaction begins with the excitation of a pair of chlorophyll molecules similar to those in the bacterial reaction center. Due to the presence of chlorophyll *a*, as opposed to bacteriochlorophyll, photosystem II absorbs light at a shorter wavelength. The pair of chlorophyll molecules at the reaction center are often referred to as P680. When the photon has been absorbed, the resulting high-energy electron is transferred to a nearby pheophytin molecule. This is above and to the right of the pair on the diagram and is coloured grey. The electron travels from the pheophytin molecule through two plastoquinone molecules, the first tightly bound, the second loosely bound. The tightly bound molecule is shown above the pheophytin molecule and is coloured red. The loosely bound molecule is to the left of this and is also coloured red. This flow of electrons is similar to that of the bacterial reaction center. Two electrons are required to fully reduce the loosely bound plastoquinone molecule to QH_2 as well as the uptake of two protons.



The difference between photosystem II and the bacterial reaction center is the source of the electron that neutralizes the pair of chlorophyll *a* molecules. In the bacterial reaction center, the electron is obtained from a reduced compound heme group in a cytochrome subunit or from a water-soluble cytochrome-c protein.

Once photoinduced charge separation has taken place, the P680 molecule carries a positive charge. P680 is a very strong oxidant and extracts electrons from two water molecules that are bound at the manganese center directly below the pair. This center, below and to the left of the pair in the diagram, contains four manganese ions, a calcium ion, a chloride ion, and a tyrosine residue. Manganese is used because it is capable of existing in four oxidation states: Mn^{2+}, Mn^{3+}, Mn^{4+} and Mn^{5+}. Manganese also forms strong bonds with oxygen-containing molecules such as water.

Every time the P680 absorbs a photon, it emits an electron, gaining a positive charge. This charge is neutralized by the extraction of an electron from the manganese center, which sits directly below it. The process of oxidizing two molecules of water requires four electrons. The water molecules that are oxidized in the manganese center are the source of the electrons that reduce the two molecules of Q to QH_2. To date, this water-splitting catalytic center cannot be reproduced by any man-made catalyst.

Photosystem I

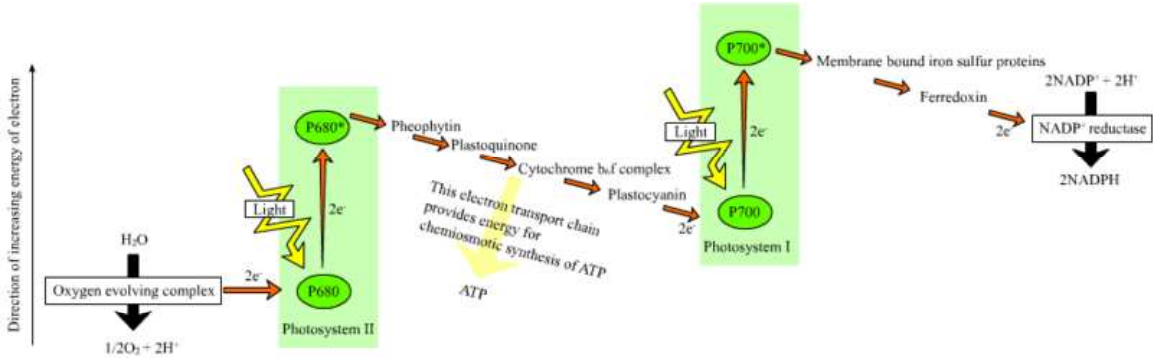
After the electron has left photosystem II it is transferred to a cytochrome b6f complex and then to plastocyanin, a blue copper protein and electron carrier. The plastocyanin complex carries the electron that will neutralize the pair in the next reaction center, photosystem I.

As with photosystem II and the bacterial reaction center, a pair of chlorophyll *a* molecules initiates photoinduced charge separation. This pair is referred to as P700. 700 Is a reference to the wavelength at which the chlorophyll molecules absorb light maximally. The P700 lies in the center of the protein. Once photoinduced charge separation has been initiated, the electron travels down a pathway through a chlorophyll α molecule situated directly above the P700, through a quinone molecule situated directly above that, through three 4Fe-4S clusters, and finally to an interchangeable ferredoxin complex. Ferredoxin is a soluble protein containing a 2Fe-2S cluster coordinated by four cysteine residues. The positive charge left on the P700 is neutralized by the transfer of an electron from plastocyanin. Thus the overall reaction catalyzed by photosystem I is:

$$Pc(Cu^{+}) + Fd_{ox} \xrightarrow{light} Pc(Cu^{2+}) + Fd_{red}$$

The cooperation between photosystems I and II creates an electron flow from H_2O to $NADP^+$. This pathway is called the 'Z-scheme' because the redox diagram from P680 to P700 resembles the letter z.

Photosynthetic electron transport chains in chloroplasts



The photosynthesis process in chloroplasts begins when an electron of P680 of PSII attains an higher-energy level. This energy is used to reduce a chain of electron acceptors that have subsequently lowered redox-potentials. This chain of electron acceptors is known as an electron transport chain. When this chain reaches PS I, an electron is again excited, creating a high redox-potential. The electron transport chain of photosynthesis is often put in a diagram called the z-scheme, because the redox diagram from P680 to P700 resembles the letter z.

The final product of PSII is plastoquinol, a mobile electron carrier in the membrane. Plastoquinol transfers the electron from PSII to the proton pump, cytochrome b6f. The ultimate electron donor of PSII is water. Cytochrome b6f proceeds the electron chain to PSI through plastocyaninmolecules. PSI is able to continue the electron transfer in two different ways. It can transfer the electrons either to plastoquinonol again, creating a cyclic electron flow, or to an enzyme called FNR creating a non-cyclic electron flow. PSI releases FNR into the stroma, where it reduces $NADP^+$ to NADPH.

Activities of the electron transport chain, especially from cytochrome b6f lead to pumping of protons from the stroma to the lumen. The resulting transmembrane proton gradient is used to make ATP via ATP synthase.

The overall process of the photosynthetic electron transport chain in chloroplasts is:

$$\mathbf{H_2O \rightarrow PS\ II \rightarrow plastoquinone \rightarrow } \boldsymbol{cytb_6f} \mathbf{\rightarrow plastocyanin \rightarrow PS\ I \rightarrow NADPH}$$

Photosystem II

PS II is an extremely complex, highly organized transmembrane structure that contains a *water-splitting complex*, chlorophylls and carotenoid pigments, a *reaction center* (P680), pheophytin (a pigment similar to chlorophyll), and two quinones. It uses the energy of sunlight to transfer electrons from water to a mobile electron carrier in the membrane called *plastoquinone*:

$$\mathbf{H_2O \rightarrow P680 \rightarrow P680^* \rightarrow plastoquinone}$$

Plastoquinone, in turn, transfers electrons to b_6f, which feeds them into PS I.

The water-splitting complex

The step $\mathbf{H_2O \rightarrow P680}$ is performed by a poorly-understood structure embedded within PS II called the *water-splitting complex* or the *oxygen-evolving complex*. It catalyzes a reaction that splits water into electrons, protons and oxygen:

$$\mathbf{2H_2O \rightarrow 4H^+ + 4e^- + O_2}$$

The electrons are transferred to special chlorophyll molecules (embedded in PS II) that are promoted to a higher-energy state by the energy of photons.

The reaction center

The excitation $\mathbf{P680 \rightarrow P680^*}$ of the reaction center pigment P680 occurs here. These special chlorophyll molecules embedded in PS II absorb the energy of photons, with maximal absorption at 680 nm. Electrons within these molecules are promoted to a higher-energy state. This is one of two core processes in photosynthesis, and it occurs with astonishing efficiency (greater than 90%) because, in addition to direct excitation by

light at 680 nm, the energy of light first harvested by *antenna proteins* at other wavelengths in the light-harvesting system is also transferred to these special chlorophyll molecules.

This is followed by the step **P680*** → **pheophytin**, and then on to **plastoquinone**, which occurs within the reaction center of PS II. High-energy electrons are transferred to plastoquinone. Plastoquinone is then released into the membrane as a mobile electron carrier.

This is the second core process in photosynthesis. The initial stages occur within *picoseconds*, with an efficiency of 100%. The seemingly impossible efficiency is due to the precise positioning of molecules within the reaction center. This is a solid-state process, not a chemical reaction. It occurs within an essentially crystalline environment created by the macromolecular structure of PS II. The usual rules of chemistry (which involve random collisions and random energy distributions) do not apply in solid-state environments.

Link of water splitting complex and chlorophyll excitation

When the chlorophyll passes the electron to pheophytin, it obtains an electron from P_{680}^*. In turn, P_{680}^* can oxidize the Z (or Y_Z) molecule. Once oxidized, the Z molecule can derive electrons from the water splitting complex.

Summary

PS II is a transmembrane structure found in all chloroplasts. It splits water into electrons, protons and molecular oxygen. The electrons are transferred to plastoquinone, which carries them to a proton pump. Molecular oxygen is released into the atmosphere.

The emergence of such an incredibly complex structure, a macromolecule that converts the energy of sunlight into potentially useful work with efficiencies that are impossible in ordinary experience, seems almost magical at first glance. Thus it is of considerable interest that essentially the same structure is found in *purple bacteria*.

Cytochrome b_6f

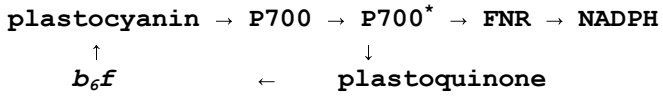
PS II and PS I are connected by a transmembrane proton pump, cytochrome b_6f complex (plastoquinol—plastocyanin reductase; EC 1.10.99.1. Electrons from PS II are carried by plastoquinone to b_6f, where they are removed in a stepwise fashion and transferred to a water-soluble electron carrier called *plastocyanin*. This redox process is coupled to the pumping of four protons across the membrane. The resulting proton gradient (together with the proton gradient produced by the water-splitting complex in PS II) is used to make ATP via ATP synthase.

The similarity in structure and function between cytochrome b_6f (in chloroplasts) and cytochrome bc_1 (*Complex III* in mitochondria) is striking. Both are transmembrane

structures that remove electrons from a mobile, lipid-soluble electron carrier (plastoquinone in chloroplasts; ubiquinone in mitochondria) and transfer them to a mobile, water-soluble electron carrier (plastocyanin in chloroplasts; cytochrome *c* in mitochondria). Both are proton pumps that produce a transmembrane proton gradient.

Photosystem I

PS I accepts electrons from plastocyanin and transfers them either to NADPH (*noncyclic electron transport*) or back to cytochrome b_6f (*cyclic electron transport*):



PS I, like PS II, is a complex, highly organized transmembrane structure that contains antenna chlorophylls, a reaction center (P700), phylloquinine, and a number of iron-sulfur proteins that serve as intermediate redox carriers.

The light-harvesting system of PS I uses multiple copies of the same transmembrane proteins used by PS II. The energy of absorbed light (in the form of delocalized, high-energy electrons) is funneled into the reaction center, where it excites special chlorophyll molecules (P700, maximum light absorption at 700 nm) to a higher energy level. The process occurs with astonishingly high efficiency.

Electrons are removed from excited chlorophyll molecules and transferred through a series of intermediate carriers to *ferredoxin*, a water-soluble electron carrier. As in PS II, this is a solid-state process that operates with essentially 100% efficiency.

There are two different pathways of electron transport in PS I. In *noncyclic electron transport*, ferredoxin carries the electron to the enzyme ferredoxin $NADP^+$ oxidoreductase that reduces $NADP^+$ to NADPH. Alternately, in *cyclic electron transport*, electrons from ferredoxin are transferred (via plastoquinone) to a proton pump, cytochrome b_6f. They are then returned (via plastocyanin) to P700.

NADPH and ATP are used to synthesize organic molecules from CO_2. The ratio of NADPH to ATP production can be adjusted by adjusting the balance between cyclic and noncyclic electron transport.

It is noteworthy that PS I closely resembles photosynthetic structures found in *green sulfur bacteria*, just as PS II resembles structures found in *purple bacteria*.

Photosynthetic electron transport chains in bacteria

PS II, PS I and cytochromeb_6f are found in chloroplasts. All plants and all photosynthetic algae contain chloroplasts, which produce NADPH and ATP by the mechanisms

described above. Essentially the same transmembrane structures are also found in *cyanobacteria*.

Unlike plants and algae, cyanobacteria are prokaryotes. They do not contain chloroplasts. Rather, they bear a striking resemblance to chloroplasts themselves. This suggests that organisms resembling cyanobacteria were the evolutionary precursors of chloroplasts. One imagines primitive eukaryotic cells taking up cyanobacteria as intracellular symbionts.

Cyanobacteria

Cyanobacteria contain structures similar to PS II and PS I in chloroplasts. Their light-harvesting system is different from that found in plants (they use *phycobilins*, rather than chlorophylls, as antenna pigments), but their electron transport chain

$$\mathbf{H_2O \rightarrow PS\ II \rightarrow plastoquinone \rightarrow b_6f \rightarrow cytochrome\ c_6 \rightarrow PS\ I \rightarrow ferredoxin \rightarrow NADPH}$$

$$\downarrow \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \uparrow$$

$$\mathbf{plastoquinone \leftarrow b_6f}$$

is essentially the same as the electron transport chain in chloroplasts. The mobile water-soluble electron carrier is cytochrome c_6 in cyanobacteria, plastocyanin in plants.

Cyanobacteria can also synthesize ATP by oxidative phosphorylation, in the manner of other bacteria. The electron transport chain is

$$\mathbf{NADH\ dehydrogenase \rightarrow plastoquinone \rightarrow b_6f \rightarrow cytochrome\ c_6 \rightarrow cytochrome\ aa_3 \rightarrow O_2}$$

where the mobile electron carriers are plastoquinone and cytochrome c_6, while the proton pumps are NADH dehydrogenase, b_6f and cytochrome aa_3.

Cyanobacteria are the only bacteria that produce oxygen during photosynthesis. The Earth's primordial atmosphere was anoxic. Organisms like cyanobacteria produced our present-day oxygen containing atmosphere.

The other two major groups of photosynthetic bacteria, purple bacteria and green sulfur bacteria, contain only a single photosystem and do not produce oxygen.

Purple bacteria

Purple bacteria contain a single photosystem that is structurally related to PS II in cyanobacteria and chloroplasts:

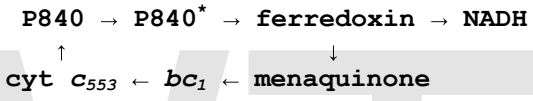
$$\text{P870} \rightarrow \text{P870}^* \rightarrow \text{ubiquinone} \rightarrow bc_1 \rightarrow \text{cytochrome } c_2 \rightarrow P870$$

This is a *cyclic* process in which electrons are removed from an excited chlorophyll molecule (*bacteriochlorophyll*; P870), passed through an electron transport chain to a proton pump (cytochrome bc_1 complex, similar but not identical to cytochrome bc_1 in chloroplasts), and then returned to the cholorophyll molecule. The result is a proton gradient, which is used to make ATP via ATP synthase. As in cyanobacteria and chloroplasts, this is a solid-state process that depends on the precise orientation of various functional groups within a complex transmembrane macromolecular structure.

In order to make NADPH, purple bacteria use an external electron donor (hydrogen, hydrogen sulfide, sulfur, sulfite, or organic molecules such as succinate and lactate) to feed electrons into a reverse electron transport chain.

Green sulfur bacteria

Green sulfur bacteria contain a photosystem that is analogous to PS I in chloroplasts:



There are two pathways of electron transfer. In *cyclic electron transfer*, electrons are removed from an excited chlorophyll molecule, passed through an electron transport chain to a proton pump, and then returned to the chlorophyll. The mobile electron carriers are, as usual, a lipid-soluble quinone and a water-soluble cytochrome. The resulting proton gradient is used to make ATP.

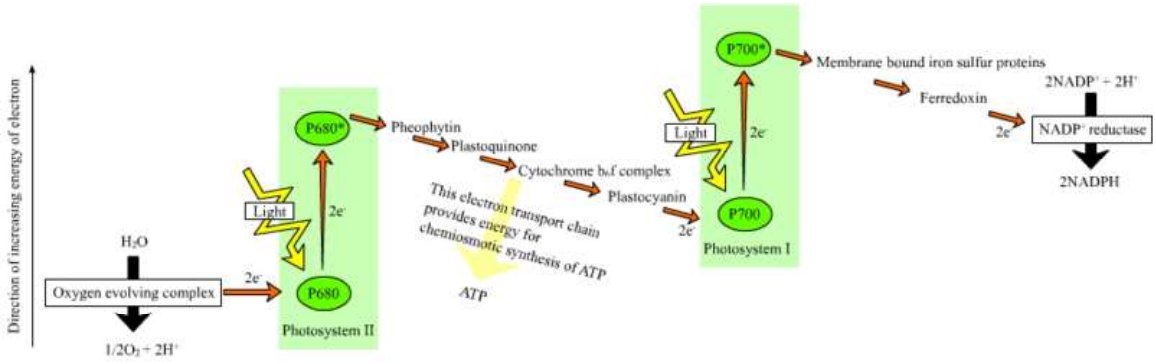
In *noncyclic electron transfer*, electrons are removed from an excited chlorophyll molecule and used to reduce NAD^+ to NADH. The electrons removed from P840 must be replaced. This is accomplished by removing electrons from H_2S, which is oxidized to sulfur (hence the name "green *sulfur* bacteria").

Purple bacteria and green sulfur bacteria occupy relatively minor ecological niches in the present day biosphere. They are of interest because of their importance in precambrian ecologies, and because they were the evolutionary precursors of modern plants.

History

The first ideas about light being used in photosynthesis were proposed by Colin Flannery in 1779 who recognized it was sunlight falling on plants that was required, although Joseph Priestly had noted the production of oxygen without the association with light in 1772. Cornelius Van Niel proposed in 1931 that photosynthesis is a case of general mechanism where a photon of light is used to photo decompose a hydrogen donor and the hydrogen being used to reduce CO_2. Then in 1939 Robin Hill showed that isolated chloroplasts would make oxygen, but not fix CO_2 showing the light and dark reactions occurred in different places. This led later to the discovery of photosystem 1 and 2.

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 (see diagram). 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.

Photodissociation

Photodissociation, **photolysis**, or **photodecomposition** is a chemical reaction in which a chemical compound is broken down by photons. It is defined as the interaction of one or more photons with one target molecule.

Photodissociation is not limited to visible light. Any photon with sufficient energy can affect the chemical bonds of a chemical compound. Since a photon's energy is inversely proportional to its wavelength, electromagnetic waves with the energy of visible light or higher, such as ultraviolet light, x-rays and gamma rays are usually involved in such reactions.

Photolysis in photosynthesis

Photolysis is part of the light-dependent reactions of photosynthesis. The general reaction of photosynthetic photolysis can be given as:

$$H_2A + 2 \text{ photons (light)} \longrightarrow 2e^- + 2H^+ + A$$

The chemical nature of "A" depends on the type of organism. In purple sulfur bacteria, hydrogen sulfide (H_2S) is oxidized to sulfur (S). In oxygenic photosynthesis, water (H_2O) serves as a substrate for photolysis resulting in the generation of free oxygen (O_2). This is the process which returns oxygen to earth's atmosphere. Photolysis of water occurs in the thylakoids of cyanobacteria and the chloroplasts of green algae and plants.

Energy transfer models

The conventional, semi-classical, model describes the photosynthetic energy transfer process as one in which excitation energy hops from light-capturing pigment molecules to reaction center molecules step-by-step down the molecular energy ladder.

The effectiveness of photons of different wavelengths depends on the absorption spectra of the photosynthetic pigments in the organism. Chlorophylls absorb light in the violet-blue and red parts of the spectrum, while accessory pigments capture other wavelengths as well. The phycobilins of red algae absorb blue-green light which penetrates deeper into water than red light, enabling them to photosynthesize in deep waters. Each absorbed photon causes the formation of an exciton (an electron excited to a higher energy state) in the pigment molecule. The energy of the exciton is transferred to a chlorophyll molecule (P680, where P stands for pigment and 680 for its absorption maximum at 680 nm) in the reaction center of photosystem II via resonance energy transfer. P680 can also directly absorb a photon at a suitable wavelength.

Photolysis during photosynthesis occurs in a series of light-driven oxidation events. The energized electron (exciton) of P680 is captured by a primary electron acceptor of the photosynthetic electron transfer chain and thus exits photosystem II. In order to repeat the reaction, the electron in the reaction center needs to be replenished. This occurs by oxidation of water in the case of oxygenic photosynthesis. The electron-deficient reaction center of photosystem II (P680*) is the strongest biological oxidizing agent yet discovered, which allows it to break apart molecules as stable as water.

The water-splitting reaction is catalyzed by the oxygen evolving complex of photosystem II. This protein-bound inorganic complex contains four manganese ions, plus calcium and chloride ions as cofactors. Two water molecules are complexed by the manganese cluster, which then undergoes a series of four electron removals (oxidations) to replenish the reaction center of photosystem II. At the end of this cycle, free oxygen (O_2) is generated and the hydrogen of the water molecules has been converted to four protons released into the thylakoid lumen.

These protons, as well as additional protons pumped across the thylakoid membrane coupled with the electron transfer chain, form a proton gradient across the membrane that drives photophosphorylation and thus the generation of chemical energy in the form of adenosine triphosphate (ATP). The electrons reach the P700 reaction center of photosystem I where they are energized again by light. They are passed down another electron transfer chain and finally combine with the coenzyme $NADP^+$ and protons outside the thylakoids to NADPH. Thus, the net oxidation reaction of water photolysis can be written as:

$$2H_2O + 2NADP^+ + 8 \text{ photons (light)} \longrightarrow 2NADPH + 2H^+ + O_2$$

The free energy change (ΔG) for this reaction is 102 kilocalories per mole. Since the energy of light at 700 nm is about 40 kilocalories per mole of photons, approximately 320 kilocalories of light energy are available for the reaction. Therefore, approximately one-third of the available light energy is captured as NADPH during photolysis and electron transfer. An equal amount of ATP is generated by the resulting proton gradient. Oxygen as a byproduct is of no further use to the reaction and thus released into the atmosphere.

Quantum models

In 2007 a quantum model was proposed by Graham Fleming, which includes the possibility that photosynthetic energy transfer might involve quantum oscillations, explaining its unusually high efficiency.

According to Fleming there is direct evidence that remarkably long-lived wavelike electronic quantum coherence plays an important part in energy transfer processes during photosynthesis, which can explain the extreme efficiency of the energy transfer because it enables the system to sample all the potential energy pathways, with low loss, and choose the most efficient one.

This approach has been further investigated by Gregory Scholes and his team at the University of Toronto, which in early 2010 published research results that indicate that some marine algae make use of quantum-coherent electronic energy transfer (EET) to enhance the efficiency of their energy harnessing.

Photolysis in the atmosphere

Photolysis also occurs in the atmosphere as part of a series of reactions by which primary pollutants such as hydrocarbons and nitrogen oxides react to form secondary pollutants such as peroxyacyl nitrates.

The two most important photodissociaton reactions in the troposphere are firstly:

$$O_3 + h\nu \rightarrow O_2 + O(^1D) \quad \lambda < 320 \text{ nm}$$

which generates an excited oxygen atom which can react with water to give the hydroxyl radical:

$$O(^1D) + H_2O \rightarrow 2OH$$

The hydroxyl radical is central to atmospheric chemistry as it initiates the oxidation of hydrocarbons in the atmosphere and so acts as a detergent.

Secondly the reaction:

$$NO_2 + h\nu \rightarrow NO + O$$

is a key reaction in the formation of tropospheric ozone.

The formation of the ozone layer is also caused by photodissociation. Ozone in the Earth's stratosphere is created by ultraviolet light striking oxygen molecules containing two oxygen atoms (O_2), splitting them into individual oxygen atoms (atomic oxygen). The atomic oxygen then combines with unbroken O_2 to create ozone, O_3. In addition,

photolysis is the process by which CFCs are broken down in the upper atmosphere to form ozone-destroying chlorine free radicals.

Astrophysics

In astrophysics, photodissociation is one of the major processes through which molecules are broken down (but new molecules are being formed). Because of the vacuum of the interstellar medium, molecules and free radicals can exist for a long time. Photodissociation is the main path by which molecules are broken down. Photodissociation rates are important in the study of the composition of interstellar clouds in which stars are formed.

Examples of photodissociation in the interstellar medium are ($h\nu$ is the scientific notation for light, specifically a photon):

$$H_2O + h\nu \rightarrow H + OH$$
$$CH_4 + h\nu \rightarrow CH_3 + H$$

Atmospheric Gamma Ray Bursts

Currently orbiting satellites detect an average of about one gamma-ray burst per day. Because gamma-ray bursts are visible to distances encompassing most of the observable universe, a volume encompassing many billions of galaxies, this suggests that gamma-ray bursts must be exceedingly rare events per galaxy.

Measuring the exact rate of Gamma Ray bursts is difficult, but for a galaxy of approximately the same size as the Milky Way, the expected rate (for long GRBs) is about one burst every 100,000 to 1,000,000 years. Only a few percent of these would be beamed towards Earth. Estimates of rates of short GRBs are even more uncertain because of the unknown beaming fraction, but are probably comparable.

A gamma-ray burst in the Milky Way, if close enough to Earth and beamed towards it, could have significant effects on the biosphere. The absorption of radiation in the atmosphere would cause photodissociation of nitrogen, generating nitric oxide that would act as a catalyst to destroy ozone.

The atmospheric photodissasociation

- N_2 -> 2N
- O_2 -> 2O
- CO_2 -> C + 2O
- H_2O -> 2H + O

would yield

- NO_2 (consumes up to 400 Ozone molecules)
- CH_2 (nominal)
- CH_4 (nominal)
- CO_2

According to a 2004 study, a GRB at a distance of about a kiloparsec could destroy up to half of Earth's ozone layer; the direct UV irradiation from the burst combined with additional solar UV radiation passing through the diminished ozone layer could then have potentially significant impacts on the food chain and potentially trigger a mass extinction. The authors estimate that one such burst is expected per billion years, and hypothesize that the Ordovician-Silurian extinction event could have been the result of such a burst.

There are strong indications that long gamma-ray bursts preferentially or exclusively occur in regions of low metallicity. Because the Milky Way has been metal-rich since before the Earth formed, this effect may diminish or even eliminate the possibility that a long gamma-ray burst has occurred within the Milky Way within the past billion years. No such metallicity biases are known for short gamma-ray bursts. Thus, depending on their local rate and beaming properties, the possibility for a nearby event to have had a large impact on Earth at some point in geological time may still be significant.

Multiple photon dissociation

Single photons in the infrared spectral range usually are not energetic enough for direct photodissociation of molecules. However, after absorption of multiple infrared photons a molecule may gain internal energy to overcome its barrier for dissociation. Multiple photon dissociation (MPD, IRMPD with infrared radiation) can be achieved by applying high power lasers, e.g. a carbon dioxide laser, or a free electron laser, or by long interaction times of the molecule with the radiation field without the possibility for rapid cooling, e.g. by collisions. The latter method allows even for MPD induced by black body radiation, a technique called Blackbody infrared radiative dissociation (BIRD).

Oxygen evolution

Oxygen evolution is the process of generating molecular oxygen through chemical reaction. Mechanisms of oxygen evolution include the oxidation of water during oxygenic photosynthesis, electrolysis of water into oxygen and hydrogen, and electrocatalytic oxygen evolution from oxides and oxoacids.

Oxygen evolution in nature

Photosynthetic oxygen evolution is the fundamental process by which breathable oxygen is generated in earth's biosphere. The reaction is part of the light-dependent reactions of photosynthesis in cyanobacteria and the chloroplasts of green algae and plants. It utilizes

the energy of light to split a water molecule into its protons and electrons for photosynthesis. Free oxygen is generated as a waste product of this reaction, and is released into the atmosphere.

Biochemical reaction

Photosynthetic oxygen evolution occurs via the light-dependent oxidation of water to molecular oxygen and can be written as the following simplified chemical reaction:

$$2H_2O \longrightarrow 4e^- + 4H^+ + O_2$$

The reaction requires the energy of four photons. The electrons from the oxidized water molecules replace electrons in the P_{680} component of photosystem II which have been removed into an electron transport chain via light-dependent excitation and resonance energy transfer onto plastoquinone. Photosytem II therefore has also been referred to as water-plastoquinone oxido-reductase. The protons are released into the thylakoid lumen, thus contributing to the generation of a proton gradient across the thylakoid membrane. This proton gradient is the driving force for ATP synthesis via photophosphorylation and coupling the absorption of light energy and oxidation of water to the creation of chemical energy during photosynthesis.

Oxygen-evolving complex

Water oxidation is catalyzed by a manganese-containing cofactor contained in photosystem II known as the oxygen evolving complex (OEC) or water-splitting complex. Manganese is an important cofactor, and calcium and chloride are also required for the reaction to occur.

X-ray crystallography studies have recently provided detailed models of the structure of the oxygen-evolving complex and its manganese cluster. Based on structural and spectroscopic experiments, oxygen evolution involves a core three-plus-one cluster of three manganese ions and one calcium ion, with one additional manganese, which are oxidized via intermediate states called *S-states*. The O-O bond of molecular oxygen is formed between manganese-ligated oxygen atoms at the most oxidized, or S4, state.

Evolution of oxygen evolution

Oxygen production during photosynthesis evolved on earth around 3.5 billion years ago. Oxygen was not only a waste product of this reaction, but was also toxic to many metabolic processes such as nitrogen fixation. Consequently, it was released into the atmosphere as a means of detoxification. This contributed to the conversion of earth's atmosphere from anaerobic to its current aerobic composition, triggering the oxygen catastrophe and the evolution of aerobic metabolism utilizing the oxygen that was released by photosynthetic organisms as part of the oxygen cycle.

History of discovery

It wasn't until the end of the 18th century that Joseph Priestley discovered by accident the ability of plants to "restore" air that had been "injured" by the burning of a candle. He followed up on the experiment by showing that air "restored" by vegetation was *"not at all inconvenient to a mouse."* He was later awarded a medal for his discoveries that: *"...no vegetable grows in vain... but cleanses and purifies our atmosphere."* Priestley's experiments were followed up by Jan Ingenhousz, a Dutch physician, who showed that "restoration" of air only worked in the presence of light and green plant parts.

Ingenhousz suggested in 1796 that CO_2 (carbon dioxide) is split during photosynthesis to release oxygen, while the carbon combined with water to form carbohydrates. While this hypothesis was attractive and reasonable and thus widely accepted for a long time, it was later proven incorrect. Graduate student C.B. Van Niel at Stanford University found that purple sulfur bacteria reduce carbon to carbohydrates, but accumulate sulfur instead of releasing oxygen. He boldly proposed that in analogy to the sulfur bacteria forming elemental sulfur from H_2S (hydrogen sulfide), plants would form oxygen from H_2O (water). In 1937, this hypothesis was corroborated by the discovery that plants are capable of producing oxygen in the absence of CO_2. This discovery was made by Robin Hill, and subsequently the light-driven release of oxygen in the absence of CO_2 was called the *Hill reaction*. Our current knowledge of the mechanism of oxygen evolution during photosynthesis was further established in experiments tracing isotopes of oxygen from water to oxygen gas.

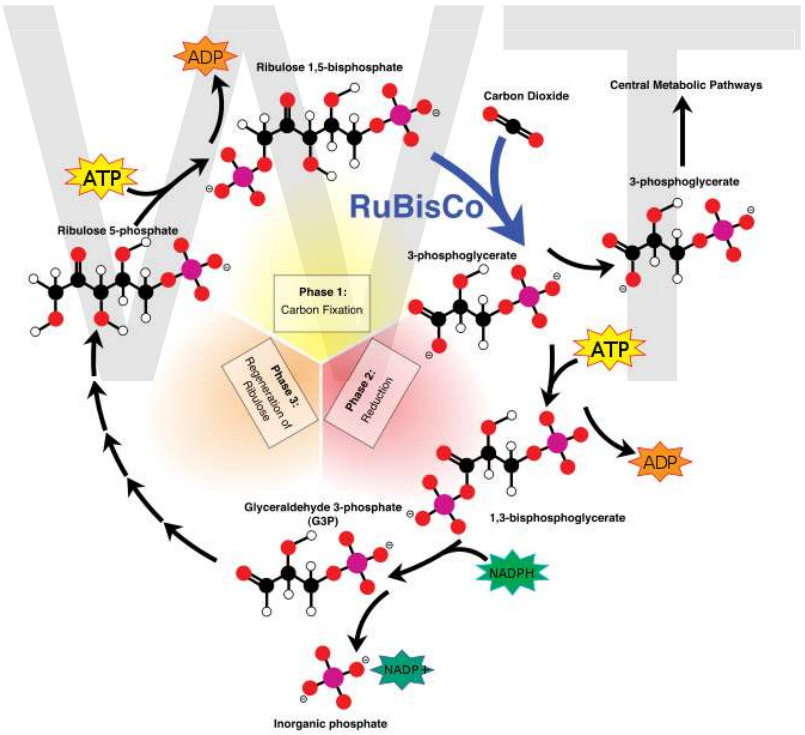
Technological oxygen evolution

Oxygen evolution occurs as a byproduct of hydrogen production via electrolysis of water. While oxygen production is not the main focus of industrial applications of water electrolysis, it becomes essential for life support systems in situations that require the generation of oxygen for air revitalization. Human exploration of regions that lack breathable oxygen, such as the deep sea or outer space, requires means of reliably generating oxygen apart from earth's atmosphere. Submarines and spacecraft utilize either an electrolytic mechanism (water or solid oxide electrolysis) or chemical oxygen generators as part of their life support equipment.

Chapter- 4

Light-independent Reactions

Calvin Cycle



Overview of the Calvin cycle and carbon fixation

The **Calvin cycle** or **Calvin–Benson cycle** or **Reductive Pentose Phosphate cycle** is a series of biochemical reactions that take place in the stroma of chloroplasts in photosynthetic organisms. It was discovered by Melvin Calvin, James Bassham and Andrew Benson at the University of California, Berkeley by using the radioactive isotope, carbon-14. It is one of the light-independent (dark) reactions, used for carbon fixation.

Overview

During photosynthesis, light energy is used in generating chemical free energy, stored in glucose. The light-independent Calvin cycle, also known (erroneously) as the "dark reaction" or "dark stage," uses the energy from short-lived electronically-excited carriers to convert carbon dioxide and water into organic compounds that can be used by the organism (and by animals that feed on it). This set of reactions is also called *carbon fixation*. The key enzyme of the cycle is called RuBisCO. In the following equations, the chemical species (phosphates and carboxylic acids) exist in equilibria among their various ionized states as governed by the pH.

The enzymes in the Calvin cycle are functionally equivalent to many enzymes used in other metabolic pathways such as gluconeogenesis and the pentose phosphate pathway, but they are to be found in the chloroplast stroma instead of the cell cytoplasm, separating the reactions. They are activated in the light (which is why the name "dark reaction" is misleading), and also by products of the light-dependent reaction. These regulatory functions prevent the Calvin cycle from being respired to carbon dioxide. Energy (in the form of ATP) would be wasted in carrying out these reactions that have no net productivity.

The sum of reactions in the Calvin cycle is the following:

$3\ CO_2 + 6\ NADPH + 5\ H_2O + 9\ ATP \rightarrow$ glyceraldehyde-3-phosphate (G3P) $+ 2\ H^+ + 6\ NADP^+ + 9\ ADP + 8\ P_i$

or

$3\ CO_2 + 6\ C_{21}H_{29}N_7O_{17}P_3 + 5\ H_2O + 9\ C_{10}H_{16}N_5O_{13}P_3 \rightarrow C_3H_5O_3\text{-}PO_3^{2-} + 2\ H^+ + 6\ NADP^+ + 9\ C_{10}H_{15}N_5O_{10}P_2 + 8\ P_i$

Hexose (six-carbon) sugars are not a product of the Calvin cycle. Although many texts list a product of photosynthesis as $C_6H_{12}O_6$, this is mainly a convenience to counter the equation of respiration, where six-carbon sugars are oxidized in mitochondria. The carbohydrate products of the Calvin cycle are three-carbon sugar phosphate molecules, or "triose phosphates," namely, glyceraldehyde-3-phosphate (G3P).

Steps of the Calvin cycle

1. The enzyme RuBisCO catalyses the carboxylation of ribulose-1,5-bisphosphate, a 5-carbon compound, by carbon dioxide (a total of 6 carbons) in a two-step reaction. The initial product of the reaction is a six-carbon intermediate so unstable that it immediately splits in half, forming two molecules of glycerate 3-phosphate, a 3-carbon compound (also: 3-phosphoglycerate, 3-phosphoglyceric acid, 3PGA).
2. The enzyme phosphoglycerate kinase catalyses the phosphorylation of 3PGA by ATP (which was produced in the light-dependent stage). 1,3-bisphosphoglycerate (glycerate-1,3-bisphosphate) and ADP are the products. (However, note that two

PGAs are produced for every CO_2 that enters the cycle, so this step utilizes two ATP per CO_2 fixed.)

3. The enzyme G3P dehydrogenase catalyses the reduction of 1,3BPGA by NADPH (which is another product of the light-dependent stage). Glyceraldehyde 3-phosphate (also G3P, GP, TP, PGAL) is produced, and the NADPH itself was oxidized and becomes $NADP^+$. Again, two NADPH are utilized per CO_2 fixed.

(Simplified versions of the Calvin cycle integrate the remaining steps, except for the last one, into one general step - the regeneration of RuBP. Also, one G3P would exit here.)

1. Triose phosphate isomerase converts all of the G3P reversibly into dihydroxyacetone phosphate (DHAP), also a 3-carbon molecule.
2. Aldolase and fructose-1,6-bisphosphatase convert a G3P and a DHAP into fructose 6-phosphate (6C). A phosphate ion is lost into solution.
3. Then fixation of another CO_2 generates two more G3P.
4. F6P has two carbons removed by transketolase, giving erythrose-4-phosphate. The two carbons on transketolase are added to a G3P, giving the ketose xylulose-5-phosphate (Xu5P).
5. E4P and a DHAP (formed from one of the G3P from the second CO_2 fixation) are converted into sedoheptulose-1,7-bisphosphate (7C) by aldolase enzyme.
6. Sedoheptulose-1,7-bisphosphatase (one of only three enzymes of the Calvin cycle that are unique to plants) cleaves sedoheptulose-1,7-bisphosphate into sedoheptulose-7-phosphate, releasing an inorganic phosphate ion into solution.
7. Fixation of a third CO_2 generates two more G3P. The ketose S7P has two carbons removed by transketolase, giving ribose-5-phosphate (R5P), and the two carbons remaining on transketolase are transferred to one of the G3P, giving another Xu5P. This leaves one G3P as the product of fixation of 3 CO_2, with generation of three pentoses that can be converted to Ru5P.
8. R5P is converted into ribulose-5-phosphate (Ru5P, RuP) by phosphopentose isomerase. Xu5P is converted into RuP by phosphopentose epimerase.
9. Finally, phosphoribulokinase (another plant-unique enzyme of the pathway) phosphorylates RuP into RuBP, ribulose-1,5-bisphosphate, completing the Calvin *cycle*. This requires the input of one ATP.

Thus, of 6 G3P produced, three RuBP (5C) are made, totaling 15 carbons, with only one available for subsequent conversion to hexose. This required 9 ATPs and 6 NADPH per 3 CO_2.

RuBisCO also reacts competitively with O_2 instead of CO_2 in photorespiration. The rate of photorespiration is higher at high temperatures. Photorespiration turns RuBP into 3PGA and 2-phosphoglycolate, a 2-carbon molecule that can be converted via glycolate and glyoxalate to glycine. Via the glycine cleavage system and tetrahydrofolate, two glycines are converted into serine +CO_2. Serine can be converted back to 3-phosphoglycerate. Thus, only 3 of 4 carbons from two phosphoglycolates can be converted back to 3PGA. It can be seen that photorespiration has very negative consequences for the plant, because, rather than fixing CO_2, this process leads to loss of

CO_2. C4 carbon fixation evolved to circumvent photorespiration, but can occur only in certain plants native to very warm or tropical climates, for example, corn.

Products of the Calvin cycle

The immediate products of one turn of the Calvin cycle are 2 glyceraldehyde-3-phosphate (G3P) molecules, 3 ADP, and 2 $NADP^+$ (ADP and $NADP^+$ are regenerated in the Light-dependent reactions). Each G3P molecule is composed of 3 carbons. In order for the Calvin cycle to continue, RuBP (ribulose 1,5-bisphosphate) must be regenerated. So, 5/6 carbon from the 2 G3P molecules are used for this purpose. Therefore, there is only 1 net carbon produced to play with for each turn. To create 1 surplus G3P requires 3 carbons, and therefore 3 turns of the Calvin cycle. To make one glucose molecule (which can be created from 2 G3P molecules) would require 6 turns of the Calvin cycle. Surplus G3P can also be used to form other carbohydrates such as starch, sucrose, and cellulose depending on what the plant needs.

Carbon fixation

Carbon fixation refers to any process through which gaseous carbon dioxide is converted into a solid compound. It mostly refers to the processes found in autotrophs (organisms that produce their own food), usually driven by photosynthesis, whereby carbon dioxide is changed into sugars. Carbon fixation can also be carried out by the process of calcification in marine, calcifying organisms such as *Emiliania huxleyi* and also by heterotrophic organisms in some circumstances.

Biological

Plants

The Calvin cycle is the most common biological method of carbon fixation.

In plants, there are three types of carbon fixation during photosynthesis:

- **C_3** plants that use the Calvin cycle for the initial steps that incorporate CO_2 into organic matter, forming a 3-carbon compound as the first stable. This form of photosynthesis occurs in the majority of terrestrial species of plants. Plants that use this pathway have a carbon isotope signature of -24 to -33‰.
- **C_4** plants that preface the Calvin cycle with reactions that incorporate CO_2 into a 4-carbon compound. C4 plants have a distinctive internal leaf anatomy. Tropical grasses, such as sugar cane and maize are C4 plants, but there are many broadleaf plants that are C4. Overall, 7600 species of terrestrial plants use C_4 carbon fixation, representing around 3% of all species. These plants have a carbon isotope signature of -16 to -10 ‰.
- CAM-plants that use Crassulacean acid metabolism as an adaptation for arid conditions. CO_2 enters through the stomata during the night and is converted into

organic acids, which release CO_2 for use in the Calvin cycle during the day, when the stomata are closed. The jade plant (*Crassula ovata*) and cacti are typical of CAM plants. Sixteen thousand species of plants use CAM. These plants have a carbon isotope signature of -20 to -10 ‰.

Microorganisms

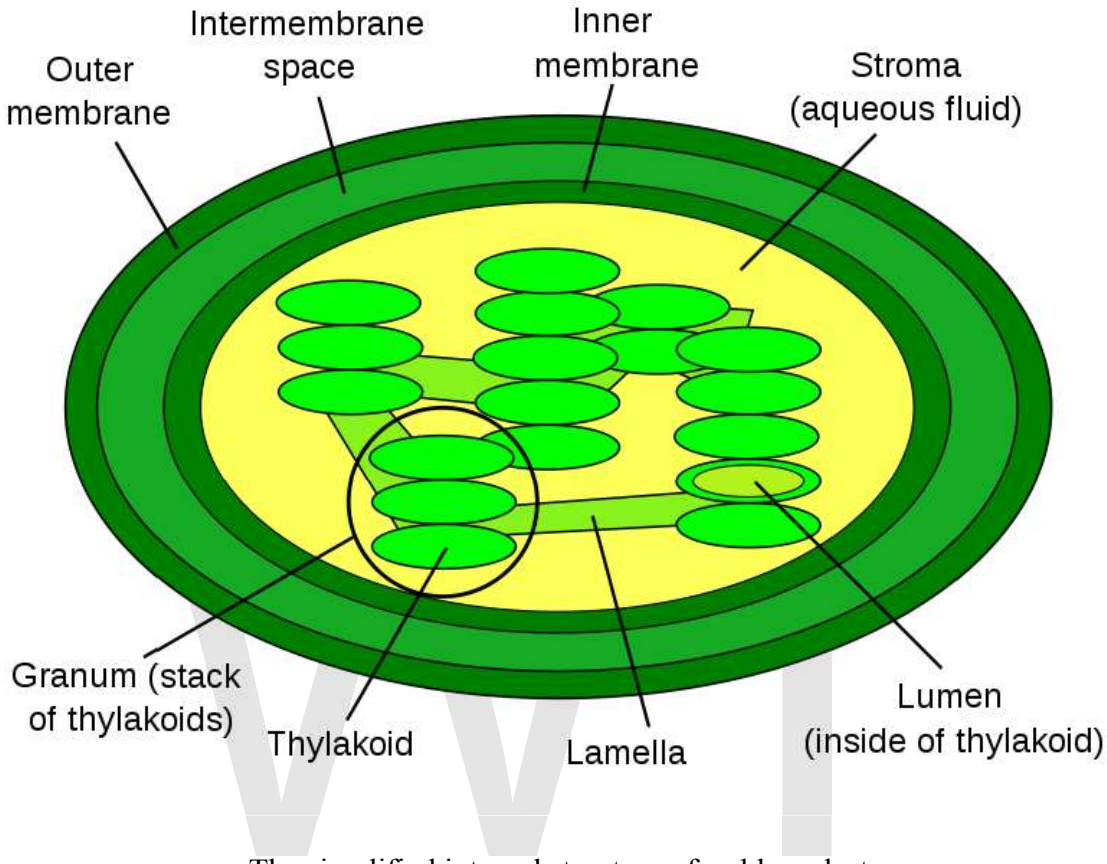
In addition to the Calvin cycle, the following alternative pathways are currently known to be used in certain autotrophic microorganisms:

- **Reverse Krebs cycle** (also known as the **reverse tricarboxylic acid cycle**, the **reverse TCA cycle**, or the **reverse citric acid cycle**). The reaction is the Citric acid cycle run in reverse and is used by photolitho-autotrophic eubacteria of the *Chlorobiales* and some chemolitho-autotrophic sulfate-reducing bacteria.
- **Reductive acetyl CoA Pathway** is found in methanogenic archaea and in acetogenic and some sulfate-reducing eubacteria as a way of fixing carbon.
- **3-Hydroxypropionate Pathway** is found in photolitho-autotrophically grown eubacteria of the genus *Chloroflexus* and, in modified form, in some chemolitho-autotrophically grown archaea as a way of fixing carbon.

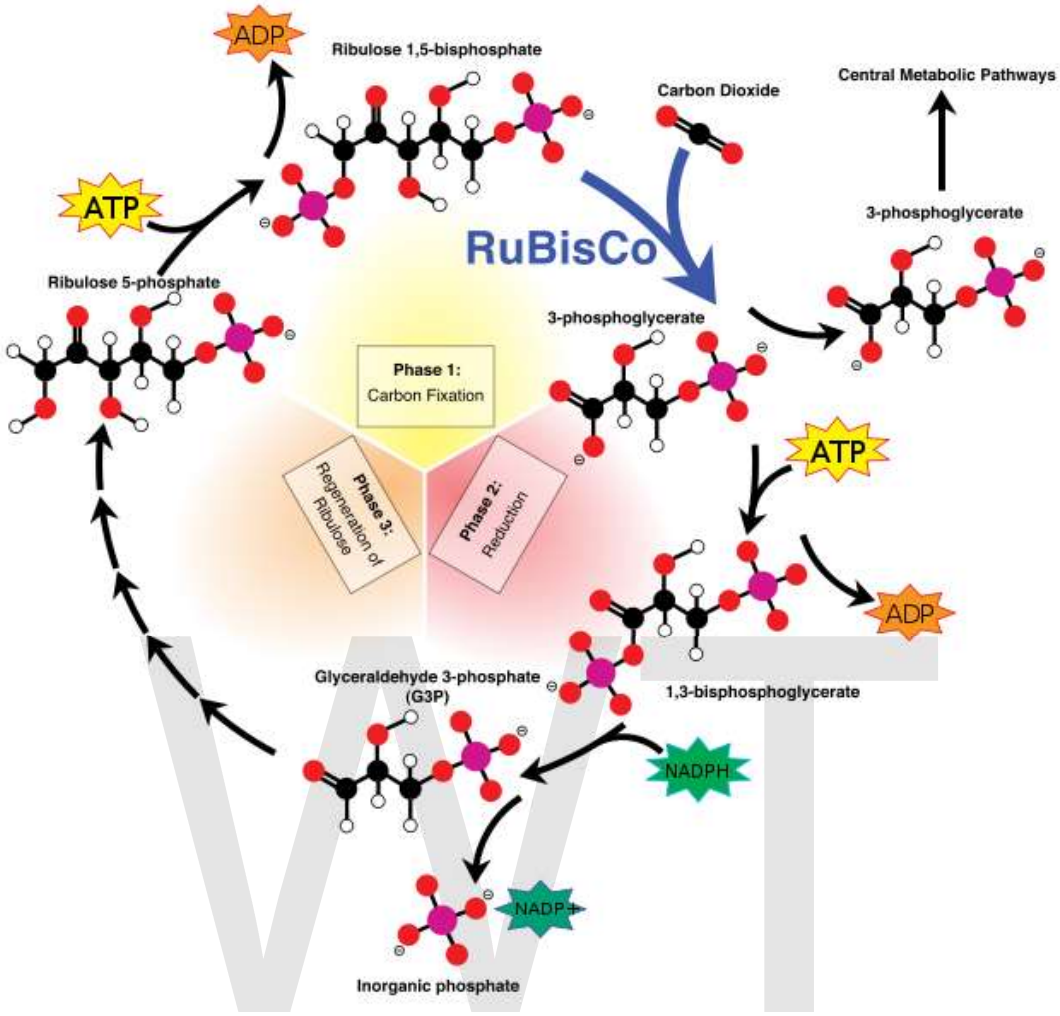
Animals

- Earl Evans and Louis Slotin discovered in 1942 that samples of liver cells are able to fix carbon dioxide into glycogen.

Light-independent reaction



The simplified internal structure of a chloroplast



Overview of the Calvin cycle and carbon fixation

The **light-independent reactions** of photosynthesis are chemical reactions that convert carbon dioxide and other compounds into glucose. These reactions occur in the stroma, the fluid-filled area of a chloroplast outside of the thylakoid membranes. These reactions take the light-dependent reactions and perform further chemical processes on them. There are three phases to the light-independent reactions, collectively called the Calvin cycle: carbon fixation, reduction reactions, and ribulose 1,5-bisphosphate (RuBP) regeneration.

Despite its name, this process occurs only when light is available. Plants do not carry out the Calvin cycle by night, they instead release sucrose into the phloem from their starch reserves. This process happens when light is available independent of the kind of photosynthesis (C3 carbon fixation, C4 carbon fixation and Crassulacean Acid Metabolism); CAM plants store malic acid in their vacuoles every night and release it by day in order to make this process work.

Coupling to other metabolic pathways

These dark reactions are closely coupled to the thylakoid electron transport chain as reducing power provided by NADPH produced in the photosystem I is actively needed. The process of photorespiration, also known as C2 cycle, is also coupled to the dark reactions as it results from an alternative reaction of the rubisco enzyme and its final

Light-dependent regulation

Despite its widespread names (both light-independent and dark reactions), these reactions do not occur in the dark or at night. There is a light-dependent regulation of the cycle enzymes, as the third step requires reduced NADP; and this process would be a waste of energy, as there is no electron flow in the dark.

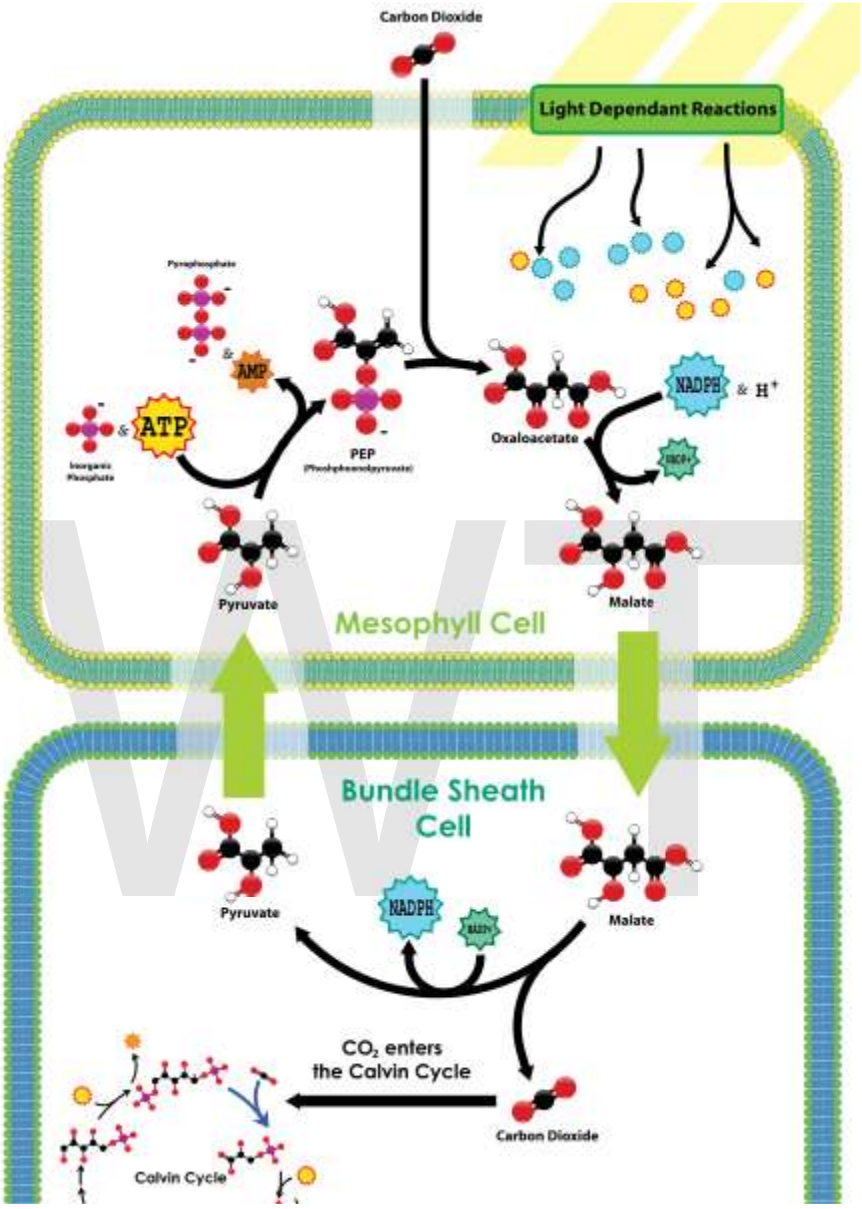
There are 2 regulation systems at work when the cycle needs to be turned on or off: thioredoxin/ferredoxin activation system, which activates some of the cycle enzymes; and the Rubisco enzime activation, which involves its own activase.

The thioredoxin/ferredoxin system activates the enzymes glyeraldehyde-3-P dehydrogenase, glyceraldehyde-3-P phosphatase, fructose-1,7-bisphosphatase, sedoheptulose-1,7-bisphosphatase and ribulose-5-phosphatase kinas, which are key points of the process. This happens when light is available, as the ferredoxin protein is reduced in the photosystem I complex of the thylakoid electron chain when electrons are circulating through it. Ferredoxin then binds to and reduces the thioredoxin protein, which activates the cycle enzymes by severing a cystine bond found in all these enzymes. This is a dynamic process as the same bond is formed again by other proteins that deactivate the enzymes. The implications of this process are that the enzymes remain mostly activated by day and are deactivated in the dark when there is no more reduced ferredoxin available.

The enzyme Rubisco has its own activation process, which involves a more complex process. A specific lysine amino acid needs to be carbamylated in order to activate the enzyme, this lysine binds to RuBP and leads to a non-functional state if left uncarbamylated. there is a specific activase enzyme, called Rubisco activase, which helps this carbamylation process by removing one proton from the lysine and making the binding of the carbon dioxide molecule possible. Even then the Rubisco enzime is not yet functional, as it needs a magnesium ion to be bound to the lysine in order to function. This magnesium ion is released from the thylakoid lumen when the inner PH drops due to the active pumping of protons from the electron flow. Rubisco activase itself is activated by increased concentrations of ATP in the stroma caused by its phosphorylation.

Carbon concentrating mechanisms

On land



Overview of C4 carbon fixation

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

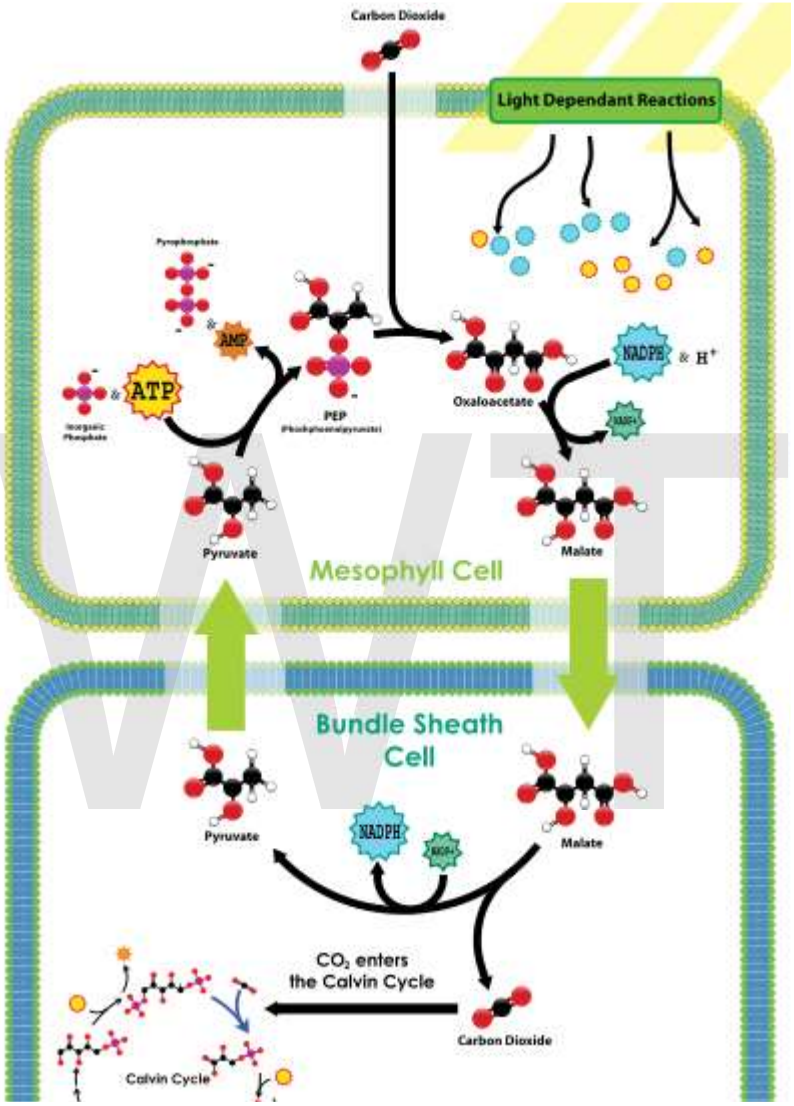
C_4 plants chemically fix carbon dioxide in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEP carboxylase and which creates the four-carbon organic acid, oxaloacetic acid. Oxaloacetic acid or malate synthesized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

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

In water

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

C_4 carbon fixation



Overview of C_4 carbon fixation

C_4 carbon fixation is one of three biochemical mechanisms, along with C_3 and CAM photosynthesis, used in carbon fixation. It is named for the 4 carbon atoms present in the first product of carbon fixation in these plants, in contrast to the 3-carbon-atom products in C_3 plants.

C_4 fixation is an elaboration of the more common C_3 carbon fixation and is believed to have evolved more recently. C_4 and CAM overcome the tendency of the enzyme RuBisCO to wastefully fix oxygen rather than carbon dioxide in what is called

photorespiration. This is achieved by using a more efficient enzyme to fix CO_2 in mesophyll cells and shuttling this fixed carbon via malate or oxaloacetate to bundle-sheath cells. In these bundle-sheath cells, RuBisCO is isolated from atmospheric oxygen and saturated with the CO_2 released by decarboxylation of the malate or oxaloacetate. These additional steps, however, require more energy in the form of ATP. Because of this extra energy requirement, C_4 plants are able to more efficiently fix carbon in only certain conditions, with the more common C_3 pathway being more efficient in other conditions.

C_4 pathway

The C_4 pathway was discovered by M. D. Hatch and C. R. Slack, in Australia, in 1966, so it is sometimes called the Hatch-Slack pathway.

In C_3 plants, the first step in the light-independent reactions of photosynthesis involves the fixation of CO_2 by the enzyme RuBisCO into 3-phosphoglycerate. However, due to the dual carboxylase / oxygenase activity of RuBisCo, an amount of the substrate is oxidized rather than carboxylated, resulting in loss of substrate and consumption of energy, in what is known as photorespiration. In order to bypass the photorespiration pathway, C_4 plants have developed a mechanism to efficiently deliver CO_2 to the RuBisCO enzyme. They utilize their specific leaf anatomy where chloroplasts exist, not only in the mesophyll cells in the outer part of their leaves but in the bundle sheath cells as well. Instead of direct fixation to RuBisCO in the Calvin cycle, CO_2 is incorporated into a 4-carbon organic acid, which has the ability to regenerate CO_2 in the chloroplasts of the bundle sheath cells. Bundle sheath cells can then utilize this CO_2 to generate carbohydrates by the conventional C_3 pathway.

The first step in the pathway is the conversion of pyruvate to PEP by the enzyme pyruvate-phosphate dikinase (pyruvate, orthophosphate dikinase). This reaction requires inorganic phosphate and ATP plus pyruvate, producing phosphoenolpyruvate, AMP, and inorganic pyrophosphate (PPi). The next step is the fixation of CO_2 into PEP by the enzyme PEP carboxylase. Both of these steps occur in the mesophyll cells:

$$\text{pyruvate} + \text{Pi} + \text{ATP} \rightarrow \text{PEP} + \text{AMP} + \text{PPi}$$
$$\text{PEP carboxylase} + \text{PEP} + CO_2 \rightarrow \text{oxaloacetate}$$

PEP carboxylase has a lower Km for CO_2 — and, hence, higher affinity — than RuBisCO. Furthermore, O_2 is a very poor substrate for this enzyme. Thus, at relatively low concentrations of CO_2, most CO_2 will be fixed by this pathway.

The product is usually converted to malate, a simple organic compound, which is transported to the bundle-sheath cells surrounding a nearby vein. Here, it is decarboxylated to produce CO_2 and pyruvate. The CO_2 now enters the Calvin cycle and the pyruvate is transported back to the mesophyll cell.

Since every CO_2 molecule has to be fixed twice, first by 4-carbon organic acid and second by RuBisCO, the C_4 pathway uses more energy than the C_3 pathway. The C_3

pathway requires 18 molecules of ATP for the synthesis of one molecule of glucose, whereas the C_4 pathway requires 30 molecules of ATP. This energy debt is more than paid for by avoiding losing more than half of photosynthetic carbon in photorespiration as occurs in some tropical plants, making it an adaptive mechanism for minimizing the loss.

There are several variants of this pathway:

1. The 4-carbon acid transported from mesophyll cells may be malate, as above, or aspartate
2. The 3-carbon acid transported back from bundle-sheath cells may be pyruvate, as above, or alanine
3. The enzyme that catalyses decarboxylation in bundle-sheath cells differs. In maize and sugarcane, the enzyme is NADP-malic enzyme; in millet, it is NAD-malic enzyme; and, in *Panicum maximum*, it is PEP carboxykinase.

C_4 leaf anatomy

The C_4 plants possess a characteristic leaf anatomy. Their vascular bundles are surrounded by two rings of cells, the inner ring, called bundle sheath cells, contain starch-rich chloroplasts lacking grana, which differ from those in mesophyll cells present as the outer ring. Hence, the chloroplasts are called dimorphic. This peculiar anatomy is called *kranz anatomy*, from the German word for wreath. The primary function of kranz anatomy is to provide a site in which CO_2 can be concentrated around RuBisCO, thereby reducing photorespiration. In order to facilitate the maintenance of a significantly higher CO_2 concentration in the bundle sheath compared to the mesophyll, the boundary layer of the kranz has a low conductance to CO_2, a property that may be enhanced by the presence of suberin.

Although most C_4 plants exhibit kranz anatomy, there are many species that operate a limited C_4 cycle without any distinct bundle sheath tissue. *Suaeda aralocaspica*, *Bienertia cycloptera* and *Bienertia sinuspersici* (all chenopods) are terrestrial plants that inhabit dry, salty depressions in the deserts of south-east Asia. These plants have been shown to operate single-cell C_4 CO_2-concentrating mechanisms, which are unique among the known C_4 mechanisms. Although the cytology of both species differs slightly, the basic principle is that fluid-filled vacuoles are employed to divide the cell into two separate areas. Carboxylation enzymes in the cytosol can, therefore, be kept separate from decarboxylase enzymes and rubisco in the chloroplasts, and a diffusive barrier can be established between the chloroplasts (which contain rubisco) and the cytosol. This enables a bundle-sheath-type area and a mesophyll-type area to be established within a single cell. Although this does allow a limited C_3 cycle to operate, it is relatively inefficient, with much leakage of CO_2 from around rubisco occurring. There is also evidence for the exhibiting of inducible C_4 photosynthesis by non-kranz aquatic macrophyte *Hydrilla verticillata* under warm conditions, although the mechanism by which CO_2 leakage from around rubisco is minimised is currently uncertain.

The evolution and advantages of the C_4 pathway

C_4 plants have a competitive advantage over plants possessing the more common C_3 carbon fixation pathway under conditions of drought, high temperatures, and nitrogen or CO_2 limitation. When grown in the same environment, at 30°C, C_3 grasses lose approximately 833 molecules of water per CO_2 molecule that is fixed, whereas C_4 grasses lose only 277 water molecules per CO_2 molecule fixed. This increased water use efficiency of C_4 grasses means that soil moisture is conserved, allowing them to grow for longer in arid environments.

C_4 carbon fixation has evolved on up to 40 independent occasions in different families of plants, making it a prime example of convergent evolution. C_4 plants arose around 25 to 32 million years ago during the Oligocene (precisely when is difficult to determine) and did not become ecologically significant until around 6 to 7 million years ago, in the Miocene Period. C_4 metabolism originated when grasses migrated from the shady forest undercanopy to more open environments, where the high sunlight gave it an advantage over the C_3 pathway. Drought was not necessary for its innovation; rather, the increased resistance to water stress was a by-product of the pathway and allowed C_4 plants to more readily colonise arid environments.

Today, C_4 plants represent about 5% of Earth's plant biomass and 1% of its known plant species. Despite this scarcity, they account for about 30% of terrestrial carbon fixation. Increasing the proportion of C4 plants on earth could assist biosequestration of CO_2 and represent an important climate change avoidance strategy. Present-day C_4 plants are concentrated in the tropics (below latitudes of 45°) where the high air temperature contributes to higher possible levels of oxygenase activity by rubisco, which increases rates of photorespiration in C_3 plants.

Plants that use C_4 carbon fixation

About 7600 species of plants use C_4 carbon fixation, which represents about 3% of all terrestrial species of plants. All these 7600 species are angiosperms. C_4 carbon fixation is less common in dicots than in monocots, with only 4.5% of dicots using the C_4 pathway, compared to 40% of monocots. Despite this, only three families of monocots utilise C_4 carbon fixation compared to 15 dicot families. Of the monocot clades containing C_4 plants, the grass (Poaceae) species use the C_4 photosynthetic pathway most. Forty-six percent of grasses are C_4 and together account for 61% of C_4 species. These include the food crops maize, sugar cane, millet, and sorghum. Of the dicot clades containing C_4 species, the order, Caryophyllales contains the most species. Of the families in the Caryophyllales, the Chenopodiaceae use C_4 carbon fixation the most, with 550 out of 1400 species using it. About 250 of the 1000 species of the related Amaranthaceae also use C_4.

Members of the sedge family Cyperaceae, and numerous families of Eudicots, including the daisies Asteraceae, cabbages Brassicaceae, and spurges Euphorbiaceae also use C_4.

Chapter- 5

Photomorphogenesis

In developmental biology, **photomorphogenesis** is light-mediated development. The photomorphogenesis of plants is often studied by using tightly-frequency-controlled light sources to grow the plants.

Germination

Light has profound effects on the development of plants. The light-mediated changes in plant growth and development are called photomorphogenesis. The most striking effects of light are observed when a germinating seedling emerges from the soil and is exposed to light for the first time.

Normally the seedling radicle (root) emerges first from the seed, and the shoot appears as the root becomes established. Later, with growth of the shoot (particularly when it merges into the light) there is increased secondary root formation and branching. This coordinated progression of developmental responses are early manifestations of correlative growth phenomena where the root affects the growth of the shoot and vice versa. To a large degree, these coordinated differential growth responses are hormone mediated.

In the absence of light, plants develop an etiolated growth pattern. Etiolation of the seedling adapts it to emerging from the soil.

Comparison of dark-grown (etiolated) and light-grown (de-etiolated) seedlings

Etiolated characteristics:

- Distinct "apical hook" (dicot) or coleoptile (monocot)
- No leaf growth

- No chlorophyll
- Rapid stem elongation
- Limited radial expansion of stem
- Limited root elongation
- Limited production of lateral roots

De-etiolated characteristics:

- Apical hook opens or coleoptile splits open
- Leaf growth promoted
- Chlorophyll produced
- Stem elongation suppressed
- Radial expansion of stem
- Root elongation promoted
- Lateral root development accelerated

The developmental changes characteristic of photomorphogenesis shown by de-etiolated seedlings, are induced by light. Typically, plants are responsive to wavelengths of light in the blue, red and far-red regions of the spectrum through the action of several different photosensory systems. The photoreceptors for red and far-red wavelengths are known as phytochromes. There are at least 5 members of the phytochrome family of photoreceptors. There are several blue light photoreceptors.

Photoreceptor systems in plants

Plants use phytochrome to detect and respond to red and far-red wavelengths.

Phytochromes are proteins with a light absorbing pigment attached (chromophore).

The chromophore is a linear tetrapyrrole called phytochromobilin.

The phytochrome apoprotein is synthesized in the Pr form. Upon binding the chromophore, the holoprotein becomes sensitive to light. If it absorbs red light it will change conformation to the biologically active Pfr form. The Pfr form can absorb red light and switch back to the Pr form.

Most plants have multiple phytochromes encoded by different genes. The different forms of phytochrome control different responses but there is also a lot of redundancy so that in the absence of one phytochrome, another may take on the missing functions.

Arabidopsis has 5 phytochromes - PHYA, PHYB, PHYC, PHYD, PHYE

Molecular analyses of phytochrome and phytochrome-like genes in higher plants (ferns, mosses, algae) and photosynthetic bacteria have shown that phytochromes evolved from prokaryotic photoreceptors that predated the origin of plants.

Blue light systems

As for the red/far-red system, plants contain multiple blue light photoreceptors which have different functions.

Based on studies with action spectra, mutants and molecular analyses, it has been determined that higher plants contain at least 4, and probably 5, different blue light photoreceptors.

Cryptochromes were the first blue light receptors to be isolated and characterized from any organism. The proteins use a flavin as a chromophore. The cryptochromes have evolved from microbial DNA-photolyase, an enzyme that carries out light-dependent repair of UV damaged DNA.

Two cryptochromes have been identified in plants.

Cryptochromes control stem elongation, leaf expansion, circadian rhythms and flowering time.

In addition to blue light, cryptochromes also perceive long wavelength UV irradiation (UV-A).

Phototropin is the blue light photoreceptor that controls phototropism. It also uses flavin as chromophore. Only one phototropin has been identified so far (NPH1). Phototropin also perceives long wavelength UV irradiation (UV-A) in addition to blue light.

Recent experiments indicate that a 4th blue light receptor exists that uses a carotenoid as a chromophore. This new photoreceptor controls blue light induction of stomatal opening. However, the gene and protein have not yet been found.

Other blue light responses exist that seem to function in plants that are missing the cryptochrome, phototropin and carotenoid photoreceptors suggesting that at least one more will be found.

Since the cryptochromes were discovered in plants, several labs have identified homologous genes and photoreceptors in a number of other organisms, including

humans, mice and flies. It appears that in mammals and flies, the cryptochromes function in entrainment of the biological clock. Indeed, in flies, a cryptochrome may be a functional part of the clock mechanism.

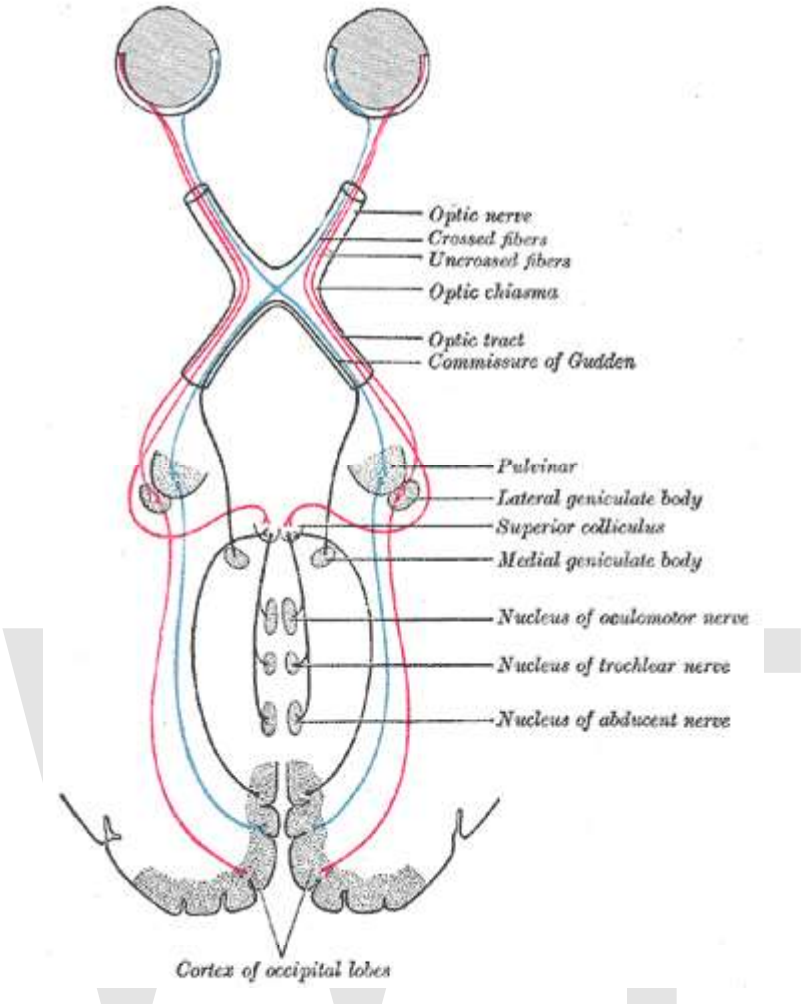
UV systems

Based on various responses to UV light, it is assumed that there are UV-specific photoreceptors....

Chapter- 6

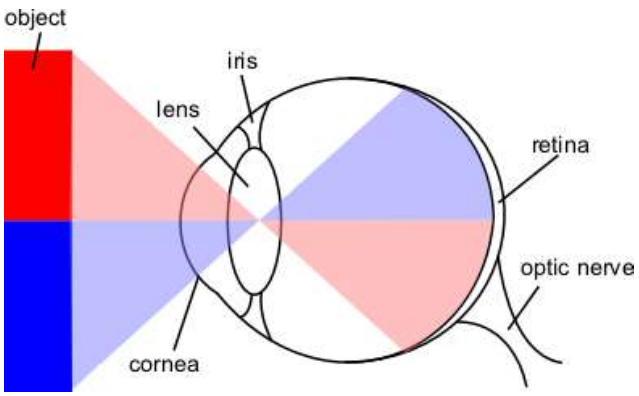
Visual System

The **visual system** is the part of the central nervous system which enables organisms to process visual detail, as well as enabling several non-image forming photoresponse functions. It interprets information from visible light to build a representation of the surrounding world. The visual system accomplishes a number of complex tasks, including the reception of light and the formation of monocular representations; the construction of a binocular perception from a pair of two dimensional projections; the identification and categorization of visual objects; assessing distances to and between objects; and guiding body movements in relation to visual objects. The psychological manifestation of visual information is known as visual perception, a lack of which is called blindness. Non-image forming visual functions, independent of visual perception, include the pupillary light reflex (PLR) and circadian photoentrainment.



The visual system includes the eyes, the connecting pathways through to the visual cortex and other parts of the brain. The illustration shows the mammalian system.

Introduction



Optical layout of the eye
The image projected onto the retina is inverted due to the optics of the eye.

Here we mostly describes the visual system of mammals, although other "higher" animals have similar visual systems. In this case, the visual system consists of:

- The eye, especially the retina
- The optic nerve
- The optic chiasma
- The optic tract
- The lateral geniculate body
- The optic radiation
- The visual cortex
- The visual association cortex.

Different species are able to see different parts of the light spectrum; for example, bees can see into the ultraviolet, while pit vipers can accurately target prey with their pit organs, which are sensitive to infrared radiation.

History

In the second half of the 19th century, many motifs of the nervous system were identified such as the neuron doctrine and brain localisation, which related to the neuron being the basic unit of the nervous system and functional localisation in the brain, respectively. These would become tenets of the fledgling neuroscience and would support further understanding of the visual system.

The notion that the cerebral cortex is divided into functionally distinct cortices now known to be responsible for capacities such as touch (somatosensory cortex), movement (motor cortex), and vision (visual cortex), was first proposed by Franz Joseph Gall in 1810. Evidence for functionally distinct areas of the brain (and, specifically, of the cerebral cortex) mounted throughout the 19th century with discoveries by Paul Broca of the language center (1861), and Gustav Fritsch and Edouard Hitzig of the motor cortex (1871). Based on selective damage to parts of the brain and the functional effects this would produce (lesion studies), David Ferrier proposed that visual function was localised to the parietal lobe of the brain in 1876. In 1881, Hermann Munk more accurately located vision in the occipital lobe, where the primary visual cortex is now known to be.

Biology of the visual system

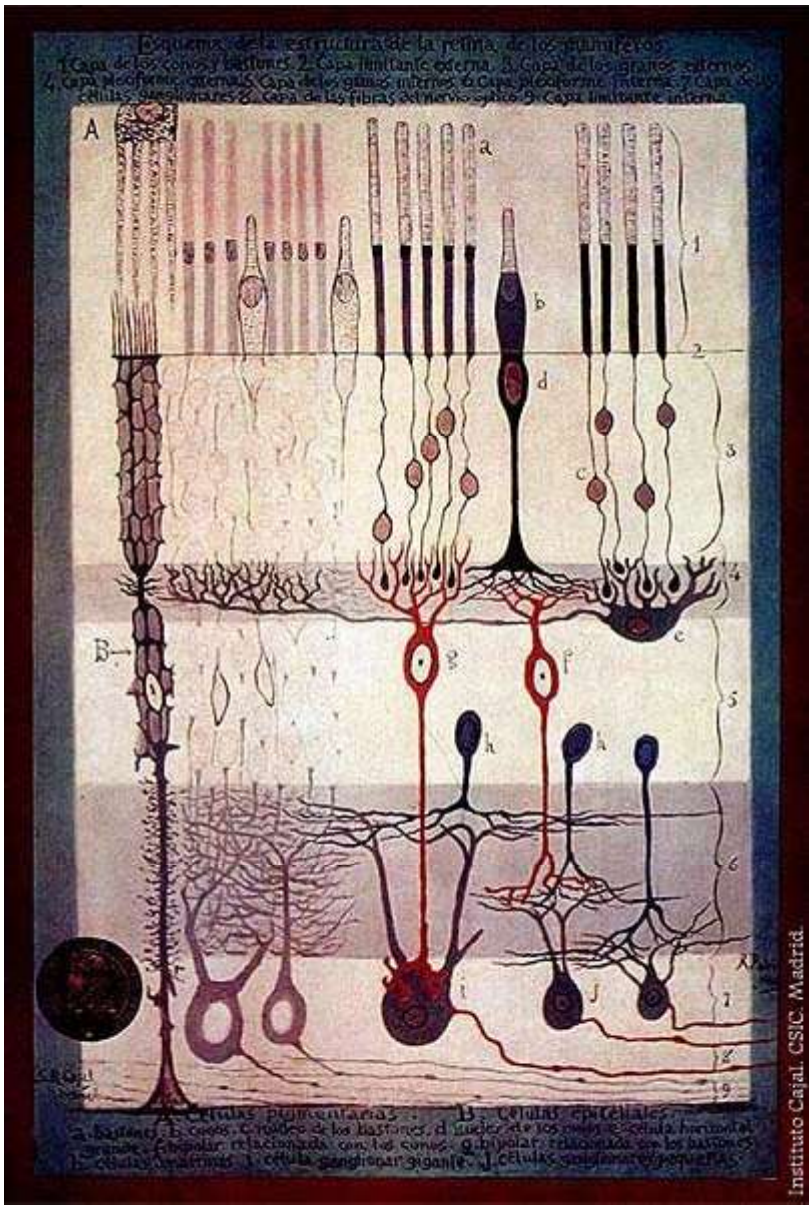
Eye

The eye is a complex biological device. The functioning of a camera is often compared with the workings of the eye, mostly since both focus light from external objects in the field of view onto a light-sensitive medium. In the case of the camera, this medium is

film or an electronic sensor; in the case of the eye, it is an array of visual receptors. With this simple geometrical similarity, based on the laws of optics, the eye functions as a transducer, as does a CCD camera.

Light entering the eye is refracted as it passes through the cornea. It then passes through the pupil (controlled by the iris) and is further refracted by the lens. The cornea and lens act together as a compound lens to project an inverted image onto the retina.

Retina



S. Ramón y Cajal, *Structure of the Mammalian Retina, 1900*

The retina consists of a large number of photoreceptor cells which contain particular protein molecules called opsins. In humans, two types of opsins are involved in conscious vision: rod opsins and cone opsins. (A third type, melanopsin in some of the retinal ganglion cells (RGC), part of the body clock mechanism, is probably not involved in conscious vision, as these RGC do not project to the lateral geniculate nucleus (LGN) but to the pretectal olivary nucleus (PON).) An opsin absorbs a photon (a particle of light) and transmits a signal to the cell through a signal transduction pathway, resulting in hyperpolarization of the photoreceptor.

Rods and cones differ in function. Rods are found primarily in the periphery of the retina and are used to see at low levels of light. Cones are found primarily in the center (or fovea) of the retina. There are three types of cones that differ in the wavelengths of light they absorb; they are usually called short or blue, middle or green, and long or red. Cones are used primarily to distinguish color and other features of the visual world at normal levels of light.

In the retina, the photoreceptors synapse directly onto bipolar cells, which in turn synapse onto ganglion cells of the outermost layer, which will then conduct action potentials to the brain. A significant amount of visual processing arises from the patterns of communication between neurons in the retina. About 130 million photoreceptors absorb light, yet roughly 1.2 million axons of ganglion cells transmit information from the retina to the brain. The processing in the retina includes the formation of center-surround receptive fields of bipolar and ganglion cells in the retina, as well as convergence and divergence from photoreceptor to bipolar cell. In addition, other neurons in the retina, particularly horizontal and amacrine cells, transmit information laterally (from a neuron in one layer to an adjacent neuron in the same layer), resulting in more complex receptive fields that can be either indifferent to color and sensitive to motion or sensitive to color and indifferent to motion. **Mechanism of generating visual signals**: The retina adapts to its change in light through the use of the rods. In the dark, the retinal has a bent shape called cis-retinal. When light is present, the retinal changes to a straight form called trans-retinal and breaks away from the opsin. This is called bleaching because the purified rhodopsin changes from violet to colorless in the light. In the dark, the rhodopsin absorbs no light therefore releasing glutamate cells which inhibit the bipolar cell. This inhibits the release of neurotransmitters to the ganglion cell. In the light, glutamate secretion ceases which no longer inhibits the bipolar cell from releasing neurotransmitters to the ganglion cell and therefore an image can be detected.

The final result of all this processing is five different populations of ganglion cells that send visual (image-forming and non-image-forming) information to the brain:

1. M cells, with large center-surround receptive fields that are sensitive to depth, indifferent to color, and rapidly adapt to a stimulus;
2. P cells, with smaller center-surround receptive fields that are sensitive to color and shape;
3. K cells, with very large center-only receptive fields that are sensitive to color and indifferent to shape or depth;

4. another population that is intrinsically photosensitive; and
5. a final population that is used for eye movements.

A 2006 University of Pennsylvania study calculated the approximate bandwidth of human retinas to be about 8960 kilobits per second, whereas guinea pig retinas transfer at about 875 kilobits.

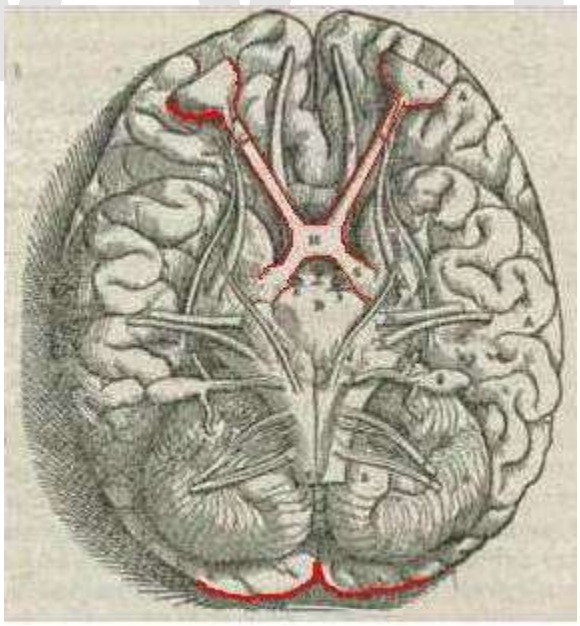
In 2007 Zaidi and co-researchers on both sides of the Atlantic studying patients without rods and cones, discovered that the novel photoreceptive ganglion cell in humans also has a role in conscious and unconscious visual perception. The peak spectral sensitivity was 481 nm. This shows that there are two pathways for sight in the retina – one based on classic photoreceptors (rods and cones) and the other, newly discovered, based on photoreceptive ganglion cells which act as rudimentary visual brightness detectors.

Photochemistry

In the visual system, **retinal**, technically called *retinene*$_1$ or "retinaldehyde", is a light-sensitive retinene molecule found in the rods and cones of the retina. Retinal is the fundamental structure involved in the transduction of light into visual signals, i.e. nerve impulses in the ocular system of the central nervous system. In the presence of light, the retinal molecule changes configuration and as a result a nerve impulse is generated.

Fibers to thalamus

Optic nerve



Information flow from the eyes (top), crossing at the optic chiasma, joining left and right eye information in the optic tract, and layering left and right visual stimuli in the lateral

geniculate nucleus. V1 in red at bottom of image. (1543 image from Andreas Vesalius' *Fabrica*)

The information about the image via the eye is transmitted to the brain along the optic nerve. Different populations of ganglion cells in the retina send information to the brain through the optic nerve. About 90% of the axons in the optic nerve go to the lateral geniculate nucleus in the thalamus. These axons originate from the M, P, and K ganglion cells in the retina, see above. This parallel processing is important for reconstructing the visual world; each type of information will go through a different route to perception. Another population sends information to the superior colliculus in the midbrain, which assists in controlling eye movements (saccades) as well as other motor responses.

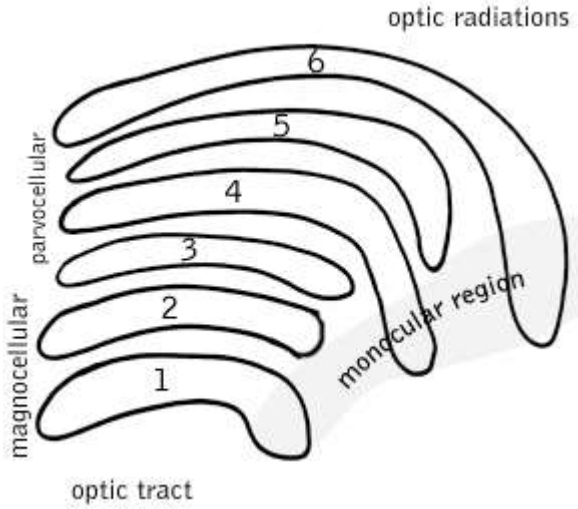
A final population of photosensitive ganglion cells, containing melanopsin, sends information via the retinohypothalamic tract (RHT) to the pretectum (pupillary reflex), to several structures involved in the control of circadian rhythms and sleep such as the suprachiasmatic nucleus (SCN, the biological clock), and to the ventrolateral preoptic nucleus (VLPO, a region involved in sleep regulation). A recently discovered role for photoreceptive ganglion cells is that they mediate conscious and unconscious vision – acting as rudimentary visual brightness detectors as shown in rodless coneless eyes.

Optic chiasm

The optic nerves from both eyes meet and cross at the optic chiasm, at the base of the hypothalamus of the brain. At this point the information coming from both eyes is combined and then splits according to the visual field. The corresponding halves of the field of view (right and left) are sent to the left and right halves of the brain, respectively, to be processed. That is, the right side of primary visual cortex deals with the left half of the *field of view* from both eyes, and similarly for the left brain. A small region in the center of the field of view is processed redundantly by both halves of the brain.

Optic tract

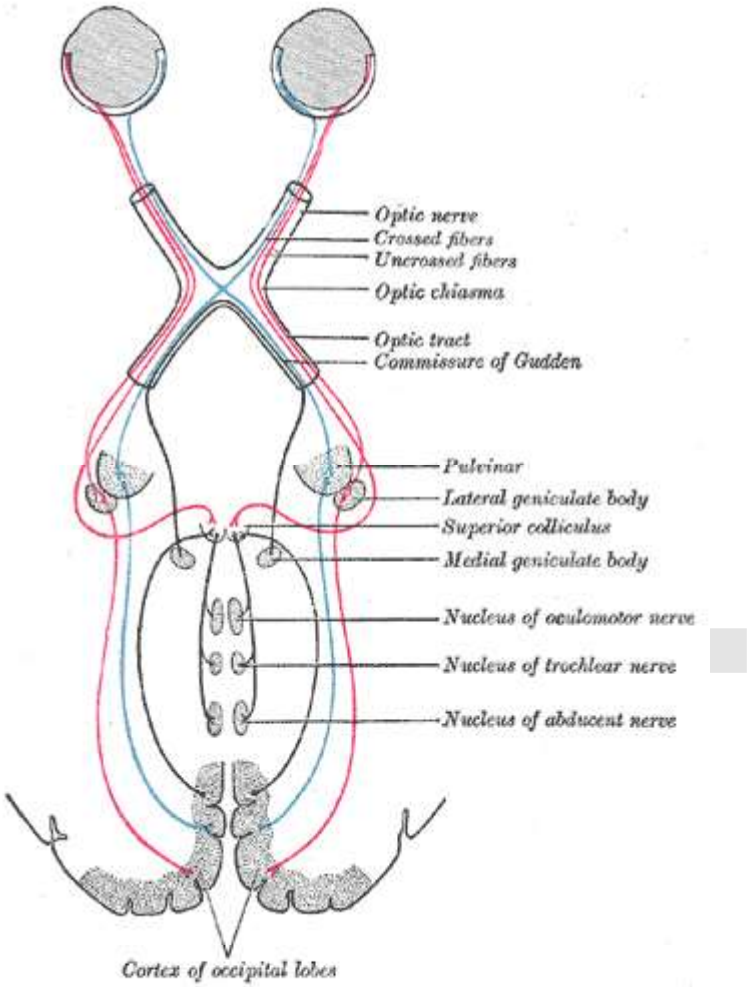
Information from the right *visual field* (now on the left side of the brain) travels in the left optic tract. Information from the left *visual field* travels in the right optic tract. Each optic tract terminates in the lateral geniculate nucleus (LGN) in the thalamus.



Six layers in the LGN

Lateral geniculate nucleus

The **lateral geniculate nucleus** (LGN) is a sensory relay nucleus in the thalamus of the brain. The LGN consists of six layers in humans and other primates starting from catarhinians, including cercopithecidae and apes. Layers 1, 4, and 6 correspond to information from the contralateral (crossed) fibers of the nasal visual field; layers 2, 3, and 5 correspond to information from the ipsilateral (uncrossed) fibers of the temporal visual field. Layer one (1) contains M cells which correspond to the M (magnocellular) cells of the optic nerve of the opposite eye and are concerned with depth or motion. Layers four and six (4 & 6) of the LGN also connect to the opposite eye, but to the P cells (color and edges) of the optic nerve. By contrast, layers two, three and five (2, 3, & 5) of the LGN connect to the M cells and P (parvocellular) cells of the optic nerve for the same side of the brain as its respective LGN. Spread out, the six layers of the LGN are the area of a credit card and about three times its thickness. The LGN is rolled up into two ellipsoids about the size and shape of two small birds' eggs. In between the six layers are smaller cells that receive information from the K cells (color) in the retina. The neurons of the LGN then relay the visual image to the primary visual cortex (V1) which is located at the back of the brain (caudal end) in the occipital lobe in and close to the calcarine sulcus The LGN is not just a simple relay station but its also a center for processing; it reecieves reciprocal input from the cortical and subcortical andreciprocal innervation from the visual cortex.



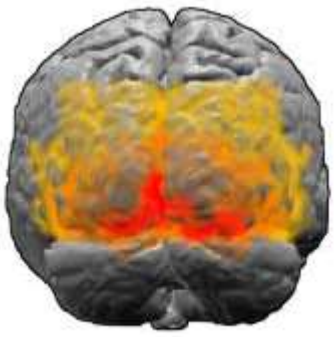
Scheme showing central connections of the optic nerves and optic tracts.

Optic radiation

The **optic radiations**, one on each side of the brain, carry information from the thalamic lateral geniculate nucleus to layer 4 of the visual cortex. The P layer neurons of the LGN relay to V1 layer 4C β. The M layer neurons relay to V1 layer 4C α. The K layer neurons in the LGN relay to large neurons called blobs in layers 2 and 3 of V1.

There is a direct correspondence from an angular position in the field of view of the eye, all the way through the optic tract to a nerve position in V1. At this juncture in V1, the image path ceases to be straightforward; there is more cross-connection within the visual cortex.

Visual cortex



Visual cortex: V1, V2, V3, V4, V5 (also called MT)

The visual cortex is the largest system in the human brain and is responsible for processing the visual image. It lies at the rear of the brain (highlighted in the image), above the cerebellum. The region that receives information directly from the LGN is called the primary visual cortex, (also called V1 and striate cortex). Visual information then flows through a cortical hierarchy. These areas include V2, V3, V4 and area V5/MT (the exact connectivity depends on the species of the animal). These secondary visual areas (collectively termed the extrastriate visual cortex) process a wide variety of visual primitives. Neurons in V1 and V2 respond selectively to bars of specific orientations, or combinations of bars. These are believed to support edge and corner detection. Similarly, basic information about color and motion is processed here.

Visual association cortex

As visual information passes forward through the visual hierarchy, the complexity of the neural representations increase. Whereas a V1 neuron may respond selectively to a line segment of a particular orientation in a particular retinotopic location, neurons in the lateral occipital complex respond selectively to complete object (e.g., a figure drawing), and neurons in visual association cortex may respond selectively to human faces, or to a particular object.

Along with this increasing complexity of neural representation may come a level of specialization of processing into two distinct pathways: the dorsal stream and the ventral stream (the Two Streams hypothesis, first proposed by Ungerleider and Mishkin in 1982). The dorsal stream, commonly referred to as the "where" stream, is involved in spatial attention (covert and overt), and communicates with regions that control eye movements and hand movements. More recently, this area has been called the "how" stream to emphasize its role in guiding behaviors to spatial locations. The ventral stream, commonly referred as the "what" stream, is involved in the recognition, identification and categorization of visual stimuli.

However, there is still much debate about the degree of specialization within these two pathways, since they are in fact heavily interconnected.

Chapter- 7

Circadian Rhythm

A **circadian rhythm** is an endogenously driven roughly 24-hour cycle in biochemical, physiological, or behavioural processes. Circadian rhythms have been widely observed, in plants, animals, fungi and cyanobacteria. The term "circadian" comes from the Latin *circa*, meaning "around", and *diem* or *dies*, meaning "day". The formal study of biological temporal rhythms such as daily, tidal, weekly, seasonal, and annual rhythms is called chronobiology. Although circadian rhythms are endogenous ("built-in", self-sustained), they are adjusted (entrained) to the environment by external cues called zeitgebers, the primary one of which is daylight.

History

The earliest known account of a circadian process dates from the 4th century BC, when Androsthenes, a ship captain serving under Alexander the Great, described diurnal leaf movements of the tamarind tree. The first modern observation of endogenous circadian oscillation was by the French scientist Jean-Jacques d'Ortous de Mairan in the 18th century; he noted that 24-hour patterns in the movement of the leaves of the plant *Mimosa pudica* continued even when the plants were kept in constant darkness.

In 1896, Patrick and Gilbert observed that during a prolonged period of sleep deprivation, sleepiness increases and decreases with a period of approximately 24 hours. In 1918, J.S. Szymanski showed that animals are capable of maintaining 24-hour activity patterns in the absence of external cues such as light and changes in temperature. Joseph Takahashi discovered the first mammalian 'clock gene' in 1994.

The term "circadian" was coined by Franz Halberg in the late 1950s.

Criteria

To be called circadian, a biological rhythm must meet these four general criteria:

1. **The rhythms repeat once a day (they have a 24-hour period).** In order to keep track of the time of day, a clock must be at the same point at the same time each day, i.e. repeat every 24 hours.
2. **The rhythms persist in the absence of external cues (endogenous).** The rhythm persists in constant conditions with a period of about 24 hours. The rationale for this criterion is to distinguish circadian rhythms from simple responses to daily external cues. A rhythm cannot be said to be endogenous unless it has been tested in conditions without external periodic input.
3. **The rhythms can be adjusted to match the local time (entrainable).** The rhythm can be reset by exposure to external stimuli (such as light and heat), a process called entrainment. The rationale for this criterion is to distinguish circadian rhythms from other imaginable endogenous 24-hour rhythms that are immune to resetting by external cues and, hence, do not serve the purpose of estimating the local time. Travel across time zones illustrates the necessity of the ability to adjust the biological clock so that it can reflect the local time and anticipate what will happen next. As their circadian clock is resetting, people will usually experience jet lag.
4. **The rhythms maintain circadian periodicity over a range of physiological temperatures (exhibit temperature compensation).** Some organisms live at a broad range of temperatures, and the thermal energy will affect the kinetics of all molecular processes in their cell(s). In order to keep track of time, the organism's circadian clock must maintain a roughly 24-hour periodicity despite the changing kinetics, a property known as temperature compensation.

Origin

Photosensitive proteins and circadian rhythms are believed to have originated in the earliest cells, with the purpose of protecting the replicating of DNA from high ultraviolet radiation during the daytime. As a result, replication was relegated to the dark. The fungus *Neurospora*, which exists today, retains this clock-regulated mechanism.

Circadian rhythms allow organisms to anticipate and prepare for precise and regular environmental changes; they have great value in relation to the outside world. The rhythmicity appears to be as important in regulating and coordinating internal metabolic processes, as in coordinating with the environment. This is suggested by the maintenance (heritability) of circadian rhythms in fruit flies after several hundred generations in constant laboratory conditions, as well as in creatures in constant darkness in the wild, and by the experimental elimination of behavioural but not physiological circadian rhythms in quail.

The simplest known circadian clock is that of the prokaryotic cyanobacteria. Recent research has demonstrated that the circadian clock of *Synechococcus elongatus* can be reconstituted *in vitro* with just the three proteins of their central oscillator. This clock has been shown to sustain a 22-hour rhythm over several days upon the addition of ATP. Previous explanations of the prokaryotic circadian timekeeper were dependent upon a DNA transcription/translation feedback mechanism.

In 1971, Ronald J. Konopka and Seymour Benzer first identified a genetic component of the biological clock using the fruit fly as a model system. Three mutant lines of flies displayed aberrant behaviour: one had a shorter period, another had a longer one, and the third had none. All three mutations mapped to the same gene, which was named "period". The same gene was identified to be defective in the sleep disorder FASPS (Familial advanced sleep phase syndrome) in human beings thirty years later, underscoring the conserved nature of the molecular circadian clock through evolution. Many more genetic components of the biological clock are now known. Their interactions result in an interlocked feedback loop of gene products resulting in periodic fluctuations that the cells of the body interpret as a specific time of the day.

A great deal of research on biological clocks was done in the latter half of the 20th century. It is now known that the molecular circadian clock can function within a single cell; i.e., it is cell-autonomous. At the same time, different cells may communicate with each other resulting in a synchronised output of electrical signaling. These may interface with endocrine glands of the brain to result in periodic release of hormones. The receptors for these hormones may be located far across the body and synchronise the peripheral clocks of various organs. Thus, the information of the time of the day as relayed by the eyes travels to the clock in the brain, and, through that, clocks in the rest of the body may be synchronised. This is how the timing of, for example, sleep/wake, body temperature, thirst, and appetite are coordinately controlled by the biological clock.

Importance in animals

Circadian rhythmicity is present in the sleeping and feeding patterns of animals, including human beings. There are also clear patterns of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities. In addition, photoperiodism, the physiological reaction of organisms to the length of day or night, is vital to both plants and animals, and the circadian system plays a role in the measurement and interpretation of day length.

> “ Timely prediction of seasonal periods of weather conditions, food availability or predator activity is crucial for survival of many species. Although not the only parameter, the changing length of the photoperiod ('daylength') is the most predictive environmental cue for the seasonal timing of physiology and behavior, most notably for timing of migration, hibernation and reproduction. ”

Impact of light–dark cycle

The rhythm is linked to the light–dark cycle. Animals, including humans, kept in total darkness for extended periods eventually function with a freerunning rhythm. Each "day", their sleep cycle is pushed back or forward, depending on whether their endogenous period is shorter or longer than 24 hours. The environmental cues that reset the rhythms each day are called *zeitgebers* (from the German, "time-givers"). It is interesting to note

that totally-blind subterranean mammals (e.g., blind mole rat *Spalax* sp.) are able to maintain their endogenous clocks in the apparent absence of external stimuli. Although they lack image-forming eyes, their photoreceptors (detect light) are still functional; as well, they do surface periodically.

Freerunning organisms that normally have one or two consolidated sleep episodes will still have them when in an environment shielded from external cues, but the rhythm is, of course, not entrained to the 24-hour light–dark cycle in nature. The sleep–wake rhythm may, in these circumstances, become out of phase with other circadian or ultradian rhythms such as metabolic, hormonal, CNS electrical, or neurotransmitter rhythms.

Recent research has influenced the design of spacecraft environments, as systems that mimic the light–dark cycle have been found to be highly beneficial to astronauts.

Arctic animals

Norwegian researchers at the University of Tromsø have shown that some Arctic animals (ptarmigan, reindeer) show circadian rhythms only in the parts of the year that have daily sunrises and sunsets. In one study of reindeer, animals at 70 degrees North showed circadian rhythms in the autumn, winter, and spring, but not in the summer. Reindeer at 78 degrees North showed such rhythms only autumn and spring. The researchers suspect that other Arctic animals as well may not show circadian rhythms in the constant light of summer and the constant dark of winter.

However, another study in northern Alaska found that ground squirrels and porcupines strictly maintained their circadian rhythms through 82 days and nights of sunshine. The researchers speculate that these two small mammals see that the apparent distance between the sun and the horizon is shortest once a day, and, thus, a sufficient signal to adjust by.

Butterfly migration

The navigation of the fall migration of the Eastern North American monarch butterfly (*Danaus plexippus*) to their overwintering grounds in central Mexico uses a time-compensated sun compass that depends upon a circadian clock in their antennae.

In plants

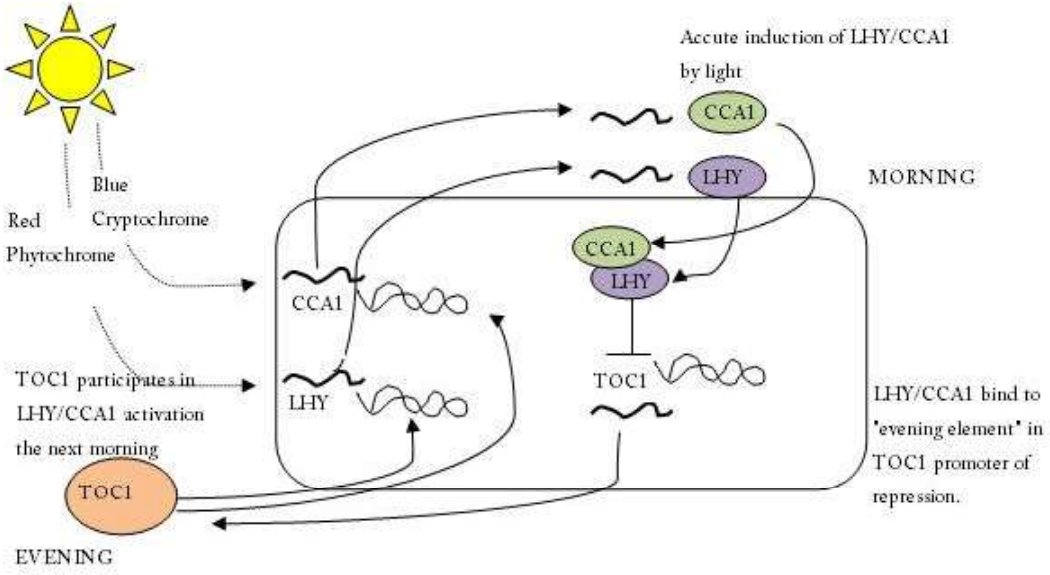


Diagram showing a small portion of the transcriptional feedback loop in *Arabidopsis*. LHY and CCA1 are considered negative elements due to its repression against TOC1 in the morning while TOC1 is considered a positive element because it results in increased transcription of LHY and CCA1 during the evening because of its accumulation.

Plant circadian rhythms tell the plant what season it is in and when to flower for the best chance of attracting insects to pollinate them and can include leaf movement, growth, germination, stomatal/gas exchange, enzyme activity, photosynthetic activity, and fragrance emission. Circadian rhythms occur as a biological rhythm with light, are endogenously generated and self sustaining, and are relatively constant over a range of ambient temperatures. Circadian rhythms feature a transcriptional feedback loop, a presence of PAS proteins, and several photoreceptors that fine-tune the clock to different light conditions. Anticipation of changes in the environment changes the physiological state that provides plants with an adaptive advantage. A better understanding of plant circadian rhythms has applications in agriculture such as helping farmers stagger crop harvests thus extending crop availability, and to secure against massive losses due to weather.

Clocks are set through signals such as light, temperature, and nutrient availability, so that the internal time matches the local time. Light is the signal and is sensed by a wide variety of photoreceptors. Red and blue light are absorbed through several phytochromes and cryptochromes. One phytochrome, phyA, is the main phytochrome in dark-grown seedlings, but rapidly degrades in light to produce Cry1. Phytochromes B–E are more stable with phyB the main phytochrome in light-grown seedlings. The cryptochrome (cry) gene is also a light-sensitive component of the circadian clock. Cryptochromes 1–2 (involved in blue–UVA) help to maintain the period length in the clock through a whole range of light conditions.

The central oscillator generates a self-sustaining rhythm and is made of two genes: CCA1 (Circadian and Clock Associated 1) and LHY (Late Elongated Hypocotyl) that encode closely related MYB transcription factors that regulate circadian rhythms in *Arabidopsis*. When CCA1 and LHY are overexpressed (under constant light or dark conditions) plants become arrhythimcal and mRNA signals reduce contributing to a negative feedback loop. CCA1 and LHY expression oscillates and peaks in early morning while TOC1 oscillates and peaks in early evening. From past observations and studies, it is hypothesised that these three components model a negative feedback loop in which over-expressed CCA1 and LHY repress TOC1 and over-expressed TOC1 is a positive regulator CCA1 and LHY.

Biological clock in mammals

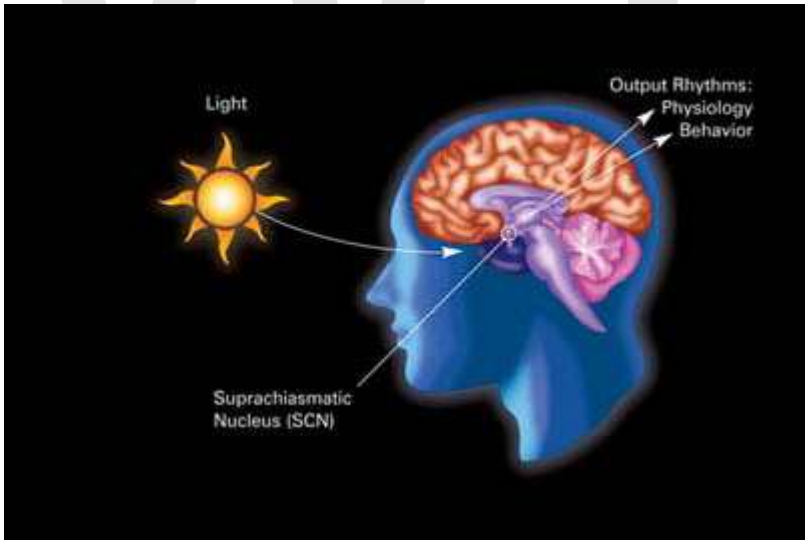


Diagram illustrating the influence of light and darkness on circadian rhythms and related physiology and behaviour through the suprachiasmatic nucleus in humans.

The primary circadian "clock" in mammals is located in the suprachiasmatic nucleus (or nuclei) (SCN), a pair of distinct groups of cells located in the hypothalamus. Destruction of the SCN results in the complete absence of a regular sleep–wake rhythm. The SCN receives information about illumination through the eyes. The retina of the eye contains

"classical" photoreceptors ("rods" and "cones"), which are used for conventional vision. But the retina also contains specialized ganglion cells which are directly photosensitive, and project directly to the SCN where they help in the entrainment of this master circadian clock.

These cells contain the photopigment melanopsin and their signals follow a pathway called the retinohypothalamic tract, leading to the SCN. If cells from the SCN are removed and cultured, they maintain their own rhythm in the absence of external cues.

The SCN takes the information on the lengths of the day and night from the retina, interprets it, and passes it on to the pineal gland, a tiny structure shaped like a pine cone and located on the epithalamus. In response, the pineal secretes the hormone melatonin. Secretion of melatonin peaks at night and ebbs during the day and its presence provides information about night-length.

Several studies have indicated that pineal melatonin feeds back on SCN rhythmicity to modulate circadian patterns of activity and other processes. However, the nature and system-level significance of this feedback are unknown.

The circadian rhythms of humans can be entrained to slightly shorter and longer periods than the Earth's 24 hours. Researchers at Harvard have recently shown that human subjects can at least be entrained to a 23.5-hour cycle and a 24.65-hour cycle (the latter being the natural solar day-night cycle on the planet Mars).

Determining the human circadian rhythm

The classic phase markers for measuring the timing of a mammal's circadian rhythm are:

- melatonin secretion by the pineal gland
- core body temperature
- plasma level of cortisol.

For temperature studies, subjects must remain awake but calm and semi-reclined in near darkness while their rectal temperatures are taken continuously. The average human adult's temperature reaches its minimum at about 05:00 (5 a.m.), about two hours before habitual wake time, though variation is great among normal chronotypes.

Melatonin is absent from the system or undetectably low during daytime. Its onset in dim light, *dim-light melatonin onset* (DLMO), at about 21:00 (9 p.m.) can be measured in the blood or the saliva. Its major metabolite can also be measured in morning urine. Both DLMO and the midpoint (in time) of the presence of the hormone in the blood or saliva have been used as circadian markers. However, newer research indicates that the melatonin *offset* may be the more reliable marker. Benloucif et al. in Chicago in 2005 found that melatonin phase markers were more stable and more highly correlated with the timing of sleep than the core temperature minimum. They found that both sleep offset and melatonin offset were more strongly correlated with the various phase markers than

sleep onset. In addition, the declining phase of the melatonin levels was more reliable and stable than the termination of melatonin synthesis.

One method used for measuring melatonin offset is to analyse a sequence of urine samples throughout the morning for the presence of the melatonin metabolite 6-sulphatoxymelatonin (aMT6s). Laberge et al. in Quebec in 1997 used this method in a study that confirmed the frequently found delayed circadian phase in healthy adolescents.

A third marker of the human pacemaker is the timing of the maximum plasma cortisol level. Klerman *et al.* in 2002 compared cortisol and temperature data to eight different analysis methods of plasma melatonin data, and found that "methods using plasma melatonin data may be considered more reliable than methods using CBT or cortisol data as an indicator of circadian phase in humans."

Outside the "master clock"

More-or-less independent circadian rhythms are found in many organs and cells in the body outside the suprachiasmatic nuclei (SCN), the "master clock". These clocks, called peripheral oscillators, are found in the oesophagus, lungs, liver, pancreas, spleen, thymus, and the skin. Though oscillators in the skin respond to light, a systemic influence has not been proven so far. There is also some evidence that the olfactory bulb and prostate may experience oscillations when cultured, suggesting that these structures may also be weak oscillators.

Furthermore, liver cells, for example, appear to respond to feeding rather than to light. Cells from many parts of the body appear to have freerunning rhythms.

Light and the biological clock

Light resets the biological clock in accordance with the phase response curve (PRC). Depending on the timing, light can advance or delay the circadian rhythm. Both the PRC and the required illuminance vary from species to species and lower light levels are required to reset the clocks in nocturnal rodents than in humans.

Lighting levels that affect the circadian rhythm in humans are higher than the levels usually used in artificial lighting in homes. According to some researchers the illumination intensity that excites the circadian system has to reach up to 1000 lux striking the retina.

In addition to light intensity, wavelength (or colour) of light is a factor in the entrainment of the body clock. Melanopsin is most efficiently excited by light from the blue part of the spectrum (420–440 nm according to some researchers while others have reported 470–485 nm). These blue wavelengths are present in virtually all light sources, therefore their elimination requires special lights or filters which appear amber.

It is thought that the direction of the light may have an effect on entraining the circadian rhythm; light coming from above, resembling an image of a bright sky, has greater effect than light entering our eyes from below.

According to a 2010 study completed by the Lighting Research Center, daylight has a direct effect on circadian rhythms and, consequently, on performance and well-being. The research showed that students who experience disruption in lighting schemes in the morning consequently experience disruption in sleeping patterns. The change in sleeping patterns may lead to negatively impacted student performance and alertness. Removing circadian light in the morning delays the dim light melatonin onset by 6 minutes a day, for a total of 30 minutes for five days.

Enforced longer cycles

Modern research under very controlled conditions has shown the human period for adults to be just slightly longer than 24 hours on average. Czeisler et al. at Harvard found the range for normal, healthy adults of all ages to be quite narrow: 24 hours and 11 minutes ± 16 minutes. The "clock" resets itself daily to the 24-hour cycle of the Earth's rotation.

The *28-hour day* is presented as a concept of time management. It builds on the fact that the week of seven days at 24 hours and a "week" of six days at 28 hours both equal a week of 168 hours. To live on the 28-hour day and six-day week would require staying awake for 19 to 20 hours and sleeping for eight to nine hours. Each "day" on this system has a unique light/dark pattern.

Studies by Nathaniel Kleitman in 1938 and by Derk-Jan Dijk and Charles Czeisler in 1994/5 have put human subjects on enforced 28-hour sleep–wake cycles, in constant dim light and with other time cues suppressed, for over a month. Because normal people cannot entrain to a 28-hour day in dim light if at all, this is referred to as a forced desynchrony protocol. Sleep and wake episodes are uncoupled from the endogenous circadian period of about 24.18 hours and researchers are allowed to assess the effects of circadian phase on aspects of sleep and wakefulness including sleep latency and other functions.

Early research into circadian rhythms suggested that most people preferred a day closer to 25 hours when isolated from external stimuli like daylight and timekeeping. Early investigators determined the human circadian period to be 25 hours or more. They went to great lengths to shield subjects from time cues and daylight, but they were not aware of the effects of indoor electric lights. The subjects were allowed to turn on light when they were awake and to turn it off when they wanted to sleep. Electric light in the evening delayed their circadian phase. These results became well-known. Researchers allowed subjects to keep electric lighting on in the evening, as it was thought at that time that a couple of 60W bulbs would not have a resetting effect on the circadian rhythms of humans. More recent research has shown that adults have a built-in day, which averages just over 24 hours, that indoor lighting does affect circadian rhythms and that most people attain their best-quality sleep during their chronotype-determined sleep periods.

Human health

Timing of medical treatment in coordination with the body clock may significantly increase efficacy and reduce drug toxicity or adverse reactions. For example, appropriately timed treatment with angiotensin converting enzyme inhibitors (ACEi) may reduce nocturnal blood pressure and also benefit left ventricular (reverse) remodelling.

A short nap during the day does not affect circadian rhythms.

A number of studies have concluded that a short period of sleep during the day, a power-nap, does not have any measurable effect on normal circadian rhythms, but can decrease stress and improve productivity.

There are many health problems associated with disturbances of the human circadian rhythm, such as seasonal affective disorder (SAD), delayed sleep phase syndrome (DSPS) and other circadian rhythm disorders. Circadian rhythms also play a part in the reticular activating system, which is crucial for maintaining a state of consciousness. In addition, a reversal in the sleep–wake cycle may be a sign or complication of uremia, azotemia or acute renal failure.

Studies have also shown that light has a direct effect on human health because of the way it influences the circadian rhythms.

Circadian rhythm and airline pilots

Due to the work nature of airline pilots, who often traverse multiple timezones and regions of sunlight and darkness in one day, and spend many hours awake both day and night, they are often unable to maintain sleep patterns that correspond to the natural human circadian rhythm; this situation can easily lead to fatigue. The NTSB cites this situation as a contributing factor to many accidents and has conducted multiple research studies in order to find methods of combating fatigue in pilots.

Disruption

Disruption to rhythms usually has a negative effect. Many travellers have experienced the condition known as jet lag, with its associated symptoms of fatigue, disorientation and insomnia.

A number of other disorders, for example bipolar disorder and some sleep disorders, are associated with irregular or pathological functioning of circadian rhythms. Recent research suggests that circadian rhythm disturbances found in bipolar disorder are positively influenced by lithium's effect on clock genes.

Disruption to rhythms in the longer term is believed to have significant adverse health consequences on peripheral organs outside the brain, particularly in the development or exacerbation of cardiovascular disease. The suppression of melatonin production associated with the disruption of the circadian rhythm may increase the risk of developing cancer.

Effect of drugs

Circadian rhythms and clock genes expressed in brain regions outside the SCN may significantly influence the effects produced by drugs such as cocaine. Moreover, genetic manipulations of clock genes profoundly affect cocaine's actions.

Chapter- 8

Bioluminescence

Flying and glowing firefly, a.k.a. *Photinus pyralis,*

Female of *Lampyris noctiluca*, the Common Glowworm.

Bioluminescence is the production and emission of light by a living organism. Its name is a hybrid word, originating from the Greek *bios* for "living" and the Latin *lumen* "light". Bioluminescence is a naturally occurring form of chemiluminescence where energy is released by a chemical reaction in the form of light emission. Fireflies, anglerfish, and other creatures produce the chemicals luciferin (a pigment) and luciferase (an enzyme). The luciferin reacts with oxygen to create light. The luciferase acts as a catalyst to speed up the reaction, which is sometimes mediated by cofactors such as calcium ions or ATP. The chemical reaction can occur either inside or outside the cell. In bacteria, the expression of genes related to bioluminescence is controlled by an operon called the Lux operon.

Bioluminescence occurs in marine vertebrates and invertebrates, as well as microorganisms and terrestrial animals. Symbiotic organisms carried within larger organisms are also known to bioluminesce.

Characteristics

Bioluminescence is a form of luminescence, or "cold light" emission; less than 20% of the light generates thermal radiation. It should not be confused with fluorescence, phosphorescence or refraction of light.

Ninety percent of deep-sea marine life are estimated to produce bioluminescence in one form or another. Most marine light-emission belongs in the blue and green light spectrum, the wavelengths that can transmit through the seawater most easily. However, certain loose-jawed fishes emit red and infrared light and the genus *Tomopteris* emits yellow bioluminescence.

Non-marine bioluminescence is less widely distributed, but a larger variety in colours is seen. The two best-known forms of land bioluminescence are fireflies and glow worms. Other insects, insect larvae, annelids, arachnids and even species of fungi have been noted to possess bioluminescent abilities.

Some forms of bioluminescence are brighter (or only exist) at night, following a circadian rhythm.

Adaptations for bioluminescence

There are five main theories for bioluminescent traits:

Counterillumination camouflage

In some squid species bacterial bioluminescence is used for counterillumination so the animal matches the overhead environmental light seen from below. In these animals, photoreceptive vesicles have been found that control the contrast of this illumination to create optiminal matching. Usually these light organs are separate from the tissue containing the bioluminescent bacteria. However, in one species Euprymna scolopes these bacteria make up an integral component of the animal's light organ. Fireflies use their light mainly for attracting the opposite sex for mating.

Attraction

Firefly larva

Bioluminescence is used as a lure to attract prey by several deep sea fish such as the anglerfish. A dangling appendage that extends from the head of the fish attracts small animals to within striking distance of the fish. Some fish, however, use a non-bioluminescent lure.

The cookiecutter shark uses bioluminescence for camouflage, but a small patch on its underbelly remains dark and appears as a small fish to large predatory fish like tuna and mackerel swimming beneath it. When these fish try to consume the "small fish", they are bitten by the shark, which gouges out small circular "cookie cutter" shaped chunks of flesh from its hosts.

Dinoflagellates have an interesting twist on this mechanism. When a predator of plankton is sensed through motion in the water, the dinoflagellate luminesces. This in turn attracts even larger predators which will consume the would-be predator of the dinoflagellate.

The attraction of mates is another proposed mechanism of bioluminescent action. This is seen actively in fireflies, which use periodic flashing in their abdomens to attract mates in the mating season. In the marine environment this has only been well-documented in certain small crustaceans called ostracod. It has been suggested that pheromones may be

used for long-distance communication, and bioluminescence used at close range to "home in" on the target.

Repulsion

Certain squid and small crustaceans use bioluminescent chemical mixtures or bioluminescent bacterial slurries in the same way as many squid use ink. A cloud of luminescence is expelled, confusing or repelling a potential predator while the squid or crustacean escapes to safety. Every species of firefly has larvae that glow to repel predators.

Communication

Communication between bacteria (quorum sensing) plays a role in the regulation of luminesence in many bacterial species. Using small extracellularly secreted molecules, they are able to adapt their behavior to only turn on genes for light production when they are at high cell densities.

Illumination

While most marine bioluminescence is green to blue, the Black Dragonfish produces a red glow. This adaptation allows the fish to see red-pigmented prey, which are normally invisible in the deep ocean environment where red light has been filtered out by the water column.

Biotechnology

Artistic rendering of bioluminescent Antarctic krill

Sepioteuthis lessoniana, one of many bioluminescent squid - 63 out of 100 genera of cuttlefish and squid contain species with the ability.

Bioluminescent organisms are a target for many areas of research. Luciferase systems are widely used in the field of genetic engineering as reporter genes. Luciferase systems have also been harnessed for biomedical research using bioluminescence imaging.

Vibrio symbiosis with numerous marine invertebrates and fish, namely the Hawaiian Bobtail Squid (*Euprymna scolopes*), are key experimental models for symbiosis, quorum sensing, and bioluminescence.

The structures of photophores, the light producing organs in bioluminescent organisms, are being investigated by industrial designers.

Proposed applications of engineered bioluminescence

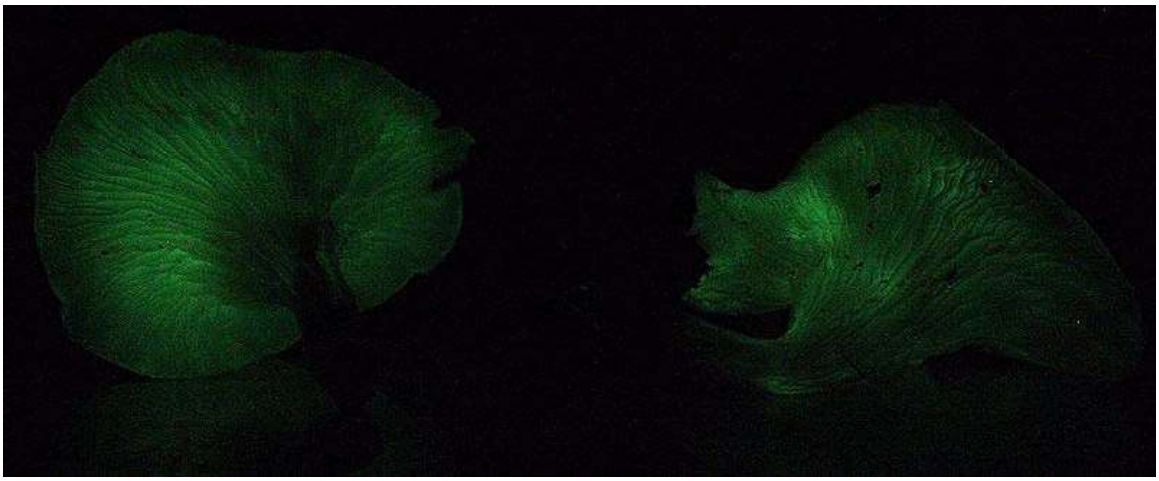
Some proposed applications of engineered bioluminescence include:

- Glowing trees to line highways to save government electricity bills
- Christmas trees that do not need lights, reducing danger from electrical fires
- Agricultural crops and domestic plants that luminesce when they need watering
- New methods for detecting bacterial contamination of meats and other foods
- Bio-identifiers for escaped convicts and mental patients
- Detecting bacterial species in suspicious corpses
- Novelty pets that bioluminesce (rabbits, mice, fish etc.)

Bioluminescent organisms

Omphalotus nidiformis

Example of a bioluminescent species of mushroom...



...glowing with the lights off.

Firefly (species unknown) with and without flash.

The fungus *Panellus stipticus* displaying bioluminescence.

All cells produce some form of bioluminescence within the electromagnetic spectrum, but most are neither visible nor noticeable to the naked eye. Every organism's bioluminescence is unique in wavelength, duration, timing and regularity of flashes. Below follows a list of organisms which have been observed to have visible bioluminescence.

Terrestrial organisms

Animals:

- certain arthropods
  - fireflies
  - click beetles
  - glow worms
    - railroad worms
  - certain mycetophilid flies
  - certain centipedes
  - certain millipedes
- a terrestrial mollusc (a tropical land snail)
  - *Quantula striata*
- annelids

Fungi:

- Mushrooms
  - Jack O'Lantern mushroom *(Omphalotus olearius)*
  - ghost fungus *(Omphalotus nidiformis)*
  - Honey mushroom
  - *Panellus stipticus*
  - several species of *Mycena*

Fish

- Anglerfish
- Cookie-cutter shark
- Flashlight fish
- Gulper eel
- Lanternfish
- Marine hatchetfish
- Midshipman fish
- Pineconefish
- Viperfish

Marine invertebrates

- many cnidarians
  - Sea pens
  - coral
  - *Aequorea victoria*, a jellyfish
- certain Ctenophores or "comb jellies"
- certain echinoderms (e.g. Ophiurida)
- certain crustaceans
  - ostracods
  - copepods
  - krill
- certain chaetognaths
- certain molluscs
  - certain clams, bivalves
  - certain nudibranchs, sea slugs
  - Octopus
    - Bolitaenidae
  - the order Teuthida
    - Colossal Squid
    - Mastigoteuthidae
    - Sepiolidae
    - Sparkling Enope Squid
    - Vampire squid

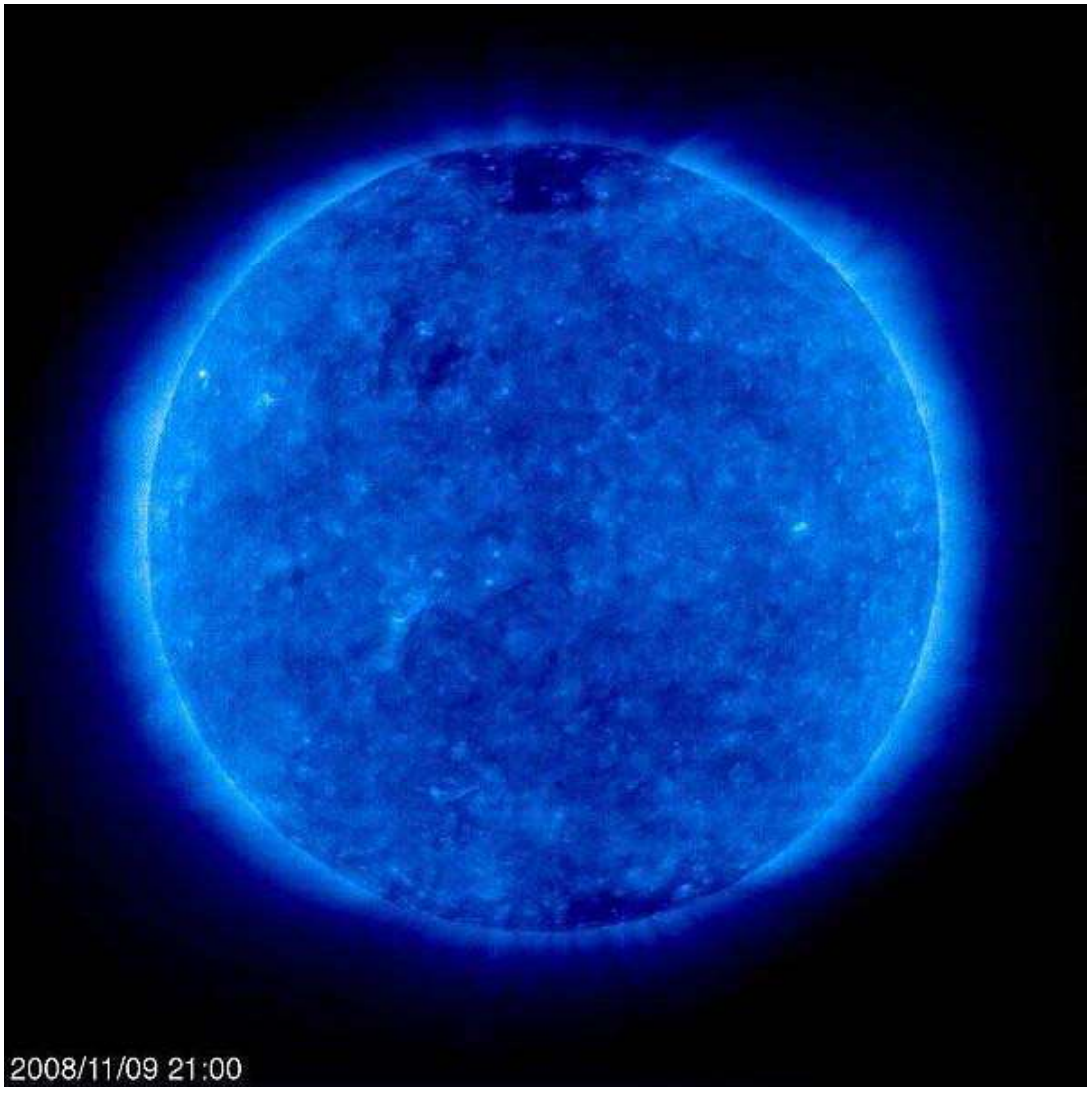
Blue ocean glow caused by myriad tiny organisms, such as Noctiluca.

Microorganisms

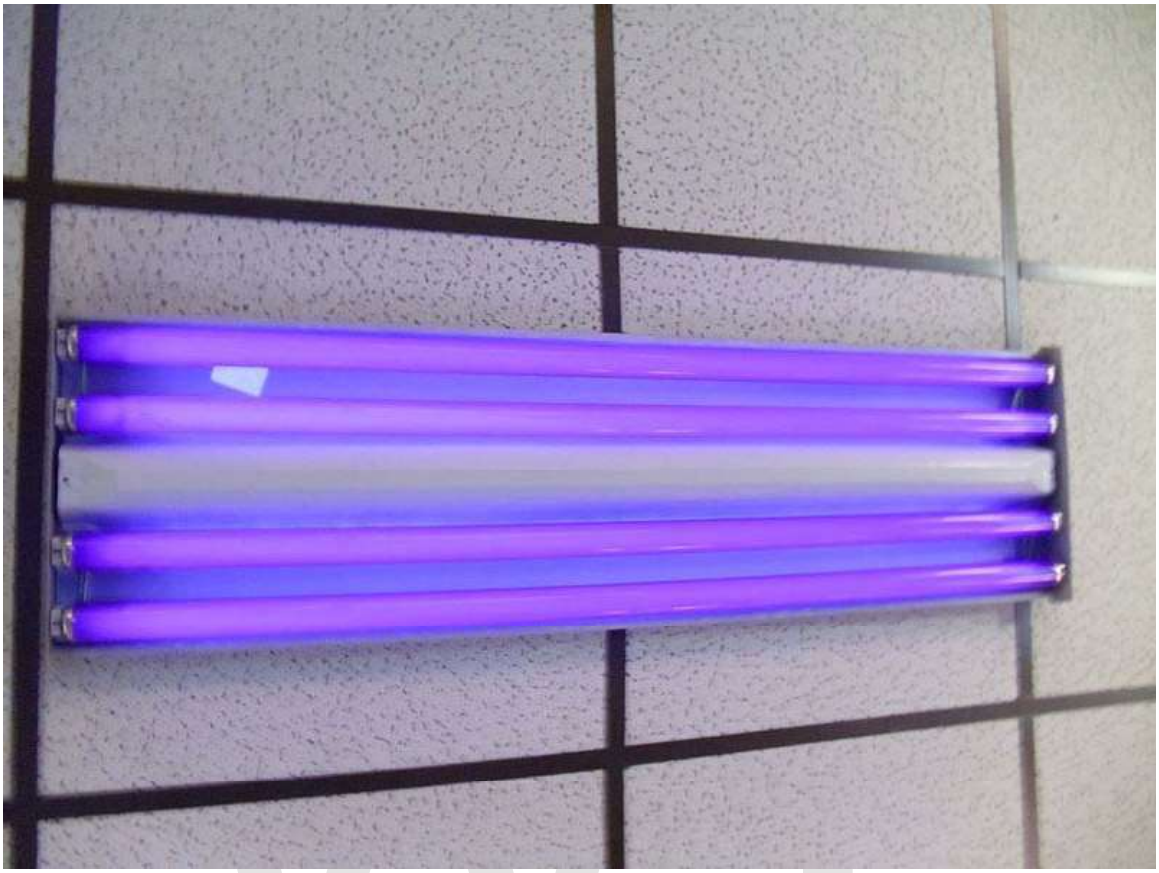
- Dinoflagellates
- Vibrionaceae (e.g. *Vibrio fischeri*, *Vibrio harveyi*, *Vibrio phosphoreum*)
- Members of the marine bacterial family Shewanellaceae, *Shewanella hanedai* and *Shewanella woodyi* have also been shown to be bioluminescent
- Fungi - A total of 71 species are bioluminescent including species of *Armillaria*, *Omphalotus*, *Mycena*, *Gerronema*, *Pleurotus*.

Chapter- 9

Ultraviolet



False-color image of the Sun's corona as seen in deep ultraviolet by the Extreme ultraviolet Imaging Telescope



Black light fluorescent tubes, a common source of long wave (UVA) ultraviolet.

Ultraviolet (**UV**) light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays, in the range 10 nm to 400 nm, and energies from 3eV to 124 eV. It is so named because the spectrum consists of electromagnetic waves with frequencies higher than those that humans identify as the color violet.

Although ultraviolet is invisible to the human eye, most people are aware of the effects of UV through the painful condition of sunburn, but the UV spectrum has many other effects, both beneficial and damaging, to human health.

UV light is found in sunlight and is emitted by electric arcs and specialized lights such as black lights. It can cause chemical reactions, and causes many substances to glow or fluoresce. Most ultraviolet is classified as non-ionizing radiation. The higher energies of the ultraviolet spectrum from about 150 nm ('vacuum' ultraviolet) are ionizing, but this type of ultraviolet is not very penetrating and is blocked by air.

Discovery

The discovery of UV radiation was intimately associated with the observation that silver salts darken when exposed to sunlight. In 1801, the German physicist Johann Wilhelm

Ritter made the hallmark observation that invisible rays just beyond the violet end of the visible spectrum were especially effective at lightening silver chloride-soaked paper. He called them "oxidizing rays" to emphasize chemical reactivity and to distinguish them from "heat rays" at the other end of the invisible spectrum. The simpler term "chemical rays" was adopted shortly thereafter, and it remained popular throughout the 19th century. The terms chemical and heat rays were eventually dropped in favour of ultraviolet and infrared radiation, respectively.

The discovery of the ultraviolet radiation below 200 nm, named vacuum ultraviolet because it is strongly absorbed by air, was made in 1893 by the German physicist Victor Schumann.

Origin of the term

The name means "beyond violet" (from Latin *ultra*, "beyond"), violet being the color of the shortest wavelengths of visible light. UV light has a shorter wavelength than violet light.

Subtypes

The electromagnetic spectrum of ultraviolet light can be subdivided in a number of ways. The draft ISO standard on determining solar irradiances (ISO-DIS-21348) describes the following ranges:

Name	Abbreviation	Wavelength range in nanometers	Energy per photon
Ultraviolet A, long wave, or black light	UVA	400 nm–315 nm	3.10–3.94 eV
Near	NUV	400 nm–300 nm	3.10–4.13 eV
Ultraviolet B or medium wave	UVB	315 nm–280 nm	3.94–4.43 eV
Middle	MUV	300 nm–200 nm	4.13–6.20 eV
Ultraviolet C, short wave, or germicidal	UVC	280 nm–100 nm	4.43–12.4 eV
Far	FUV	200 nm–122 nm	6.20–10.2 eV
Vacuum	VUV	200 nm–100 nm	6.20–12.4 eV
Low	LUV	100 nm–88 nm	12.4–14.1 eV
Super	SUV	150 nm–10 nm	8.28–124 eV
Extreme	EUV	121 nm–10 nm	10.2–124 eV

In photolithography and laser technology, the term *deep ultraviolet* or *DUV* refers to wavelengths below 300 nm. Extreme Ultraviolet stands here for discrete spectral ranges

of around 13.5 nm (in future planned also 6.x nm) of about 2 % bandwidth. In fields like analytics and life sciences, the acronym "XUV" is used for Extreme Ultraviolet for characterizing the broader spectral range, such as to distinguish from EUV. XUV is separated from x-rays and VUV, by the fact that the photoelectron ionization of innershell electrons is the - by orders of magnitudes - dominating photon-matter interaction effect. This is in contrast to x-rays, where scatter is relevant and VUV where the interaction is mainly with outer ("chemical active") electrons of the atoms and molecules.

"Vacuum UV" is so named because it is absorbed strongly by air and is, therefore, used in a vacuum. In the long-wave limit of this region, roughly 150–200 nm, the principal absorber is the oxygen in air. Work in this region can be performed in an oxygen-free atmosphere, pure nitrogen being commonly used, which avoids the need for a vacuum chamber.

Sources of UV

Natural sources of UV

The sun emits ultraviolet radiation in the UVA, UVB, and UVC bands. The Earth's ozone layer blocks 97-99% of this UV radiation from penetrating through the atmosphere. Of the ultraviolet radiation that reaches the Earth's surface, 98.7% is UVA. (UVC and more energetic radiation is responsible for the generation of the ozone layer, and formation of the ozone there). Extremely hot stars emit proportionally more UV radiation than the sun; the star R136a1 has a thermal energy of 4.57 eV, which falls in the near-UV range.

Ordinary glass is partially transparent to UVA but is opaque to shorter wavelengths, whereas Silica or quartz glass, depending on quality, can be transparent even to vacuum UV wavelengths. Ordinary window glass passes about 90% of the light above 350 nm, but blocks over 90% of the light below 300 nm.

The onset of vacuum UV, 200 nm, is defined by the fact that ordinary air is opaque at shorter wavelengths. This opacity is due to the strong absorption of light of these wavelengths by oxygen in the air. Pure nitrogen (less than about 10 ppm oxygen) is transparent to wavelengths in the range of about 150–200 nm. This has wide practical significance now that semiconductor manufacturing processes are using wavelengths shorter than 200 nm. By working in oxygen-free gas, the equipment does not have to be built to withstand the pressure differences required to work in a vacuum. Some other scientific instruments, such as circular dichroism spectrometers, are also commonly nitrogen-purged and operate in this spectral region.

Extreme UV is characterized by a transition in the physics of interaction with matter: Wavelengths longer than about 30 nm interact mainly with the chemical valence electrons of matter, whereas wavelengths shorter than that interact mainly with inner shell electrons and nuclei. The long end of the EUV/XUV spectrum is set by a prominent He^+ spectral line at 30.4 nm. XUV is strongly absorbed by most known materials, but it is

possible to synthesize multilayer optics that reflect up to about 50% of XUV radiation at normal incidence. This technology has been used to make telescopes for solar imaging; it was pioneered by the NIXT and MSSTA sounding rockets in the 1990s; (current examples are SOHO/EIT and TRACE) and for nanolithography (printing of traces and devices on microchips).

"Black light"

A black light, or Wood's light, is a lamp that emits long wave UV radiation and very little visible light. They are sometimes referred to as a "UV light". Fluorescent black lights are typically made in the same fashion as normal fluorescent lights except that only one phosphor is used, and the clear glass envelope of the bulb may be replaced by a deep-bluish-purple glass called Wood's glass, a nickel-oxide–doped glass, which blocks almost all visible light above 400 nanometres. The color of such lamps is often referred to in the trade as "blacklight blue" or "BLB." This is to distinguish these lamps from "bug zapper" blacklight ("BL") lamps that do not have the blue Wood's glass. The phosphor typically used for a near 368 to 371 nanometre emission peak is either europium-doped strontium fluoroborate ($SrB_4O_7F:Eu^{2+}$) or europium-doped strontium borate ($SrB_4O_7:Eu^{2+}$) while the phosphor used to produce a peak around 350 to 353 nanometres is lead-doped barium silicate ($BaSi_2O_5:Pb^{+}$). "Blacklight Blue" lamps peak at 365 nm.

While "black lights" do produce light in the UV range, their spectrum is confined to the longwave UVA region. Unlike UVB and UVC, which are responsible for the direct DNA damage that leads to skin cancer, black light is limited to lower-energy, longer waves and does not cause sunburn. However, UVA is capable of causing damage to collagen fibers and destroying vitamins A and D in skin.

A black light may also be formed by simply using Wood's glass instead of clear glass as the envelope for a common incandescent bulb. This was the method used to create the very first black light sources. Though it remains a cheaper alternative to the fluorescent method, it is exceptionally inefficient at producing UV light (less than 0.1% of the input power), owing to the black body nature of the incandescent light source. Incandescent UV bulbs, due to their inefficiency, may also become dangerously hot during use. More rarely still, high-power (hundreds of watts) mercury-vapor black lights that use a UV-emitting phosphor and an envelope of Wood's glass can be found. These lamps are used mainly for theatrical and concert displays, and also become very hot during normal use.

Some UV fluorescent bulbs specifically designed to attract insects use the same near-UV emitting phosphor as normal blacklights, but use plain glass instead of the more expensive Wood's glass. Plain glass blocks less of the visible mercury emission spectrum, making them appear light-blue to the naked eye. These lamps are referred to as "blacklight" or "BL" in most lighting catalogs.

Ultraviolet light can also be generated by some light-emitting diodes.

Ultraviolet fluorescent lamps

Fluorescent lamps without a phosphorescent coating to convert UV to visible light, emit ultraviolet light with two peaks at 253.7 nm and 185 nm due to the peak emission of the mercury within the bulb. Eighty-five to ninety percent of the UV produced by these lamps is at 253.7 nm, while only five to ten percent is at 185 nm. Germicidal lamps use quartz (glass) doped with an additive to block the 185 nm wavelength. With the addition of a suitable phosphorescent coating, they can be modified to produce a UVA, UVB, or visible light spectrum (all fluorescent tubes used for domestic and commercial lighting are mercury (Hg) UV emission bulbs at heart).

Such low-pressure mercury lamps are used extensively for disinfection, and in standard form have an optimum operating temperature of about 30 degrees Celsius. Use of a mercury amalgam allows operating temperature to rise to 100 degrees Celsius, and UVC emission to about double or triple per unit of light-arc length. These low-pressure lamps have a typical efficiency of approximately thirty to thirty-five percent, meaning that for every 100 watts of electricity consumed by the lamp, it will produce approximately 30-35 watts of total UV output.

Ultraviolet LEDs

Light-emitting diodes (LEDs) can be manufactured to emit light in the ultraviolet range, although practical LED arrays are very limited below 365 nm. LED efficiency at 365 nm is about 5-8%, whereas efficiency at 395 nm is closer to 20%, and power outputs at these longer UV wavelengths are also better. Such LED arrays are beginning to be used for UV curing applications, and are already successful in digital print applications and inert UV curing environments. Power densities approaching 3,000 mW/cm^2 (30 kW/m^2) are now possible, and this, coupled with recent developments by photoinitiator and resin formulators, makes the expansion of LED-cured UV materials likely.

Ultraviolet lasers

UV laser diodes and UV solid-state lasers can be manufactured to emit light in the ultraviolet range. Wavelengths available include 262, 266, 349, 351, 355, and 375 nm. Ultraviolet lasers have applications in industry (laser engraving), medicine (dermatology and keratectomy), secure communications, and computing (optical storage). They can be made by applying frequency conversion to lower-frequency lasers, or from Ce:LiSAF crystals (cerium doped with lithium strontium aluminum fluoride), a process developed in the 1990s at Lawrence Livermore National Laboratory.

Gas-discharge lamps

Argon and deuterium lamps are often used as stable sources, either windowless or with various windows such as magnesium fluoride.

Detecting and measuring UV radiation

Ultraviolet detection and measurement technology can vary with the part of the spectrum under consideration. While some silicon detectors are used across the spectrum, and in fact the US NIST has characterized simple silicon diodes that work with visible light too, many specializations are possible for different applications. Many approaches seek to adapt visible light-sensing technologies, but these can suffer from unwanted response to visible light and various instabilities. A variety of solid-state and vacuum devices have been explored for use in different parts of the UV spectrum. Ultraviolet light can be detected by suitable photodiodes and photocathodes, which can be tailored to be sensitive to different parts of the UV spectrum. Sensitive ultraviolet photomultipliers are available.

Near UV

Between 200-400 nm, a variety of detector options exist.

Vacuum UV

Technology for VUV instrumentation has been largely driven by solar physics for many decades and more recently some photolithography applications for semiconductors. While optics can be used to remove unwanted visible light that contaminates the VUV, in general, detectors can be limited by their response to non-VUV radiation, and the development of "solar-blind" devices has been an important area of research. Wide-gap solid-state devices or vacuum devices with high-cutoff photocathodes can be attractive compared to silicon diodes. Recently, a diamond-based device flew on the LYRA

Human health-related effects of UV radiation

Beneficial effects

Vitamin D

UVB exposure induces the production of vitamin D in the skin at a rate of up to 1,000 IUs per minute. The majority of positive health effects are related to this vitamin. It has regulatory roles in calcium metabolism (which is vital for normal functioning of the nervous system, as well as for bone growth and maintenance of bone density), immunity, cell proliferation, insulin secretion, and blood pressure.

Aesthetics

Too little UVB radiation may lead to a lack of vitamin D. Too much UVB radiation may lead to direct DNA damage, sunburn, and skin cancer. An appropriate amount of UVB (which varies according to skin color) leads to a limited amount of direct DNA damage. This is recognized and repaired by the body, then melanin production is increased, which

leads to a long-lasting tan. This tan occurs with a 2-day lag phase after irradiation, but it is much less harmful and is longer-lasting than the one obtained from UVA.

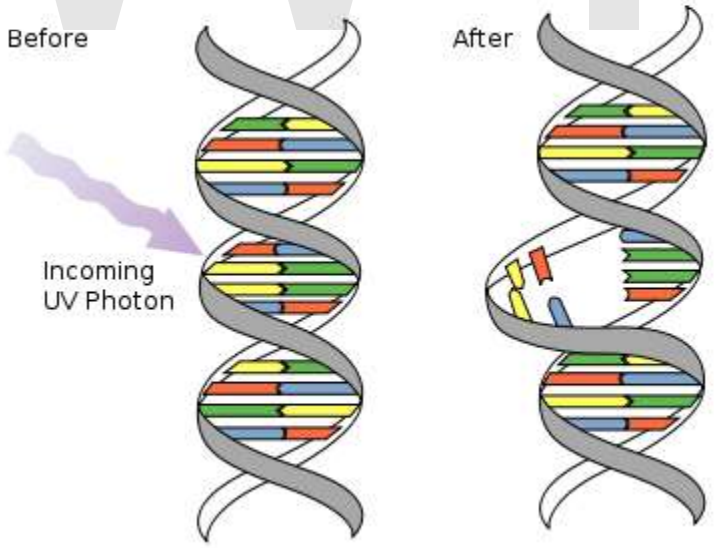
Medical applications

Ultraviolet radiation has other medical applications, in the treatment of skin conditions such as psoriasis and vitiligo. UVA radiation has been much used in conjunction with psoralens (PUVA treatment) for psoriasis, although this treatment is less used now because the combination produces dramatic increases in skin cancer, and because treatment with UVB radiation by itself is more effective. In cases of psoriasis and vitiligo, UV light with wavelength of 311 nm is most effective.

Harmful effects

An overexposure to UVB radiation can cause sunburn and some forms of skin cancer. However the most deadly form - malignant melanoma - is mostly caused by the indirect DNA damage (free radicals and oxidative stress). In humans, prolonged exposure to solar UV radiation may result in acute and chronic health effects on the skin, eye, and immune system. This can be seen from the absence of a UV-signature mutation in 92% of all melanoma.

UVC rays are the highest energy, most dangerous type of ultraviolet light. Little attention has been given to UVC rays in the past since they are filtered out by the atmosphere. However, their use in equipment such as pond sterilization units may pose an exposure risk, if the lamp is switched on outside of its enclosed pond sterilization unit.



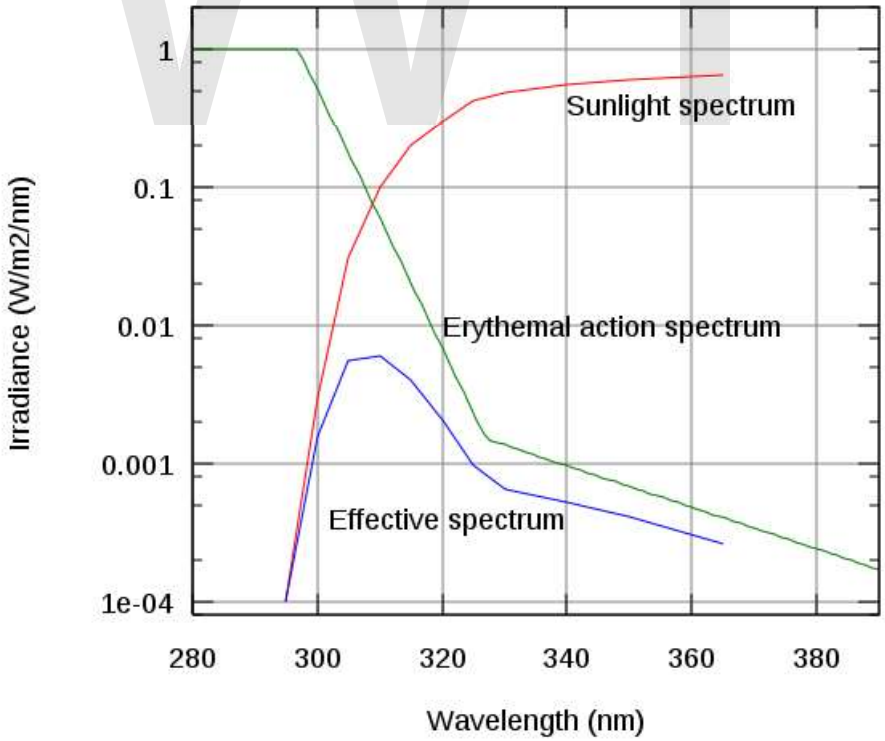
Ultraviolet photons harm the DNA molecules of living organisms in different ways. In one common damage event, adjacent thymine bases bond with each other, instead of

across the "ladder". This "thymine dimer" makes a bulge, and the distorted DNA molecule does not function properly.

Skin

> “Ultraviolet (UV) irradiation present in sunlight is an environmental human carcinogen. The toxic effects of UV from natural sunlight and therapeutic artificial lamps are a major concern for human health. The major acute effects of UV irradiation on normal human skin comprise sunburn inflammation erythema, tanning, and local or systemic immunosuppression.”
>
> — Matsumura and Ananthaswamy , (2004)

UVA, UVB, and UVC can all damage collagen fibers and, therefore, accelerate aging of the skin. Both UVA and UVB destroy vitamin A in skin, which may cause further damage. In the past, UVA was considered less harmful, but today it is known it can contribute to skin cancer via indirect DNA damage (free radicals and reactive oxygen species). It penetrates deeply, but it does not cause sunburn. UVA does not damage DNA directly like UVB and UVC, but it can generate highly reactive chemical intermediates, such as hydroxyl and oxygen radicals, which in turn can damage DNA. Because it does not cause reddening of the skin (erythema), it cannot be measured in SPF testing. There is no good clinical measurement for blockage of UVA radiation, but it is important for sunscreen to block both UVA and UVB. Some scientists blame the absence of UVA filters in sunscreens for the higher melanoma risk found for sunscreen users.



The reddening of the skin due to the action of sunlight depends both on the amount of sunlight and on the sensitivity of the skin ("erythemal action spectrum") over the UV spectrum.

UVB light can cause direct DNA damage. The radiation excites DNA molecules in skin cells, causing aberrant covalent bonds to form between adjacent cytosine bases, producing a dimer. When DNA polymerase comes along to replicate this strand of DNA, it reads the dimer as "AA" and not the original "CC". This causes the DNA replication mechanism to add a "TT" on the growing strand. This mutation can result in cancerous growths, and is known as a "classical C-T mutation". The mutations caused by the direct DNA damage carry a UV signature mutation that is commonly seen in skin cancers. The mutagenicity of UV radiation can be easily observed in bacterial cultures. This cancer connection is one reason for concern about ozone depletion and the ozone hole. UVB causes some damage to collagen, but at a very much slower rate than UVA.

As a defense against UV radiation, the amount of the brown pigment melanin in the skin increases when exposed to moderate (depending on skin type) levels of radiation; this is commonly known as a sun tan. The purpose of melanin is to absorb UV radiation and dissipate the energy as harmless heat, blocking the UV from damaging skin tissue. UVA gives a quick tan that lasts for days by oxidizing melanin that was already present, and triggers the release of the melanin from melanocytes. UVB yields a tan that takes roughly 2 days to develop because it stimulates the body to produce more melanin. The photochemical properties of melanin make it an excellent photoprotectant. Older and more widespread sunscreen chemicals cannot dissipate the energy of the excited state as efficiently as melanin, and, therefore, the penetration of these sunscreen ingredients into the lower layers of the skin may increase the amount of free radicals and reactive oxygen species (ROS). In recent years, improved filtering substances have come into use in commercial sunscreen lotions that do not significantly degrade or lose their capacity to protect the skin as the exposure time increases (*photostable* substances).

Sunscreen prevents the direct DNA damage that causes sunburn. Most of these products contain an SPF rating to show how well they block UVB rays. The SPF rating, however, offers no data about UVA protection. In the US, the Food and Drug Administration is considering adding a star rating system to show UVA protection. A similar system is already used in some European countries. Some sunscreen lotions now include compounds such as titanium dioxide, which helps protect against UVA rays. Other UVA blocking compounds found in sunscreen include zinc oxide and avobenzone.

Sunscreen safety debate

Medical organizations recommend patients protect themselves from UV radiation by using sunscreen. Five sunscreen ingredients have been shown to protect mice against skin tumors.

However, some sunscreen chemicals produce potentially harmful substances if they are illuminated while in contact with living cells. The amount of sunscreen that penetrates

through the stratum corneum may or may not be large enough to cause damage. In one study of sunscreens, the authors write:

The question whether UV filters acts on or in the skin has so far not been fully answered. Despite the fact that an answer would be a key to improve formulations of sun protection products, many publications carefully avoid addressing this question.

In an experiment by Hanson et al. published in 2006, the amount of harmful reactive oxygen species (ROS) was measured in untreated and in sunscreen treated skin. In the first 20 minutes, the film of sunscreen had a protective effect and the amount of ROS was smaller. After 60 minutes, however, the amount of absorbed sunscreen was so high, the amount of ROS was higher in the sunscreen treated skin than in the untreated skin.

Such effects can be avoided by using newer generations of filter substances or combinations that maintain their UV protective properties even after several hours of solar exposure. Sunscreen products containing photostable filters like drometrizole trisiloxane, bisoctrizole, or bemotrizinol have been available for many years throughout the world, but are not yet available in the U.S., whereas another high-quality filter, ecamsule, has also been available in the U.S. since 2006.

Eye

High intensities of UVB light are hazardous to the eyes, and exposure can cause *welder's flash* (photokeratitis or arc eye) and may lead to cataracts, pterygium, and pinguecula formation.

UV light is absorbed by molecules known as chromophores, which are present in the eye cells and tissues. Chromophores absorb light energy from the various wavelengths at different rates - a pattern known as absorption spectrum. If too much UV light is absorbed, eye structures such as the cornea, the lens and the retina can be damaged.

Protective eyewear is beneficial to those who are working with or those who might be exposed to ultraviolet radiation, particularly short wave UV. Given that light may reach the eye from the sides, full coverage eye protection is usually warranted if there is an increased risk of exposure, as in high altitude mountaineering. Mountaineers are exposed to higher than ordinary levels of UV radiation, both because there is less atmospheric filtering and because of reflection from snow and ice.

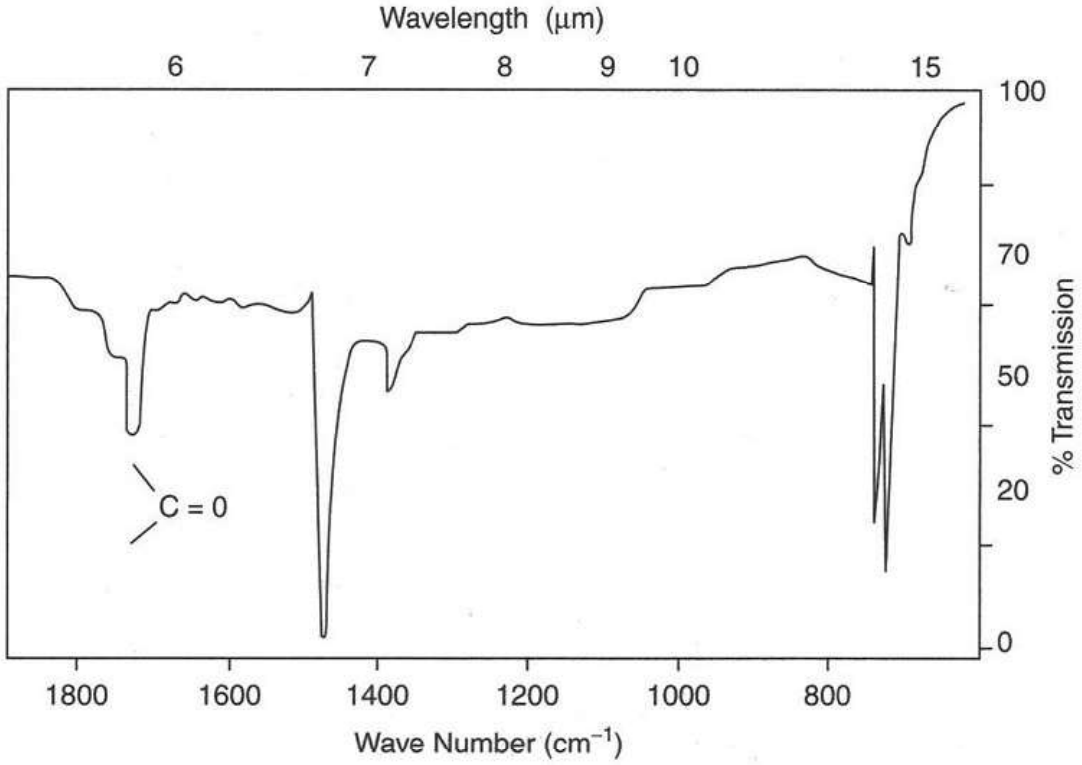
Ordinary, untreated eyeglasses give some protection. Most plastic lenses give more protection than glass lenses, because, as noted above, glass is transparent to UVA and the common acrylic plastic used for lenses is less so. Some plastic lens materials, such as polycarbonate, inherently block most UV. There are protective treatments available for eyeglass lenses that need it, which will give better protection. But even a treatment that *completely* blocks UV will not protect the eye from light that arrives around the lens.

Degradation of polymers, pigments and dyes

UV damaged polypropylene rope (left) and new rope (right)

Many polymers used in consumer products are degraded by UV light, and need addition of UV absorbers to inhibit attack, especially if the products are exposed to sunlight. The problem appears as discoloration or fading, cracking, and, sometimes, total product disintegration if cracking has proceeded sufficiently. The rate of attack increases with exposure time and sunlight intensity.

It is known as UV degradation, and is one form of polymer degradation. Sensitive polymers include thermoplastics, such as polypropylene, polyethylene, and poly(methyl methacrylate) as well as speciality fibers like aramids. UV absorption leads to chain degradation and loss of strength at sensitive points in the chain structure. They include tertiary carbon atoms, which in polypropylene occur in every repeat unit. Aramid rope must be shielded with a sheath of thermoplastic if it is to retain its strength.The impact of UV on polymers is used in nanotechnology, transplantology, X-ray lithography and others fields for modification of properties (roughness, hydrophobicity) of polymer surface. For example, it is known about smoothing effect of vacuum ultraviolet (VUV) on a poly(methyl methacrylate) surface.



IR spectrum showing carbonyl absorption due to UV degradation of polyethylene

In addition, many pigments and dyes absorb UV and change colour, so paintings and textiles may need extra protection both from sunlight and fluorescent bulbs, two common sources of UV radiation. Old and antique paintings such as watercolour paintings, for example, usually must be placed away from direct sunlight. Common window glass provides some protection by absorbing some of the harmful UV, but valuable artifacts need extra shielding. Many museums place black curtains over watercolour paintings and ancient textiles, for example. Since watercolours can have very low pigment levels, they need extra protection from UV light.

Blockers and absorbers

Ultraviolet Light Absorbers (UVAs) are molecules used in organic materials (polymers, paints, etc.) to absorb UV light to reduce the UV degradation (photo-oxidation) of a material. A number of different UVAs with different absorption properties exist. UVAs can disappear over time, so monitoring of UVA levels in weathered materials is necessary.

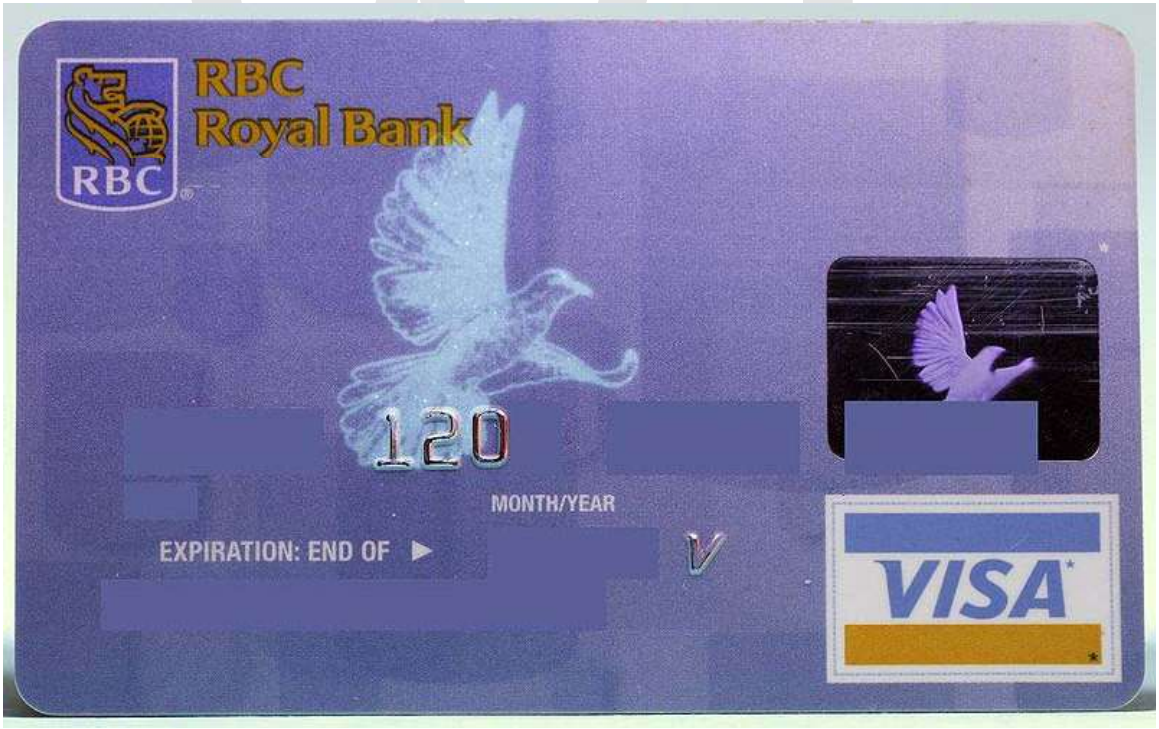
In sunscreen, ingredients that absorb UVA/UVB rays, such as avobenzone and octyl methoxycinnamate, are known as absorbers. They are contrasted with physical "blockers" of UV radiation such as titanium dioxide and zinc oxide.

Applications of UV

By wavelength:

- **13.5 nm**: Extreme Ultraviolet Lithography
- **230-400 nm**: Optical sensors, various instrumentation
- **230-365 nm**: UV-ID, label tracking, barcodes
- **240-280 nm**: Disinfection, decontamination of surfaces and water (DNA absorption has a peak at 260 nm)
- **250-300 nm**: Forensic analysis, drug detection
- **270-300 nm**: Protein analysis, DNA sequencing, drug discovery
- **280-400 nm**: Medical imaging of cells
- **300-400 nm**: Solid-state lighting
- **300-365 nm**: Curing of polymers and printer inks
- **300-320 nm**: Light therapy in medicine
- **350-370 nm**: Bug zappers (flies are most attracted to light at 365 nm)

Security



A bird appears on many Visa credit cards when held under a UV light source

To help prevent counterfeiters, sensitive documents (e.g., credit cards, driver's licenses, passports) may also include a UV watermark that is visible only under a UV-emitting light. Passports issued by most countries usually contain UV sensitive inks and security threads. Visa stamps and stickers on passports of visitors contain large detailed seals invisible under normal light, but strongly visible under UV illumination. Passports issued

by many nations have UV sensitive watermarks on all pages. Currencies of various countries' banknotes have an image, as well as many multicolored fibers, that are visible only under ultraviolet light.

Some brands of pepper spray will leave an invisible chemical (UV dye) that is not easily washed off on a pepper sprayed attacker, which would help police identify them later.

Forensics

UV is an investigative tool at the crime scene helpful in locating and identifying bodily fluids (semen, blood, bile etc.). E.g., ejaculated fluids or saliva are detected by high-power UV, irrespective of the structure or colour of the surface the fluid is deposited upon.

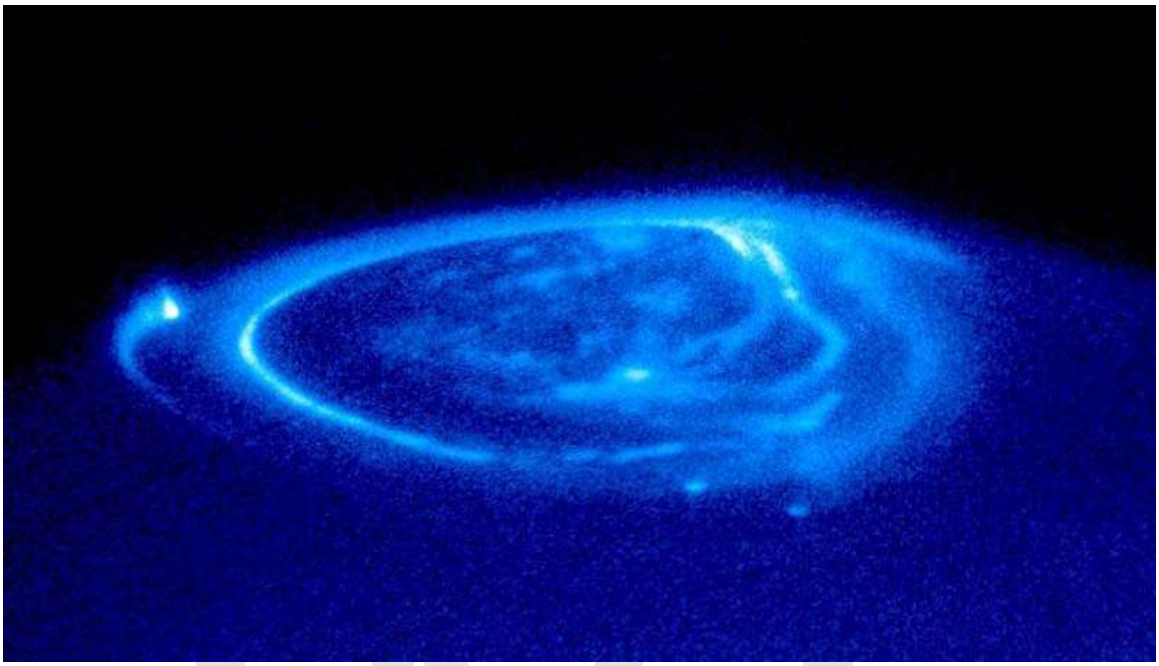
Fluorescent lamps

Fluorescent lamps produce UV radiation by ionising low-pressure mercury vapour. A phosphorescent coating on the inside of the tubes absorbs the UV and converts it to visible light.

The main mercury emission wavelength is in the UVC range. Unshielded exposure of the skin or eyes to mercury arc lamps that do not have a conversion phosphor is quite dangerous.

The light from a mercury lamp is predominantly at discrete wavelengths. Other practical UV sources with more continuous emission spectra include xenon arc lamps (commonly used as sunlight simulators), deuterium arc lamps, mercury-xenon arc lamps, metal-halide arc lamps, and tungsten-halogen incandescent lamps.

Astronomy



Aurora at Jupiter's north pole as seen in ultraviolet light by the Hubble Space Telescope.

In astronomy, very hot objects preferentially emit UV radiation. Because the ozone layer blocks many UV frequencies from reaching telescopes on the surface of the Earth, most UV observations are made from space.

Biological surveys and pest control

Some animals, including birds, reptiles, and insects such as bees, can see near-ultraviolet light. Many fruits, flowers, and seeds stand out more strongly from the background in ultraviolet wavelengths as compared to human color vision. Scorpions glow or take on a yellow to green color under UV illumination, thus assisting in the control of these arachnids. Many birds have patterns in their plumage that are invisible at usual wavelengths but observable in ultraviolet, and the urine and other secretions of some animals, including dogs, cats, and human beings, is much easier to spot with ultraviolet. Urine trails of rodents can be detected by pest control technicians for proper treatment of infested dwellings.

Butterflies use ultraviolet as a communication system for sex recognition and mating behavior.

Many insects use the ultraviolet wavelength emissions from celestial objects as references for flight navigation. A local ultraviolet emissor will normally disrupt the navigation process and will eventually attract the flying insect.

Entomologist using a UV light for collecting beetles in the Paraguayan Chaco.

Ultraviolet traps called bug zappers are used to eliminate various small flying insects. They are attracted to the UV light, and are killed using an electric shock, or trapped once they come into contact with the device. Different designs of ultraviolet light traps are also used by entomologists for collecting nocturnal insects during faunistic survey studies.

Spectrophotometry

UV/VIS spectroscopy is widely used as a technique in chemistry, to analyze chemical structure, the most notable one being conjugated systems. UV radiation is often used in visible spectrophotometry to determine the fluorescence of a given sample. In biological research, UV light is used for quantification of nucleic acids.

Sanitary compliance

UV lamps including newer LEDs (light-emitting diode) aid in the detection of organic mineral deposits that remain on surfaces where periodic cleaning and sanitizing may not be properly accomplished. Both urine and phosphate soaps are easily detected using UV inspection. Pet urine deposits in carpeting or other hard surfaces can be detected for accurate treatment and removal of mineral tracers and the odor-causing bacteria that feed on proteins within. Many hospitality industries use UV lamps to inspect for unsanitary

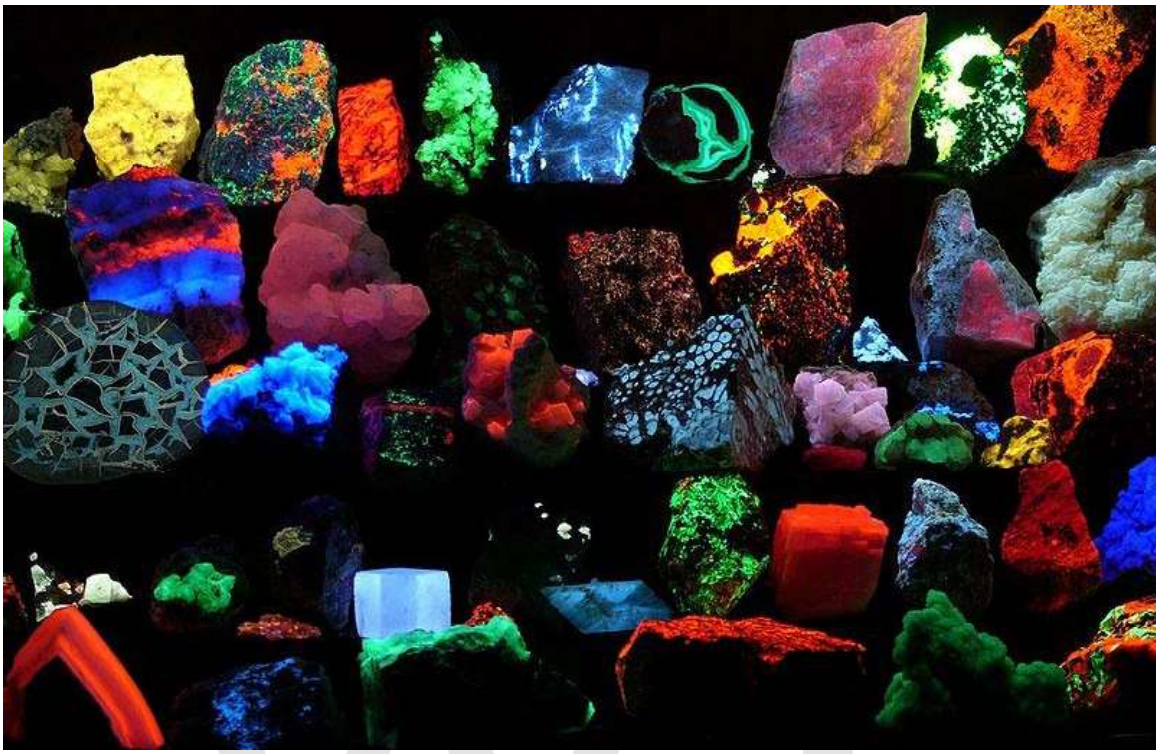
bedding to determine lifecycle for mattress restoration as well as general performance of the cleaning staff. A perennial news feature for many television news organizations involves an investigative reporter's using a similar device to reveal unsanitary conditions in hotels, public toilets, hand rails, and such.

Air purification

Using a catalytic reaction from titanium dioxide and UV light exposure, a strong oxidative effect occurs on any organic objects that pass through the media, converting otherwise irritating pathogens, pollens, and mold spores into harmless inert byproducts. The cleansing mechanism of UV is a photochemical process. The contaminants that pollute the indoor environment are almost entirely based upon organic or carbon-based compounds. These compounds break down when exposed to high-intensity UV at 240 to 280 nm. Short-wave ultraviolet light can destroy DNA in living microorganisms and break down organic material found in indoor air. UVC's effectiveness is directly related to intensity and exposure time.

UV light has also been shown (by KJ Scott *et al*) as effective in reducing gaseous contaminants such as carbon monoxide and VOCs. Scott and his colleagues demonstrated that the correct mixture of UV lamps radiating at 184 and 254 nm can remove LOW concentrations hydrocarbons and carbon monoxide, if the lamps are held in a radiation chamber (a box or drum) and the air is recycled between the room and the reaction chamber. This arrangement prevents the introduction of ozone into the treated air. Alternatively, air may be treated by passing by a=a single UV source operating at 184 nm and subsequent catalysis with iron oxide. The iron oxides remove the ozone produced by the UV lamp.

Analyzing minerals



A collection of mineral samples brilliantly fluorescing at various wavelengths as seen while being irradiated by UV light.

Ultraviolet lamps are also used in analyzing minerals and gems, and in other detective work including authentication of various collectibles. Materials may look the same under visible light, but fluoresce to different degrees under ultraviolet light, or may fluoresce differently under short wave ultraviolet versus long wave ultraviolet.

Authentication

In other detective work including authentication of various collectibles and art, and detecting counterfeit currency absent of marker dyes. Materials may look the same under visible light, but fluoresce to different degrees under ultraviolet light, or may fluoresce differently under short-wave ultraviolet versus long-wave ultraviolet.

Chemical markers

UV fluorescent dyes are used in many applications (for example, biochemistry and forensics). The Green Fluorescent Protein (GFP) is often used in genetics as a marker. Many substances, such as proteins, have significant light absorption bands in the ultraviolet that are of use and interest in biochemistry and related fields. UV-capable spectrophotometers are common in such laboratories.

Photochemotherapy

Exposure to UVA light while the skin is hyper-photosensitive by taking psoralens is an effective treatment for psoriasis called PUVA. Due to the potential of psoralens to cause damage to the liver, PUVA may be used only a limited number of times over a patient's lifetime.

Phototherapy

Exposure to UVB light, in particular, the 310 nm narrowband UVB range, is an effective long-term treatment for many skin conditions like psoriasis, vitiligo, eczema, and others. UVB phototherapy does not require additional medications or topical preparations for the therapeutic benefit; only the light exposure is needed. However, phototherapy can be effective when used in conjunction with certain topical treatments such as anthralin, coal tar, and Vitamin A and D derivatives, or systemic treatments such as methotrexate and soriatane.

Typical treatment regimes involve short exposure to UVB rays 3 to 5 times a week at a hospital or clinic, and up to 30 or more sessions may be required before results are noticeable. Almost all of the conditions that respond to UVB light are chronic problems, so continuous treatment is required to keep those problems in check. Home UVB systems are common solutions for those whose conditions respond to treatment. Home systems permit patients to treat themselves every other day (the ideal treatment regimen for most) without the frequent, costly trips to the office/clinic and back.

Side-effects may include itching and redness of the skin due to UVB exposure, and possibly sunburn, if patients do not minimize exposure to natural UV rays during treatment days. Cataracts can frequently develop if the eyes are not protected from UVB light exposure. There is no link between an increase in the patient's risk for skin cancer and the proper use of UVB phototherapy]].. "Proper use" is generally defined as reaching the "Sub-Erythemic Dose" (S.E.D.), the maximum amount of UVB your skin can receive *without* burning.

Certain fungal growths under the toenail can be treated using a specific wavelength of UV delivered from a high-power LED (light-emitting diode) and can be safer than traditional systemic drugs.

Photolithography

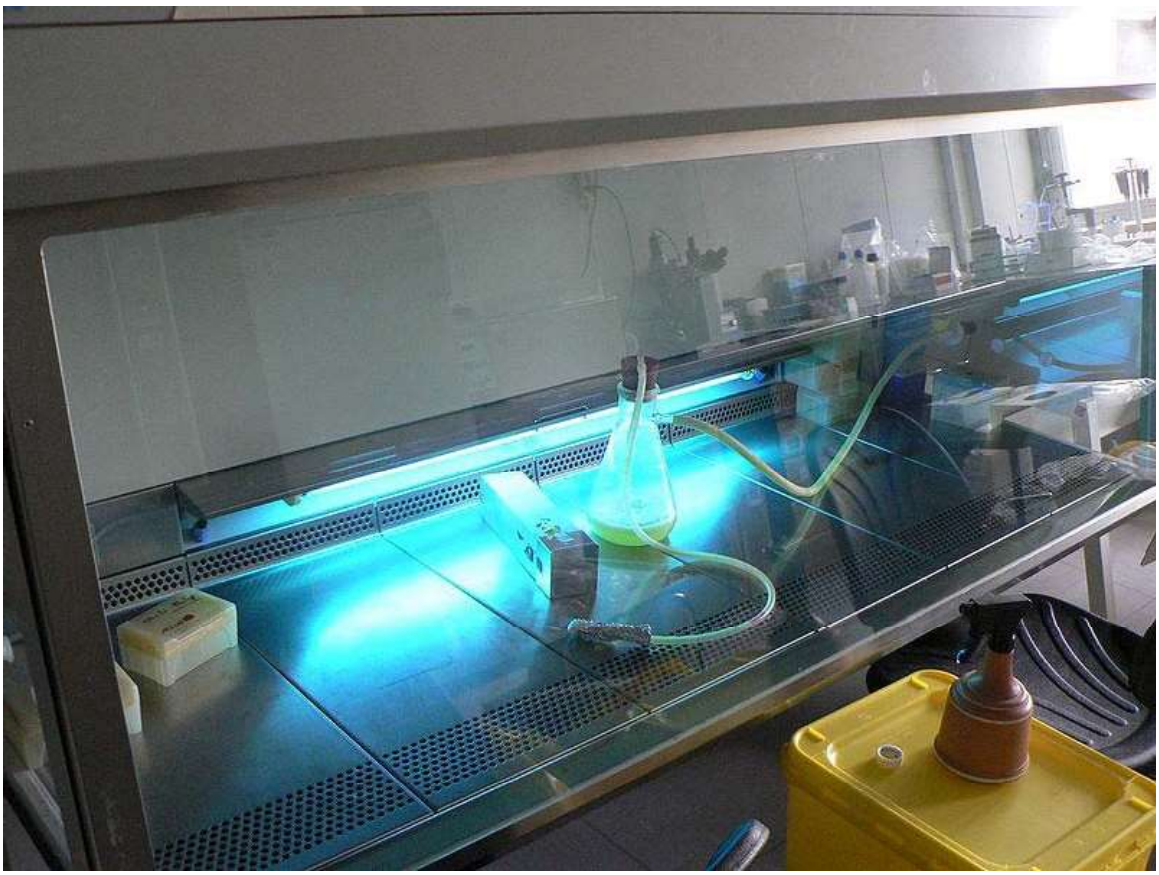
Ultraviolet radiation is used for very fine resolution photolithography, a procedure wherein a chemical called a photoresist is exposed to UV radiation that has passed through a mask. The light causes chemical reactions to occur in the photoresist, and, after development (a step that removes either the exposed or the unexposed photoresist), a pattern determined by the mask remains on the sample. Steps may then be taken to "etch" away areas of the sample where no photoresist remains.

UV radiation is used extensively in the electronics industry because photolithography is used in the manufacture of semiconductors, integrated circuit components, and printed circuit boards.

Checking electrical insulation

An application of UV is to detect corona discharge (often called "corona") on electrical apparatus. Degradation of insulation in electrical apparatus or pollution causes corona, wherein a strong electric field ionizes the air and excites nitrogen molecules, causing the emission of ultraviolet radiation. The corona degrades the insulation level of the apparatus. Corona produces ozone and to a lesser extent nitrogen oxide, which may subsequently react with water in the air to form nitrous acid and nitric acid vapour in the surrounding air.

Sterilization



A low pressure mercury vapor discharge tube floods the inside of a hood with shortwave UV light when not in use, sterilizing microbiological contaminants from irradiated surfaces.

Ultraviolet lamps are used to sterilize workspaces and tools used in biology laboratories and medical facilities. Commercially available low-pressure mercury-vapor lamps emit about 86% of their light at 254 nanometers (nm), which coincides very well with one of

the two peaks of the germicidal effectiveness curve (i.e., effectiveness for UV absorption by DNA). One of these peaks is at about 265 nm and the other is at about 185 nm. Although 185 nm is better absorbed by DNA, the quartz glass used in commercially available lamps, as well as environmental media such as water, are more opaque to 185 nm than 254 nm (C. von Sonntag et al., 1992). UV light at these germicidal wavelengths causes adjacent thymine molecules on DNA to dimerize; if enough of these defects accumulate on a microorganism's DNA, its replication is inhibited, thereby rendering it harmless (even though the organism may not be killed outright). However, since microorganisms can be shielded from ultraviolet light in small cracks and other shaded areas, these lamps are used only as a supplement to other sterilization techniques.

Disinfecting drinking water

UV radiation can be an effective viricide and bactericide. Disinfection using UV radiation is commonly used in wastewater treatment applications and is finding an increased usage in drinking water treatment. Many bottlers of spring water use UV disinfection equipment to sterilize their water. Solar water disinfection is the process of using PET bottles and sunlight to disinfect water.

New York City has approved the construction of a 2-billion-US-gallon-per-day (7,600,000 m^3/d) ultraviolet drinking water disinfection facility. There are also several facilities under construction and several in operation that treat waste water with several stages of filters, hydrogen peroxide, and UV light to bring the water up to drinking standards. One such facility exists in Orange County, California. NASA has examined the use of this technology, using titanium dioxide as catalyst, for breaking down harmful products in spacecraft waste water.

It used to be thought that UV disinfection was more effective for bacteria and viruses, which have more exposed genetic material, than for larger pathogens that have outer coatings or that form cyst states (e.g., Giardia) that shield their DNA from the UV light. However, it was recently discovered that ultraviolet radiation can be somewhat effective for treating the microorganism Cryptosporidium. The findings resulted in the use of UV radiation as a viable method to treat drinking water. Giardia in turn has been shown to be very susceptible to UV-C when the tests were based on infectivity rather than excystation. It has been found that protists are able to survive high UV-C doses but are sterilized at low doses.

Solar water disinfection (SODIS) has been extensively researched in Switzerland and has proven ideal to treat small quantities of water cheaply using natural sunlight. Contaminated water is poured into transparent plastic bottles and exposed to full sunlight for six hours. The sunlight treats the contaminated water through two synergetic mechanisms: UV-A irradiation and increased water temperature. If the water temperatures rises above 50 °C (120 °F), the disinfection process is three times faster.

Food processing

As consumer demand for fresh and "fresh-like" food products increases, the demand for nonthermal methods of food processing is likewise on the rise. In addition, public awareness regarding the dangers of food poisoning is also raising demand for improved food processing methods. Ultraviolet radiation is used in several food processes to kill unwanted microorganisms. UV light can be used to pasteurize fruit juices by flowing the juice over a high-intensity ultraviolet light source. The effectiveness of such a process depends on the UV absorbance of the juice.

Fire detection

Ultraviolet detectors generally use either a solid-state device, such as one based on silicon carbide or aluminium nitride, or a gas-filled tube as the sensing element. UV detectors that are sensitive to UV light in any part of the spectrum respond to irradiation by sunlight and artificial light. A burning hydrogen flame, for instance, radiates strongly in the 185- to 260-nanometer range and only very weakly in the IR region, whereas a coal fire emits very weakly in the UV band yet very strongly at IR wavelengths; thus, a fire detector that operates using both UV and IR detectors is more reliable than one with a UV detector alone. Virtually all fires emit some radiation in the UVC band, whereas the Sun's radiation at this band is absorbed by the Earth's atmosphere. The result is that the UV detector is "solar blind", meaning it will not cause an alarm in response to radiation from the Sun, so it can easily be used both indoors and outdoors.

UV detectors are sensitive to most fires, including hydrocarbons, metals, sulfur, hydrogen, hydrazine, and ammonia. Arc welding, electrical arcs, lightning, X-rays used in nondestructive metal testing equipment (though this is highly unlikely), and radioactive materials can produce levels that will activate a UV detection system. The presence of UV-absorbing gases and vapors will attenuate the UV radiation from a fire, adversely affecting the ability of the detector to detect flames. Likewise, the presence of an oil mist in the air or an oil film on the detector window will have the same effect.

Herpetology

Reptiles need long wave UV light for de novo synthesis of vitamin D. Vitamin D is needed to metabolize calcium for bone and egg production. Thus, in a typical reptile enclosure, a fluorescent UV lamp should be available for vitamin D synthesis. This should be combined with the provision of heat for basking, either in the same or by another lamp.

Curing of electronic potting resins

Electronic components that require clear transparency for light to exit or enter (photo voltaic panels and sensors) can be potted using acrylic resins that are cured using UV light energy. The advantages are low VOC emissions and rapid curing.

Curing of inks, adhesives, varnishes and coatings

Certain inks, coatings, and adhesives are formulated with photoinitiators and resins. When exposed to the correct energy and irradiance in the required band of UV light, polymerization occurs, and so the adhesives harden or cure. Usually, this reaction is very quick, a matter of a few seconds. Applications include glass and plastic bonding, optical fiber coatings, the coating of flooring, UV Coating and paper finishes in offset printing, and dental fillings. Curing of decorative finger nail "gels".

An industry has developed around the manufacture of UV sources for UV curing applications. This includes UV lamps, UV LEDs, and Excimer Flash lamps. Fast processes such as flexo or offset printing require high-intensity light focused via reflectors onto a moving substrate and medium; and high-pressure Hg (mercury) or Fe (iron, doped)-based bulbs are used, which can be energized with electric arc or microwaves. Lower-power sources (fluorescent lamps, LED) can be used for static applications, and, in some cases, small high-pressure lamps can have light focused and transmitted to the work area via liquid-filled or fiber-optic light guides.

Sun tanning

Sun tanning describes a darkening of the skin in a natural physiological response stimulated by exposure to ultraviolet radiation from sunshine (or a sunbed). With excess exposure to the sun, a suntanned area can also develop sunburn. The increased production of melanin is triggered by the direct DNA damage. This kind of damage is recognized by the body and as a defense against UV radiation the skin produces more melanin. Melanin dissipates the UV energy as harmless heat, and therefore it is an excellent photoprotectant. Melanin protects against the direct DNA damage and against the indirect DNA damage. Sunscreen protects only against the direct DNA damage, but increases the indirect DNA damage. Some studies suggest that this may be the cause of the higher incidence of melanoma found in sunscreen users compared to non-users.

Erasing EPROM modules

Some EPROM (erasable programmable read-only memory) modules are erased by exposure to UV radiation. These modules often have a transparent glass (quartz) window on the top of the chip that allows the UV radiation in. These have been largely superseded by EEPROM and flash memory chips in most devices.

Preparing low surface energy polymers

UV radiation is useful in preparing low surface energy polymers for adhesives. Polymers exposed to UV light will oxidize, thus raising the surface energy of the polymer. Once the surface energy of the polymer has been raised, the bond between the adhesive and the polymer is stronger.

Reading otherwise illegible papyruses

Using multi-spectral imaging it is possible to read illegible papyruses, such as the burned papyruses of the Villa of the Papyri or of Oxyrhynchus, or the Archimedes palimpsest. The technique involves taking pictures of the illegible papyruses using different filters in the infrared or ultraviolet range, finely tuned to capture certain wavelengths of light. Thus, the optimum spectral portion can be found for distinguishing ink from paper on the papyrus surface.

Lasers

Ultraviolet lasers have applications in industry (laser engraving), medicine (dermatology and keratectomy), free air secure communications and computing (optical storage). They can be made by applying frequency conversion to lower-frequency lasers, or from Ce:LiSAF crystals (cerium doped with lithium strontium aluminum fluoride), a process developed in the 1990s at Lawrence Livermore National Laboratory.

UV solar cells and UV degradation of solar cells

Japan's National Institute of Advanced Industrial Science and Technology (AIST) has succeeded in developing a transparent solar cell that uses ultraviolet light to generate electricity but allows visible light to pass through it. Most conventional solar cells use visible and infrared light to generate electricity. In contrast, the innovative new solar cell uses ultraviolet radiation. Used to replace conventional window glass, the installation surface area could be large, leading to potential uses that take advantage of the combined functions of power generation, lighting and temperature control.

Also PEDOT-PSS solar cells is an ultraviolet (UV) light-selective and -sensitive photovoltaic cell easily fabricated.

On the other hand, a nanocrystalline layer of Cu_2O in the construction of photovoltaic cells increases their ability to utilize UV radiations for photocurrent generation.

Nondestructive testing

UV light of a specified spectrum and intensity is used to stimulate fluorescent dyes so as to highlight defects in a broad range of materials. These dyes may be carried into surface-breaking defects by capillary action (liquid penetrant inspection) or they may be bound to ferrite particles caught in magnetic leakage fields in ferrous materials (magnetic particle inspection).

Evolutionary significance

Evolution of early reproductive proteins and enzymes is attributed in modern models of evolutionary theory to ultraviolet light. UVB light causes thymine base pairs next to each

other in genetic sequences to bond together into thymine dimers, a disruption in the strand that reproductive enzymes cannot copy. This leads to frameshifting during genetic replication and protein synthesis, usually killing the organism. As early prokaryotes began to approach the surface of the ancient oceans, before the protective ozone layer had formed, blocking out most wavelengths of UV light, they almost invariably died out. The few that survived had developed enzymes that verified the genetic material and broke up thymine dimer bonds, known as base excision repair enzymes. Many enzymes and proteins involved in modern mitosis and meiosis are similar to excision repair enzymes, and are believed to be evolved modifications of the enzymes originally used to overcome UV light.

Chapter- 10

Light Therapy

Bright light therapy is a common treatment for seasonal affective disorder and for circadian rhythm disorders.

Light therapy or **phototherapy** (classically referred to as **heliotherapy**) consists of exposure to daylight or to specific wavelengths of light using lasers, light-emitting diodes, fluorescent lamps, dichroic lamps or very bright, full-spectrum light, usually controlled with various devices. The light is administered for a prescribed amount of time and, in some cases, at a specific time of day.

Commercially, the common use of the term is associated with the treatment of skin, sleep disorder and some psychiatric disorders. Light therapy directed at the skin is used to treat acne vulgaris and neonatal jaundice. Light therapy which strikes the retina of the eyes is used to treat circadian rhythm disorders such as delayed sleep phase syndrome and can also be used to treat seasonal affective disorder, with some support for its use also with non-seasonal psychiatric disorders.

The medical (mainstream and complementary and alternative medicine) applications of light therapy also include pain management, accelerated wound healing, hair growth, acupuncture, improvement in blood properties and blood circulation, and sinus-related diseases and disorders. Many of these use low level laser therapy and red light therapy in the 620–660 nm range.

The National Center for Complementary and Alternative Medicine has listed "Light Therapy" as a practice that involves "veritable forms of energy that include those involving electromagnetic fields".

History

Indian medical literature dating to 1500 BC describes a treatment combining herbs with natural sunlight to treat non-pigmented skin areas. Buddhist literature from about 200 AD and 10th-century Chinese documents made similar references.

Danish physician Nils Finsen is believed to be the father of modern phototherapy. He developed the first artificial light source for this purpose, and used his invention to treat lupus vulgaris. He received the Nobel Prize in Physiology or Medicine in 1903.

Since then a large array of treatments have been developed from the use of controlled light. Though the popular consumer understanding of "light therapy" is associated with treating seasonal affective disorder, other applications, growing in recognition include the application of low level laser, red light, near-infrared and ultraviolet lights for pain management, hair growth, skin treatments, accelerated wound healing. The modalities include light-based acupuncture, directing light on painful areas, blood irradiation therapy and photodynamic therapy.

Skin related

Acne vulgaris

Sunlight was long known to improve acne, and this was thought to be due to antibacterial and other effects of the ultraviolet spectrum which cannot be used as a long-term treatment due to the likelihood of skin damage.

It was found that some of the visible violet light present in sunlight (in the range 415–430 nm) activates a porphyrin (Coproporphyrin III) in *Propionibacterium acnes* which

damages and ultimately kills the bacteria by releasing singlet oxygen. A total of 320 J/cm^2 of light within this range renders the bacteria non-viable.

The use of light therapy for three consecutive days has been shown to reduce the bacteria in the pores by 99.9%. Since there are few porphyrins naturally found in the skin, the treatment is believed safe except in patients with porphyria; although eye protection is used due to light-sensitive chemicals in the retina. The light is usually created by superluminous LEDs. This form of treatment has been approved by the FDA for some lightwave systems . Overall improvements of on average 76% for 80% of patients occurs over three months; most studies show that it performs better than benzoyl peroxide and the treatment is far better tolerated. However, approximately 10% of users see no improvement.

Psoriasis and eczema

A feature of psoriasis is localized inflammation mediated by the immune system. UV radiation is known to suppress the immune system and reduce inflammatory responses. Light therapy for skin conditions like psoriasis or eczema use UV-A (315–400 nm wavelength) or UV-B (280–315 nm wavelength) light waves. UV-A, combined with a drug taken orally, is known as PUVA treatment. Narrow band UV-B is the 310 nm wavelength and is given as a light therapy treatment rather than full spectrum UV-B.

Tanning

Tanning is caused by the effects of two different spectrums of ultraviolet radiation: UV-A and UV-B.

Wound healing

Lightwave therapy has been suggested for use in healing of wounds. Some say that low-level laser therapy does not appear to be effective, while others find that it can be effective. Lightwave therapy is used clinically in many areas outside the United States including Canada, Europe and Asia.

Photodynamic therapy

Visible blue light is used with aminolevulinic acid for the treatment of actinic keratosis. This is not a U.S. FDA-approved treatment for acne vulgaris.

Mood and sleep related

Light boxes

The production of the hormone melatonin, a sleep regulator, is inhibited by light and permitted by darkness as registered by photosensitive ganglion cells in the retina. To

some degree, the reverse is true for serotonin, which has been linked to mood disorders. Hence, for the purpose of manipulating melatonin levels or timing, light boxes providing very specific types of artificial illumination to the retina of the eye are effective.

Light therapy either uses a lightbox which emits up to 10,000 lux of light, much brighter than a customary incandescent lamp, or a lower intensity of specific wavelengths of light from the blue (470 nm) to the green (525 nm) areas of the visible spectrum. Newer light therapy devices use LED technology, making them much smaller and more convenient for users. A 1995 study showed that green light therapy at doses of 350 lux produces melatonin suppression and phase shifts equivalent to 10,000 lux bright light therapy and another study published in May 2010 suggests that the blue light often used for SAD treatment should perhaps be replaced by green or white illumination.

In treatment, the patient's eyes are to be at a prescribed distance from the light source with the light striking the retina. This does not require looking directly into the light.

Seasonal affective disorder

While full sunlight is preferred for seasonal affective disorder (SAD), light boxes may be effective for the treatment of the condition. Light boxes for seasonal affective disorder are designed to filter out most UV light, which can cause eye and skin damage. The U.S. Food and Drug Administration has not approved the use of light boxes to treat SAD due to unclear results in clinical trials, but light therapy is still seen as the main form of treatment for SAD. Direct sunlight, reflected into the windows of a home or office by a computer-controlled mirror device called a heliostat, has also been used as a type of light therapy for the treatment of SAD.

It is possible that response to light therapy for SAD could be season dependent.

Non-seasonal depression

Light therapy has also been suggested in the treatment of non-seasonal depression and other psychiatric disturbances, including major depressive disorder, bipolar disorder and postpartum depression. A meta-analysis by the Cochrane Collaboration concluded that "For patients suffering from non-seasonal depression, light therapy offers modest though promising antidepressive efficacy."

Circadian rhythm sleep disorders

Chronic CRSD

In the management of circadian rhythm disorders such as delayed sleep phase syndrome (DSPS), the timing of light exposure is critical. For DSPS, the light must be provided to the retina as soon after spontaneous awakening as possible to achieve the desired effect, as shown by the phase response curve for light in humans. Some users have reported success with lights that turn on shortly *before* awakening (dawn simulation). Morning use

may also be effective for non-24-hour sleep-wake syndrome, while evening use is recommended for advanced sleep phase syndrome.

Situational CRSD

Light therapy has been tested for individuals on shift work, and for jet lag.

Neonatal jaundice

A newborn infant undergoing white-light phototherapy to treat neonatal jaundice.

Light therapy is used to treat cases of neonatal jaundice through the isomerization of the bilirubin and consequently transformation into compounds that the newborn can excrete via urine and stools. A common treatment of neonatal jaundice is the Bili light.

Parkinson's disease

Bright light therapy may ease Parkinson's disease by reducing patients' tremors.

Safety

Ultraviolet light causes progressive damage to human skin. This is mediated by genetic damage, collagen damage, as well as destruction of vitamin A and vitamin C in the skin

and free radical generation. Ultraviolet light is also known to be a factor in formation of cataracts. Researchers have questioned whether limiting blue light exposure could reduce the risk of age-related macular degeneration.

Modern phototherapy lamps used in the treatment of seasonal affective disorder and sleep disorders either filter out or do not emit ultraviolet light and are considered safe and effective for the intended purpose, as long as photosensitizing drugs are not being taken at the same time and in the absence of any existing eye conditions. Light therapy is a mood altering treatment, and just as with drug treatments, there is a possibility of triggering a manic state from a depressive state, causing anxiety and other side effects. While these side effects are usually controllable, it is recommended that patients undertake light therapy under the supervision of an experienced clinician, rather than attempting to self-medicate.

It is reported that bright light therapy may activate the production of reproductive hormones, such as testosterone, luteinizing hormone, follicle-stimulating hormone, and estradiol.

There are few absolute contraindications to light therapy, although there are some circumstances in which caution is required. These include when a patient has a condition that might render his or her eyes more vulnerable to phototoxicity, has a tendency toward mania, has a photosensitive skin condition, or is taking a photosensitizing herb (such as St. John's wort) or medication. Patients with porphyria should avoid most forms of light therapy. Patients on certain drugs like methotrexate or chloroquine should use caution with light therapy as there is a chance that these drugs could cause porphyria.

Side effects

Side effects of light therapy for sleep phase disorders include jumpiness or jitteriness, headache, and nausea. Some nondepressive physical complaints (such as poor vision and skin rash or irritation) may improve with light therapy.