The Light Reactions of Photosynthesis

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ABSTRACT Historically, the role of light in photosynthesis has been ascribed either to a photolysis of carbon dioxide or to a photolysis of water and a resultant rearrangement of constituent atoms into molecules of oxygen and glucose (or formaldehyde). The discovery of photophosphorylation demonstrated that photosynthesis includes a light-induced phosphorus metabolism that precedes, and is independent from, a photolysis of water or CO₂. ATP formation could best be accounted for not by a photolytic disruption of the covalent bonds in CO₂ or water but by the operation of a light-induced electron flow that results in a release of free energy which is trapped in the pyrophosphate bonds of ATP.

Photophosphorylation is now divided into (a) a noncyclic type, in which the formation of ATP is coupled with a light-induced electron transport from water to ferredoxin and a concomitant evolution of oxygen and (b) a cyclic type which yields only ATP and produces no net change in the oxidation-reduction state of any electron donor or acceptor. Reduced ferredoxin formed in (a) serves as an electron donor for the reduction of NADP by an enzymatic reaction that is independent of light. ATP, from both cyclic and noncyclic photophosphorylation, and reduced NADP jointly constitute the assimilatory power for the conversion of CO₂ to carbohydrates (3 moles of ATP and 2 moles of reduced NADP are required per mole of CO₂).

Investigations, mainly with whole cells, have shown that photosynthesis in green plants involves two photosystems, one (System II) that best uses light of “short” wavelength (λ < 655 nm) and another (System I) that best uses light of “long” wavelength (λ > 655 nm). Cyclic photophosphorylation in chloroplasts involves a System I photoreaction. Noncyclic photophosphorylation is widely held to involve a collaboration of two photoreactions: a short-wavelength photoreaction belonging to System II and a long-wavelength photoreaction belonging to System I. Recent findings, however, indicate that noncyclic photophosphorylation may include two short-wavelength, System II, photoreactions that operate in series and are joined by a “dark” electron-transport chain to which is coupled a phosphorylation site.

Early concepts

The first hypothesis about the role of light in photosynthesis came very appropriately from Jan Ingenhousz, who some years earlier had made the epochal discovery that it is “the influence of the light of the sun upon the plant” (1) that is responsible for the “restorative” effect of vegetation on “bad” air—an observation first made in 1771 by Joseph Priestley without reference to light (2). In 1796, Ingenhousz wrote that the green plant absorbs from “carbonic acid in the sunshine, the carbon, throwing out at that time the oxygen alone, and keeping the carbon to itself as nourishment” (3).

The idea that light liberates oxygen by photodecomposing CO₂ had, with some modifications, persisted for well over a century. It seemed to have had a special attraction for some of the most illustrious chemists in their day, e.g., von Baeyer (4) and Willstätter (5); its last great contemporary protagonist was Otto Warburg (6). After de Saussure (7) showed that water is a reactant in photosynthesis, the CO₂ cleavage hypothesis readily accounted for the deceptively simple overall photosynthesis equation (Eq. 1): the C₂H₄O₂: proportions in the carbohydrate product fitted the idea that the carbon from the photodecomposition of CO₂ recombines with the elements of water.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2
\] (i)

A different hypothesis, one that profoundly influenced research in photosynthesis, was put forward by van Niel (8). After elucidating the nature of bacterial photosynthesis, he proposed (8) that bacterial and plant photosynthesis are special cases of a general process in which light energy is used to photodecompose a hydrogen donor, H₂A, with the released hydrogen in turn reducing CO₂ by dark, enzymic reactions:

\[
\text{CO}_2 + 2\text{H}_2\text{A} \rightarrow (\text{CH}_2\text{O}) + \text{H}_2\text{O} + 2\text{A}
\] (ii)

The hypothesis envisaged that in plant photosynthesis H₂A is water, whereas in green sulfur bacteria (for example) H₂A is H₂S, with the results that oxygen becomes the by-product of plant photosynthesis and elemental sulfur the by-product of bacterial photosynthesis.

In later formulations (9, 10) van Niel no longer considered the photodecomposition of water as being unique to plant photosynthesis but postulated that “the photochemical reaction in the photosynthetic process of green bacteria, purple bacteria, and green plants represents, in all cases, a photodecomposition of water” (10). According to this concept, the distinction between plant and bacterial photosynthesis turned on the events that followed the photodecomposition of water into H and OH. H was used for CO₂ reduction and OH formed a complex with an appropriate acceptor. In plant photosynthesis, the acceptor was regenerated when the complex was decomposed by liberating molecular oxygen. In bacterial photosynthesis, oxygen was not liberated and the acceptor could be regenerated only when the OH-acceptor complex was reduced by the special hydrogen donor, H₂A, that is always required in bacterial photosynthesis.

The concept of photodecomposition of CO₂ or photodecomposition of water provided, each in its own historical period, a broad, general perspective on the role of light in the overall events of photosynthesis. In the last two decades, however, the focus in photosynthesis research shifted toward the isolation, identification, and characterization of the specific reactions and mechanisms by which light energy drives the photosynthetic process. This approach has led to new perspectives on the mechanisms by which light energy is
used in photosynthesis and to a finding, inadmissible under either of the two earlier hypotheses, that the photosynthetic apparatus can convert light energy into a stable form of chemical energy, independently of the splitting of either water or CO₂.

First products of photosynthesis: experiments with whole cells

In chemical terms, an insight into the role of light in photosynthesis could come from identification of the first chemically defined products that are formed under the influence of light. In the 19th century (Fig. 1) this approach established that starch is the first product of photosynthesis in chloroplasts (11)—a conclusion that was later revised in favor of soluble carbohydrates (ref. 12). In the modern period, the powerful new techniques of ¹⁴C (ref. 13), paper chromatography (14), and radioautography (15) aided in the identification of phosphoglyceric acid (PGA) as the first stable product of photosynthesis, formed only after a few seconds of illumination (16). Aside from PGA, phosphate esters of two sugars, ribulose and sedoheptulose, were soon added to the list of early products of photosynthesis in green cells (17, 18).

The discovery of PGA and other early intermediates of CO₂ assimilation led Calvin and his associates (19, 20) to the formulation of a photosynthetic carbon cycle (reductive pentose phosphate cycle), which was convincingly identified with the dark phase of photosynthesis. The chief importance of the carbon cycle to the understanding of the role of light in photosynthesis lay in revealing which of its component enzymic reactions require an input of energy-rich chemical intermediates that must be formed by the light reactions. As summarized in Fig. 2, the carbon cycle shows that the conversion of 1 mole of CO₂ to the level of hexose phosphate requires 3 moles of ATP and 2 moles of reduced NADP. Accordingly, the need for light energy in photosynthesis by green plants could now be traced to those photochemical reactions that generate ATP and NADPH₂.

The occurrence of phosphorylated compounds among the early products of CO₂ assimilation suggested that light-induced phosphorus assimilation may, in fact, precede carbon assimilation but experimental evidence for this conclusion was lacking in whole cells. When Calvin’s group investigated the photoassimilation of phosphorus by Scenedesmus cells with the aid of carrier-free KH₂¹⁴PO₄, they found that the shortest exposure to light gave the lowest incorporation of ¹⁴P into ATP and, conversely, that the highest incorporation of ³²P into ATP occurred on short, dark exposure (21). The first compound to be labeled proved to be not the expected ATP but again PGA (21). Other investigations of direct photoassimilation of phosphorus by intact cells also gave results that were at best suggestive [see review (22)]. In short, experiments with whole cells proved, for reasons discussed below, incapable of yielding evidence for an independent light-induced phosphorus metabolism. Its occurrence in photosynthesis was discovered not in whole cells but in isolated chloroplasts.

First products of photosynthesis: experiments with isolated chloroplasts

Chloroplasts were once widely believed to be the site of complete photosynthesis but this view was not supported by critical evidence (23, 24) and was largely abandoned after Hill (25, 26) demonstrated that isolated chloroplasts could evolve oxygen but could not assimilate CO₂. [The failure of isolated chloroplasts to fix CO₂ was also reported with the sensitive ¹⁴CO₂ technique (27).] In the oxygen-producing reaction, which became known as the Hill reaction, isolated chloroplasts evolved oxygen only in the presence of artificial oxidants with distinctly positive oxidation-reduction potentials, e.g., ferric oxalate, ferricyanide, benzoquinone.

The Hill reaction established that the photoproduction of oxygen by chloroplasts is basically independent of CO₂ assimilation. This provided strong support for the view that the source of photosynthetic oxygen is water. *Left in doubt was the role of chloroplasts in the energy-storing reactions needed for CO₂ assimilation. The photochemical generation by isolated chloroplasts of a strong reductant capable of reducing CO₂ was deemed unlikely on experimental and theoretical grounds (26, 28). The first experiments with the sensitive ³²P technique to test the ability of isolated chloroplasts to form ATP, on illumination, also gave negative results (29).

A different perspective on the photosynthetic capacity of isolated chloroplasts began to emerge in 1951 when three laboratories (30–32), independently and simultaneously, found that isolated chloroplasts could photoreduce NADP despite its strongly electronegative redox potential (Eₘ = −320 mV, at pH 7). This finding was followed by several other developments which drastically altered the then prevalent ideas about the photosynthetic capacity of isolated chloroplasts. These developments (Fig. 1) will now be discussed in chronological order.

In 1954, a reinvestigation of photosynthesis in isolated chloroplasts by different methods yielded evidence for a light-dependent assimilation of CO₂ (ref. 33). Chloroplasts isolated from spinach leaves assimilated ¹⁴CO₂ to the level of carbohydrates, including starch, with a simultaneous evolution of oxygen (34, 35). When the conversion of ¹⁴CO₂ by isolated chloroplasts to sugars and starch was confirmed and extended in other laboratories (36–40), the capacity of chloroplasts to carry on complete extracellular photosynthesis was no longer open to question.

Because of the earlier negative results, special experimental safeguards were deemed necessary to establish that chloroplasts alone, without other organelles or enzyme systems and with light as the only energy source, were capable of a total synthesis of carbohydrates from CO₂. The chloroplasts were washed and, to eliminate a possible source of chemical energy and metabolites, their isolation was performed not as formerly

* Contrary to a widely held belief, this conclusion was not unequivocally documented by experiments with ¹⁸O ([see A. H. Brown and A. W. Frenkel, Annu. Rev. Plant Physiol., 4, 53 (1953)].
in isotonic sugar solutions (25) but in isotonic sodium chloride (33, 35). In comparison with the parent leaves, washed saline chloroplasts gave low rates of CO₂ assimilation (35)—a situation similar to the first reconstruction of other cellular processes in vitro, e.g., fermentation (41, 42), protein synthesis (43), and polymerization of DNA (44). Crucial for the documentation of complete photosynthesis in isolated chloroplasts were not high rates but the fact that their newly found CO₂ assimilation was reproducible and yielded the same intermediate and final products as photosynthesis by intact cells. More recently, when CO₂ assimilation ceased to be a matter of dispute and the same experimental safeguards were no longer needed, much higher rates of CO₂ assimilation by isolated chloroplasts were obtained with modified procedures (45-48).

Since neither ATP nor reduced NADP was added to isolated chloroplasts that fixed CO₂, it was clear that these energy-rich compounds were being photochemically generated from their respective precursors within the chloroplasts. Chloroplasts were already known to photoreduce added NADP but nothing was known about their ability (or that of any other photosynthetic structures) to form ATP at the expense of light energy. A renewed attack on this problem in isolated chloroplasts was therefore undertaken.

**Discovery of photosynthetic phosphorylation**

The likelihood of detecting a direct role of light in ATP formation was much greater in isolated chloroplasts than in intact cells. Intact cells contain only catalytic amounts of the precursor adenosine phosphates (AMP, ADP) and these, because of permeability barriers, could not be increased by external additions. By contrast, in experiments with isolated chloroplasts, it was possible to supply these normally catalytic substances in substrate amounts and, with the aid of labeled inorganic phosphate, determine chemically their light-induced conversion to ATP.

In 1954, work with the same spinach chloroplast preparations that fixed CO₂ led to the discovery that they were also able to convert light energy into chemical energy and trap it in the pyrophosphate bonds of ATP (49, 33). Several unique features distinguished this photosynthetic phosphorylation (photophosphorylation), as the process was named, from substrate-level phosphorylation in fermentation and oxidative phosphorylation in respiration: (a) ATP formation occurred in the chlorophyll-containing lamellae and was independent of other enzyme systems or organelles (including mitochondria, which were previously considered necessary for photosynthetic ATP formation, ref. 51); (b) no energy-rich substrate, other than absorbed photons, served as a source of energy; (c) no oxygen was produced or consumed; (d) ATP formation was not accompanied by a measurable electron transport involving any external electron donor or acceptor (49, 33, 50). The light-induced ATP formation could be expressed by the equation:

\[ \text{hv} \cdot \text{ADP} + \text{n} \cdot \text{P}_\text{i} \rightarrow \text{n} \cdot \text{ATP} \] (iii)

When photophosphorylation in chloroplasts was followed by evidence of a similar phenomenon in cell-free preparations of such diverse types of photosynthetic organisms as photosynthetic bacteria (52) and algae (53, 54), it became evident that photophosphorylation is not peculiar to plants containing chloroplasts but is a major ATP-forming process in nature that supplies ATP for the biosynthetic reactions in all types of photosynthesis.

Soon after the demonstration of photosynthetic phosphorylation in isolated chloroplasts, attempts were made to evaluate its physiological significance. Since, as with other cellular processes when first reproduced in vitro, the rates of photosynthetic phosphorylation were low, there was little inclination at first to accord this process quantitative importance as a photosynthetic mechanism for converting light into chemical energy (55). With further improvement in experimental methods (which included the use of broken chloroplasts with lowered permeability barriers), rates of photosynthetic phosphorylation increased 170 times (56) and more (57) over those originally described (33).

The improved rates of photosynthetic phosphorylation were equal to, or greater than, the maximum known rates of carbon assimilation in intact leaves. It appeared, therefore, that isolated chloroplasts retain, without substantial loss, the enzymic apparatus for photosynthetic phosphorylation—a conclusion in harmony with evidence that the phosphorylating system was tightly bound in the water-insoluble lamellar portion of the chloroplasts.

**Role of light in photophosphorylation**

Once the main features of photophosphorylation were firmly established, the next objective was to explain its mechanism, particularly its absolute dependence on illumination (33, 50). On the one hand, photophosphorylation was independent of such classical manifestations of photosynthesis as oxygen evolution and CO₂ assimilation; on the other hand, it seemed unlikely that light was involved in the formation of ATP itself, a reaction universally occurring in all cells independently of photosynthesis. Light energy, therefore, had to be used in photophosphorylation before ATP synthesis and in a manner unrelated to CO₂ assimilation or oxygen evolution. The most probable mechanism for such a role seemed to be a light-induced electron flow (58, 59).

It is often difficult for the student of photosynthesis today to realize that before the discovery of photophosphorylation the concept of a light-induced electron transport had no substantial basis in photosynthesis research. The idea that photon energy is used in photosynthesis to transfer electrons rather than cumbersome atoms had a few proponents at various times, for example Katz (60) and Levitt (61) but, as the literature before the late 1950s shows, it did not become a viable concept in photosynthesis—it was merely one of several specu-
relative ideas based on model systems. The situation changed with light-induced ATP formation. ATP is formed in nonphotosynthetic cells at the expense of energy released by electron transport. The idea that ATP may also be formed in photosynthesis through a special light-induced electron flow mechanism in chloroplasts now had a high probability that could be experimentally tested.

The electron flow hypothesis (58, 59) envisaged that a chlorophyll molecule, on absorbing a quantum of light, becomes excited and promotes an electron to an outer orbital with a higher energy level. This high-energy electron is then transferred to an adjacent electron acceptor molecule, a catalyst (A) with a strongly electronegative oxidation–reduction potential. The transfer of an electron from excited chlorophyll to this first acceptor is the energy conversion step proper and terminates the photochemical phase of the process. By transforming a flow of photons into a flow of electrons, it constitutes a mechanism for generating a strongly electronegative reductant at the expense of the excitation energy of chlorophyll.

Once the strongly electronegative reductant is formed, no further input of energy is needed. Subsequent electron transfers within the chloroplast liberate energy, since they constitute an electron flow from the electron acceptor to electron acceptors (thought to include chloroplast cytochromes) with more electropositive redox potentials (58, 59). Several of the exergonic electron transfer steps, particularly those involving cytochromes, were thought to be coupled with phosphorylation. At the end of one cycle, the electron originally emitted by the excited chlorophyll molecule returns to the electron-deficient chlorophyll molecule and the quantum absorption process is repeated. A mechanism of this kind would account for the observed lack of any oxidation–reduction change in any external electron donor or acceptor. Because of the envisaged cyclic pathway traversed by the emitted electron, the process was named cyclic photophosphorylation (58, 59).

A cyclic electron flow that is driven by light and that liberates chemical energy, used for the synthesis of the pyrophosphate bond of ATP, is unique to photosynthetic cells. The idea has been discussed elsewhere (58, 59) that cyclic photophosphorylation may be a primitive manifestation of photosynthetic activity—an activity that is the common denominator of plant and bacterial photosynthesis.

Noncyclic photophosphorylation

As already alluded to, there was at first no experimental evidence linking photophosphorylation to the photo-reduction of NADP by chloroplasts. In fact, these two photochemical activities of chloroplasts appeared to be antagonistic (62). It was therefore wholly unexpected when a second type of photophosphorylation was discovered in 1957 (ref. 63) that provided direct experimental evidence for a coupling between photoreduction of NADP and the synthesis of ATP. Here, in contrast to cyclic photophosphorylation, ATP formation was stoichiometrically coupled with a light-driven transfer of electrons from water to NADP (or to a nonphysiological electron acceptor such as ferricyanide) and a concomitant evolution of oxygen. Moreover, ATP formation in this coupled system greatly increased the rate of electron transfer from water to ferricyanide (63–65) or to NADP (66) and the rate of the concomitant oxygen evolution. It thus became apparent that the electron transport system of chloroplasts functions more effectively when it is coupled, as it would be under physiological conditions, to the synthesis of ATP. The conventional Hill reaction (25, 26) now appeared to measure a non-coupled electron transport, severed from its normally coupled phosphorylation (63).

In extending the electron flow concept to this new reaction, it was envisaged (58, 59) that a chlorophyll molecule excited by a captured photon transfers an electron to NADP (or to ferricyanide). It was postulated that electrons thus removed from chlorophyll are replaced by electrons from water (OH−, at pH 7) with a resultant evolution of oxygen. In this manner, light would induce an electron flow from OH− to NADP and a coupled phosphorylation. Because of the unidirectional or noncyclic nature of this electron flow, this process was named noncyclic photophosphorylation (58, 59).

Role of ferredoxin

Further progress in elucidating the role of light in chloroplast reactions came from investigations of the mechanism of NADP reduction and of the identity of the catalyst in cyclic photophosphorylation by chloroplasts.

Investigations of the mechanism of NADP reduction and cyclic photophosphorylation in chloroplasts led to the recognition of the key role of the iron–sulfur protein, ferredoxin. The name ferredoxin was introduced in 1962 by Mortenson et al. (67) and did not, at first, concern photosynthesis. It referred to a nonheme, iron-containing protein which they isolated from Clostridium pasteurianum, an anaerobic bacterium devoid of chlorophyll and normally living in the soil at a depth to which sunlight does not penetrate. A connection between ferredoxin and photosynthesis was established, also in 1962, when C. pasteurianum ferredoxin was crystallized and found to mediate the photo-reduction of NADP by spinach chloroplasts (68). In this reaction, Clostridium ferredoxin replaced a native chloroplast protein (known by different names) that up to then was thought to be peculiar to photosynthetic cells. Its replaceability by the bacterial ferredoxin and other considerations led to renaming the chloroplast protein ferredoxin (68). A discussion of the history of ferredoxin, its occurrence and properties, is given elsewhere (69, 70).

Ferredoxin is now known to play a key role in photosynthesis—a role that includes (but is not limited to) a catalytic function in both cyclic and noncyclic photophosphorylation. Ferredoxin is an electron carrier protein whose reversible oxidation–reduction is accompanied by characteristic changes in its absorption spectrum (Fig. 3). From the standpoint of electron transport, the most notable finding (68) was the strongly electronegative oxidation–reduction potential of ferredoxin, close to that of hydrogen gas (Em = −420 mV, at pH 7) and about 100 mV more electronegative than that of NADP. Thus, ferredoxin (in its reduced state) emerged as the strongest chemically defined reductant that is photochemically generated by, and is isolable from, chloroplasts.

The existence of stronger reductants in chloroplasts has been suggested on the basis of observations (71–73) that
chloroplasts photoreduce nonphysiological dyes, some of which have polarographically measured oxidation–reduction potentials that are more negative than that of ferredoxin. However, without evidence that such reductants exist in chloroplasts, these suggestions still remain speculative. Recent reports (74, 75) of the isolation of a “ferredoxin reducing substance (FRS)” from chloroplasts have not been confirmed (76).

The mechanism of NADP reduction by chloroplasts was resolved (77) into (a) a photochemical reduction of ferredoxin followed by two “dark” steps, (b) reoxidation of ferredoxin by ferredoxin-NADP reductase, a chloroplast flavoprotein enzyme, isolated in crystalline form (78), and (c) reoxidation of the reduced ferredoxin-NADP reductase by NADP. Thus, what was formerly called photoreduction of NADP turned out to be a photoreduction of ferredoxin, followed by electron transfer to the flavin component of ferredoxin-NADP reductase and thence by hydrogen transfer to NADP (two reducing equivalents are transferred to NADP⁺ in the form of a hydride ion, H⁻).

\[
\text{Illuminated chloroplasts} \rightarrow \text{ferredoxin} \rightarrow \text{ferredoxin-NADP reductase} \rightleftharpoons \text{NADP}
\]

The role assigned to ferredoxin as the terminal electron acceptor in the photochemical events that lead to NADP reduction was further documented when the photoreduction of substrate amounts of ferredoxin was found to be accompanied by stoichiometric oxygen evolution and ATP formation (79). Earlier formulations of noncyclic photosphorylation (63, 64) were now further refined to show that the omission of NADP did not affect ATP formation. The true equation for noncyclic photosphorylation became:

\[
4 \text{Ferredoxin}_{\text{ox}} + 2\text{ADP} + 2\text{P}_1 + 2\text{H}_2\text{O} \rightarrow 4 \text{Ferredoxin}_{\text{red}} + 2\text{ATP} + \text{O}_2 + 4\text{H}^+ \quad (\text{a})
\]

Turning to cyclic photosphorylation in chloroplasts, an unsolved question was its puzzling dependence on an added catalyst, e.g., menadione (80) or phenazine methosulfate (81), a substance that is foreign to living cells. No such additions were required for cyclic photosphorylation in freshly prepared bacterial chromatophores (82, 83). Moreover, unlike bacterial cyclic photosphorylation, the process in chloroplasts was not sensitive to such characteristic inhibitors of phosphorylation as antimycin A, at low concentrations (84).

A possible explanation of this dependence was that chloroplasts lose a soluble constituent like ferredoxin in the process of chloroplast isolation. Evidence was indeed obtained later for cyclic photosphorylation that is dependent only on catalytic amounts of ferredoxin and proceeds without the addition of any other catalyst of photosphorylation (85, 86). Moreover, when catalyzed by ferredoxin, cyclic photosphorylation in chloroplasts became for the first time sensitive to inhibition by low concentrations of antimycin A and oligomycin (69), and resembled in this respect cyclic photosphorylation in bacteria (84).

Sensitivity to antimycin A and to other inhibitors provided also a sharp distinction between ferredoxin-catalyzed cyclic and noncyclic photosphorylations. Low concentrations of antimycin A, oligomycin, and other inhibitors which sharply inhibited cyclic photosphorylation had no effect on noncyclic photosphorylation (87, 69). By contrast, low concentrations of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) and α-phenanthroline, which sharply inhibited noncyclic photosphorylation, actually stimulated cyclic photosphorylation (87).

There are now several other lines of evidence that point to ferredoxin as the endogenous catalyst of cyclic photosphorylation in chloroplasts: (a) As expected of a true catalyst, ferredoxin stimulates cyclic photosphorylation at low concentrations (1 × 10⁻⁴ M), comparable on a molar basis to those of the other known catalysts of the process; (b) when light intensity is restricted, ferredoxin catalyzes ATP formation more effectively than any other catalyst; (c) in adequate light, cyclic photosphorylation by ferredoxin produces ATP at a rate comparable with the maximum rates of photosynthesis in vivo (87).

Ferredoxin emerged, therefore, as the physiological catalyst of cyclic and noncyclic photosphorylation of chloroplasts. Other substances that catalyze cyclic or noncyclic photosphorylation in isolated chloroplasts appear to act as substitutes for ferredoxins. It is noteworthy that recent evidence (Shanmugam and Arnon, Biochim. Biophys. Acta, in press) suggests that cyclic photosphorylation in bacterial chromatophores may also be catalyzed by a ferredoxin of a kind that is membrane-bound.

To recapitulate, cyclic and noncyclic photosphorylation account for the basic feature of photosynthesis, i.e., conversion of light energy into chemical energy. Noncyclic photosphorylation generates part of the needed ATP and all of the reductant in the form of reduced ferredoxin, which in turn serves as the electron donor for the reduction of NADP by an enzymic reaction that is independent of light. Cyclic photosphorylation provides the remainder of the required ATP (literature reviewed in ref. 88). Jointly, cyclic and noncyclic photosphorylation generate all of the assimilatory power, made up of ATP and reduced NADP, that is required for CO₂ assimilation.

Two photosystems in plant photosynthesis

Parallel and, in the main, unrelated to investigations of cyclic and noncyclic photosphorylation in isolated chloroplasts were investigations, chiefly at the cellular level, on the effects
of monochromatic light on plant photosynthesis. This work has led to wide agreement (see reviews, 89, 90) that photosynthesis in green plants includes two photosystems, one involving light reactions that proceed best in "short" wavelength (λ < 700 nm) light (System II) and another—known as System I—that proceeds best in "long" wavelength (λ > 700 nm) light. It became important, therefore, to determine the relation of cyclic and noncyclic photophosphorylation to these two photosystems.

Soon after ferredoxin-catalyzed cyclic photophosphorylation was discovered, it was found to proceed most effectively in long-wavelength light associated with System I. By contrast, noncyclic photophosphorylation (and oxygen evolution) was found to proceed best in short-wavelength light and to come to an almost complete halt at wavelengths above 700 nm (87). Expressed on the basis of equal absorption by chloroplasts of light at each wavelength, the sharp decline or "red drop" of noncyclic photophosphorylation in far-red light (714 nm) was in marked contrast to the sharp increase or "red rise" of cyclic photophosphorylation at the longer wavelengths (Fig. 4). Thus, on the basis of their response to monochromatic light, noncyclic photophosphorylation was identified with System II and cyclic photophosphorylation with System I.

Fig. 4 also shows that the wavelength dependence of the phosphorylation associated with electron flow from an artificial electron donor (reduced 2,6-dichlorophenol indophenol) to NADP (91) resembles the cyclic system. This similarity suggests that electron transport involved in the photoreduction of NADP by DPIP4 proceeds via a portion of System I and is not involved in the photoreduction of NADP by water via System II (92, 93). In this view (further elaborated below), ferredoxin-NADP could be reduced either by water via System II or by an artificial electron donor via System I. System I identified with cyclic, and System II identified with noncyclic, electron transport could thus be regarded as parallel processes in chloroplasts, each basically capable of proceeding independently of the other (92, 93).

The idea that Systems I and II operate in parallel runs counter to a still widely held concept (90, 94)—one that our own laboratory embraced in 1961 (ref. 95) and abandoned in 1965 (ref. 92)—that the two photosystems must operate in series and, through such collaboration, bring about the light-induced reduction of ferredoxin-NADP by water. A detailed discussion of the relative merits of each concept is not possible within the space allotted to this survey. The aim here will be to cover in broad outline some recent findings pertaining to electron transport in chloroplasts and the interpretation our laboratory placed on them. It may be pertinent to note that different laboratories are already in considerable agreement about some of the new facts even when they differ about interpretations.

### Three light reactions

Until recently there was general agreement that System II included only one short-wavelength light reaction. But recent experiments in our laboratory on electron transport in isolated chloroplasts have yielded evidence (96–104) for two short-wavelength photoreactions (Ia and Ib) in the noncyclic electron transport from water to ferredoxin-NADP. These two photoreactions of System II are linked in series; together with the parallel single photoreaction of System I they form, in our view, the three light reactions of plant photosynthesis (Fig. 5).

According to this concept, in System II (Fig. 5, left) photoreaction Ib oxidizes water and reduces Component 550 (C550), while photoreaction Ia oxidizes plastocyanin (PC) and reduces ferredoxin. These two light reactions are joined by a "dark" electron transfer chain which includes (but is not limited to) cytochrome b56 and is coupled to the noncyclic phosphorylation site.

Component 550 is a newly discovered photoreactive chloroplast component, distinct from cytochromes, which undergoes photoreduction by electrons from water (or a substitute electron donor) only when chloroplasts are illuminated by System II (short-wavelength) light (96–98). The photoreduction of Component 550 is measured by a decrease in absorbance at 550 nm (546 nm at 77°C) but its chemical nature is otherwise still unknown. The existence of Component 550 has now been confirmed by Erixon and Butler (105, 106), Boardman et al. (107), and Bendall and Sofrova (108). Erixon and Butler (106) have also added the significant observation that Component 550 acts as the previously postulated quencher (Q) of fluorescence of System II.

Plastocyanin is a copper-containing protein in chloroplasts discovered by Katoh (109) and implicated in noncyclic electron transport from water to NADP (110–113). Cytochrome b56 is one of the three cytochromes native to chloroplasts, the other two being cytochromes f and b. Cytochrome b56 has been associated with chloroplast fragments enriched in System II (refs. 114–116). Some investigators (117–119) have proposed that it serves as an electron donor to cytochrome f in an electron transport chain that joins Systems II and I. In this formulation, plastocyanin is a requirement for the photooxidation by System I of both cytochromes b56 and f:

\[
H_2O \rightarrow h_{P(II)} \rightarrow Q \rightarrow \text{cyt. } b_{56} \rightarrow \text{c yt. } f \rightarrow \text{PC} \\
\rightarrow h_{P(II)} \rightarrow \text{Fd} \rightarrow \text{NADP}
\]

Our recent experiments show that removal of plastocyanin from chloroplasts abolished their capacity to photoxidize cytochrome b56 but not that of cytochrome f. The addition of plastocyanin restored the photooxidation of cytochrome...
ferredoxin

in electron acceptors: The new photoreduction according impossible according renewal System by demonstration activities and experiments to participate absorbed by P700 chlorophyll a II.

older hypothesis, ferredoxin and cytochrome components of cyclic electron carriers of cytochrome f, by itself by (short-wavelength) light, 550, no effect b559 (ref. 100). Discussed elsewhere (93) are the roles of Cl−, manganese, and plastoquinone (PQ) (Knaff and Arnon, ref. 96).

Cytochromes b6 and f have previously been assigned to cyclic photophosphorylation (92, 93) and are now also considered components of System I. The present concept places cytochrome f and P700 in System I and, in contrast to the older hypothesis, predicts that neither would be required for the photoreduction of ferredoxin-NADP by water via System II. [P700 represents a small component part of the total chlorophyll a and has in situ an absorption peak at 700 nm; P700 is considered to act as the terminal trap for the light energy absorbed by the “bulk” chlorophyll of System I and to participate directly in photochemical reactions (120).] Experimental verification of this prediction was sought from experiments with chloroplast fragments (101, 104). The concept of three photoreactions arranged in two parallel photosystems was greatly strengthened when chloroplasts were subdivided into separate fragments with properties and activities corresponding to those ascribed here to System II and System I, respectively (101, 104). An especially pertinent demonstration was the photoreduction of ferredoxin-NADP by water by the use of chloroplast fragments devoid of System I activity and free of functional P700 and cytochrome f. The isolation of such chloroplast fragments of System II is conceivable according to the new hypothesis but theoretically impossible according to the old hypothesis.

Photoreduction of primary electron acceptors at 77°K

The new scheme for electron transport envisages two primary electron acceptors: Component 550 in photoreaction II b and ferredoxin in photoreaction II a and in the single photoreaction of System I. A primary electron acceptor would be expected to undergo reduction by an electron transferred solely as a result of photon capture at temperatures low enough to inhibit chemical reactions. This expectation was fulfilled for photoreaction II b when the photoreduction of Component 550 was indeed found to occur at liquid-nitrogen temperature (Fig. 6).

Evidence for the photoreduction of ferredoxin at 77°K was sought by electron paramagnetic resonance spectroscopy—a technique that, unlike absorption spectrophotometry, would permit detection of ferredoxin reduction without the interference of chlorophyll or chloroplast cytochromes. When whole spinach chloroplasts were illuminated at 77°K from 10 to 50

![Diagram](image.png)

**Fig. 5.** Scheme for three light reactions in plant photosynthesis. Explanation in text; fp stands for ferredoxin-NADP reductase. Discussed elsewhere (93) are the roles of Cl−, manganese, and plastoquinone (PQ) (Knaff and Arnon, ref. 96).

![Diagram](image.png)

**Fig. 6.** Light-induced absorbance changes of Component 550 in the region 540–560 nm at −189°C (5 mM FeCy; reference wavelength, 538 nm, Knaff and Arnon, ref. 98).
Few questions about dyeing as NADP reduce the versy and of interaction as depicted to ferredoxin-NADP were similar. Osmotically disrupting photoactive, primary photosynthesis in plants was evidenced by the inability (without added ferredoxin) to reduce NADP photochemically with either water or reduced dye as the hydrogen donor.

Quantum efficiency of photosynthesis
Few questions in photosynthesis have received more intensive theoretical and experimental study and led to more controversy than the efficiency of the quantum conversion process. The extensive literature on this subject, which is beyond the scope of this article, concerns quantum efficiency of complete photosynthesis in whole cells, as measured by oxygen evolution or CO₂ fixation. A different approach to the bioenergetics of photosynthesis emerged from the electron flow theory that was invoked to explain cyclic and noncyclic photophosphorylation. Transfer of one electron from excited chlorophyll to a primary electron acceptor, Component 550 or ferredoxin, is depicted as a one-quantum photochemical act. Since two photoacts are involved in transfer of an electron from water to ferredoxin-NADP (Fig. 5), the theoretical quantum requirement for System II becomes two quanta per electron or eight quanta per molecule of O₂ (Eq. 9). Likewise, with only one photoact in System I, its light-induced electron transport would have a theoretical quantum requirement of one quantum per electron.

Fig. 8 shows experimental measurements close to two quanta per electron for System II and one quantum per electron for System I. The quantum requirement measurements in Fig. 8 demonstrate again the contrasting responses of Systems II and I to monochromatic light: increasing quantum efficiency for System II and decreasing quantum efficiency for System I at the shorter wavelengths and a reverse situation at the longer wavelengths. Similar results, despite many variations in conditions and experimental design, have been obtained by other investigators (123–129).

Since the assimilation of one molecule of CO₂ to the level of hexose via the reductive pentose phosphate cycle requires two molecules of NADPH₂ and three molecules of ATP (Fig. 2), it becomes possible to arrive at the maximum theoretical quantum efficiency of photosynthesis from measurements of the respective quantum efficiencies for cyclic and noncyclic electron transport. A requirement of two quanta per electron for System I means that eight quanta would be required to produce two NADPH₂ and two ATP (accompanied by evolution of one O₂) via noncyclic photophosphorylation. Two additional quanta assigned to generate one ATP via cyclic photophosphorylation would give a total requirement of about ten quanta per molecule of CO₂ assimilated or O₂ liberated—a value in good agreement with measurements of the overall quantum efficiency of photosynthesis in intact cells.

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Light Reactions of Photosynthesis

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