CHAPTER 1

Dissecting Oxygenic Photosynthesis: The Evolution of the "Z"-Scheme for Thylakoid Reactions

Govindjee[1] and Lars Olof Björn[2, 3]

[1]University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

E-mail: gov@illinois.edu

[2]School of Life Science, The Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, South China Normal University, Guangzhou 510631, China

[3]Lund University, Department of Biology, Sölvegatan 35, SE-22362 Lund, Sweden

ABSTRACT

From a primitive beginning we have come to our present understanding of what is going on in the thylakoid membranes. We start our overview with the determination of the size of the photosynthetic unit. This unit gave the concept of light absorbing antenna and the reaction centers, where the primary photochemistry takes place. We continue with the determination of the maximum quantum yield and the realization that two light reactions and two photosystems are involved in oxygenic photosynthesis. This is then followed by the discovery of the "period four oxygen clock" and the structure of the oxygen-evolving center. After a brief mention of the various protein components in the thylakoid membrane and a comparison of the two photosystem complexes, we provide here a summary and the evolution of the so-called "Z" scheme for the electron flow from water to the pyridine nucleotide $NADP^+$, nicotinamide adenine dinucleotide phosphate. We conclude with a picture of the thylakoid membrane domains in the chloroplast, and the intricate machinery where all the reactions from water to $NADP^+$ reduction takes place. Our chapter is not a review, but a viewpoint that we favour.

Keywords: Electron Transfer Pathway; Emerson Enhancement Effect; Oxygen Clock; Photosynthetic Unit; Red Drop in Photosynthesis; Two Light Reactions; Two Photosystems; Z-Scheme of Photosynthesis

INTRODUCTION

Photosynthesis is a very complicated process, and in particular, this holds for the kind of photosynthesis carried out by plants, algae and cyanobacteria, i.e., oxygen-producing (oxygenic) photosynthesis (Rabinowitch and Govindjee 1969; Blankenship 2002). Initially, it was thought that oxygen was released by the photochemical splitting of carbon dioxide:

$$CO_2 + \text{light} \Rightarrow C^* + O_2 \quad \text{(the asterisk on C indicates an activated state)}$$

Then the carbon would combine with water to form carbohydrate:

$$C^* + 6H_2O \Rightarrow C_6H_{12}O_6$$

This is the origin of the term carbohydrate, still in use, despite the fact that this scheme has been proven to be wrong. For the description and historical evaluation of this early and erroneous hypothesis, see Nickelsen (2010). In the following section we shall discuss the 'photosynthetic unit (i.e., the existence of a large number of light-harvesting pigment molecules and a very few reaction center molecules where the primary photochemistry takes place); the minimum quantum requirement, i.e., the minimum number of photons of light needed for the evolution of one molecule of oxygen (its inverse being the maximum quantum yield, i.e., the number of oxygen molecules evolved per photon of light absorbed); and the two-light reaction and two-pigment scheme of photosynthesis. We conclude with a picture of the thylakoid membrane domains in the chloroplast, the intricate machinery where all the reactions from water to NADP reduction take place.

We refer the readers to Govindjee (2000) for an earlier overview of the concepts discussed here; Govindjee and Krogmann (2004) for a timeline of oxygenic photosynthesis; and Orr and Govindjee (2010) for an entry into all literature on photosynthesis at all levels.

PHOTOSYNTHETIC UNITS

Engelmann (1884) was one of the first to quantitate the relationship between absorption of light and photosynthesis.The story of how the complex photosynthetic machinery has been dissected and its components (at least many of them, now well characterized) started with a paper by Blackman (1905), in which he studied the rate of photosynthesis in different combinations of light intensity and carbon dioxide concentration. He drew the conclusion that the photosynthetic process could be divided into a "light reaction" and a "dark reaction", and that the "dark reaction" limits the rate when the light is strong enough. (The "dark reaction", in fact, as it turned out, a series of a great number of reaction steps, had often been referred to, in the past, by many as the "Blackman reaction".)

In two very important papers, Emerson and Arnold (1932a, 1932b) conclusively separated and characterized the photochemical step and the Blackman reaction. They built on earlier observations by Richard Willstätter (Nobel laureate in Chemistry, 1915) and Otto Warburg (Nobel laureate in Physiology or Medicine, 1931) in which they had found that if the light driving photosynthesis was divided into short pulses, the amount of photosynthesis per total time of the experiment was not very much decreased, while the amount of photosynthesis during the time in light was increased. Taking the technical possibilities of the time into account, we marvel at the ingenuity of the apparatus that Emerson and Arnold (1932a) describe (see the historical appreciation by Myers (1994). With their apparatus they could deliver very short and intense repetitive light flashes, and study how photosynthesis varied when intensity, flash duration, and flash interval were varied. Using the unicellular green alga, *Chlorella*, they found that as the flash intensity increased they approached light saturation, which could not be overcome even if the flash interval was increased (so that the average amount of light per unit time was decreased). They drew the conclusion that with strong flashes they would activate all "photosynthetic units", which

would then, during the dark interval, use the collected energy to drive the "Blackman reaction" and complete photosynthesis. By comparing the maximum yield of oxygen per flash with the content of chlorophyll, they could calculate that one photosynthetic unit contains about 2400 chlorophyll molecules (Fig. 1.1).

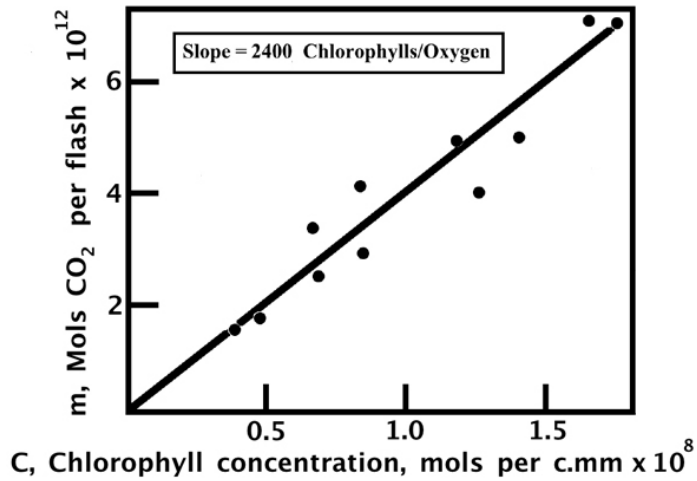


Fig. 1.1 The Photosynthetic Unit in the green alga *Chlorella* (1932). With sufficiently strong and short light flashes, and with appropriate optimal dark periods between repetitive flashes, the amount of oxygen given off (considered equivalent to carbon dioxide uptake) did not depend on the flash intensity, but only on the amount of chlorophyll in algal suspensions illuminated by the flashes. The slope of the line (C/m) in the diagram corresponded to 2400 molecules of chlorophyll per molecule of oxygen evolved. Thus the chlorophyll content of one photosynthetic unit (PSU) was considered to be 2400 per oxygen molecule evolved (or carbon dioxide taken up). This number should be divided by four, since four electrons must be moved to produce one oxygen molecule, so the photosynthetic unit in this experiment may be regarded as containing 600 chlorophyll molecules per electron transferred. However, since we know now that there are two pigment systems and two light reactions, this number would be 300 chlorophylls per electron transferred. Redrawn from Emerson and Arnold (1932b).

Emerson and Arnold (1932b) wrote:

> *"We need only suppose that for every 2480 molecules of chlorophyll there is present in the cell one unit capable of reducing one molecule of carbon dioxide each time it is suitably activated by light."*

It is obvious to us that although Emerson & Arnold had measured oxygen, they talked about CO_2 since they were still using, for description, the early ideas of oxygen coming from activated CO_2. However, it was in this experiment that the concept of what we now call the antenna and the reaction center was born. The unit could very well be what was called the 'photoenzyme', and in today's language, we may equate it to the 'reaction center' with the caveat that the ~2400 chlorophyll molecules be divided by at least 8, the minimum number of photons needed to evolve one oxygen molecule. Soon thereafter, Arnold and Kohn (1934) called this unit "chlorophyll unit". On the other hand, Kohn (1936) suggested that the absorbing unit was ~500 Chls, since he assumed that 4 photons were needed for the evolution of one molecule of oxygen. However, Gaffron and Wohl (1936) really understood these experiments and calculated that in a *Chlorella* suspension that Emerson and Arnold (1932a, 1932b) had used, where oxygen evolution begins immediately upon exposing the samples to low light, it would have taken an average of an hour or more to collect several photons (4-12) on the same chlorophyll molecule necessary for evolving one molecule of oxygen. Gaffron and Wohl (1936) explained this paradox by suggesting that there must be 'funneling'

of quanta absorbed by different molecules on to one common 'center' where the chemistry would begin. We now know that it is not the photons that are being transferred but the "excitation energy", or the excitons (see Clegg *et al.* 2010). We also know that 2400 is not a magic number in all photosynthetic organisms. Schmid and Gaffron (1968) showed that in several algae, cyanobacteria and higher plants, the number of chlorophyll molecules evolved per oxygen, under different physiological conditions, was in multiples of approximately 300: ~300, ~600, ~1200, ~1800, ~2400 and ~4800.

The method of dividing light into flashes in order to characterize light-driven processes introduced by Emerson and Arnold (1932a, 1932 b) has been used by others. For example, Björn (1965) used it to characterize steps in the differentiation of plastids, and Sundqvist (1969) used it to study steps in the formation of chlorophyll. But more importantly, it was used in a more elaborate form, as single flashes, with appropriate dark times, by Joliot *et al.* (1969) and Kok *et al.* (1970) to further characterize the oxygen evolution steps of photosynthesis. We shall return to this later.

QUANTUM YIELDS AND ACTION SPECTRA OF OXYGENIC PHOTOSYNTHESIS

Transformation of two water molecules, plus one molecule of carbon dioxide, to one molecule of oxygen and a one-carbon equivalent of carbohydrate, involves the transfer of 4 electrons (2 electrons per oxygen atom). The Stark-Einstein equivalence law (Stark 1908, Einstein 1912) states that one photon reacts with one molecule, so splitting the carbon dioxide to C* and O_2, according to the old and naive theory mentioned earlier, would need only one photon, provided it had sufficient energy. However, Cornelis B. van Niel (see e.g., van Niel 1941) had, by comparison of non-oxygenic (referred to as 'anoxygenic') bacterial photosynthesis with the oxygenic plant, algal and cyanobacterial photosynthesis, come to the conclusion that both could be summarized with one formula:

$$2\ H_2A + CO_2 + \text{light} \Rightarrow 2A + CH_2O + H_2O$$

where, in the case of anoxygenic photosynthesis, A stands for, e.g., S, and in the case of oxygenic photosynthesis for O (or ½ O_2), implying that the oxygen released comes from water and not from carbon dioxide. This makes it likely that one photon moves one electron, so that 4 photons would be required for the release of one molecule of oxygen and assimilation of one molecule of carbon dioxide.

Otto Warburg and coworkers reported that this, so-called, minimum quantum requirement of photosynthesis (the ratio of minimum number of photons required per oxygen evolved) was 4, or in later publications, under certain conditions even a little less (see e.g., Warburg *et al.* 1950). Warburg wanted to harmonize the thermodynamic requirement of at least 3 red photons with the Stark-Einstein one-to-one relation between photons and molecules. He worked out a theory by which three photons produced three oxygen molecules with the help of extra energy; this extra energy was obtained by reoxidation of assimilate, using up two of the oxygen molecules produced (see e.g., Damaschke *et al.* 1953, Warburg 1958). In contrast to Warburg's minimum values of 3-4 photons per oxygen, many investigators obtained values that were in the range of 8-12 (see e.g., Arnold 1935, 1949; Magee *et al.* 1939). Emerson and Lewis (1941) discovered that one of the reasons for Warburg's low minimum quantum requirement (inversely high maximum quantum yield) was the CO_2 burst in the first minutes of illumination that Warburg had counted as oxygen. Emerson and Chalmers (1955) substantiated this error in Warburg's work in a long and a thorough paper. Emerson and Lewis (1943), using the green alga *Chlorella* as Warburg had used, determined the minimum number of photons to be ~11 per oxygen evolved, and the same result was obtained later for the diatom *Navicula* by Tanada (1951) and the red alga *Porphyridium* by Brody and Emerson (1959). (See Emerson, 1958, for a review.) Emerson died in a plane crash

on February 4, 1959 (see Rabinowitch 1961), and Warburg continued to publish low values of 3-4. Rajni Govindjee *et al.* (1968) showed that the minimum quantum number for oxygen evolution was 8-12 even in young synchronous cultures of *Chlorella* and that too under conditions specified by Warburg to give much lower values. In his final paper, before his death in 1970, Warburg *et al.* (1969) experimentally obtained a minimum value of 12 photons per oxygen evolved, but, by an unusual 'photolyte theory', he calculated the number to be 3-4 (see Govindjee 1999a) for the description and explanation of these results). An overview of many measurements of photosynthetic quantum yields in various plants has been published by Skillman (2008). Further, Kärin Nickelsen and Govindjee (personal communication 2010; in preparation) deal with the history of the quantum yield controversy between Otto Warburg and his doctoral student Robert Emerson. Robert Emerson and his coworkers should have faced the same dilemma, which had misled Warburg: Why would a photon move less than one electron? Stated in other words, why should one need 2 or more photons to move one electron from water to CO_2? A curious observation that Emerson and Lewis (1943) made in *Chlorella* began to unfold the explanation. The quantum yield was not the same for all wavelengths; there was a dip at about 500 nm (clearly due to shielding by yellow pigments that were not active as antenna pigments, as well as due to low efficiency of excitation energy transfer from carotenoids to chlorophyll *a* (see Govindjee 1999b) and a smaller one at 660 nm; but most significantly, the quantum yield dropped abruptly at wavelengths above 682 nm.

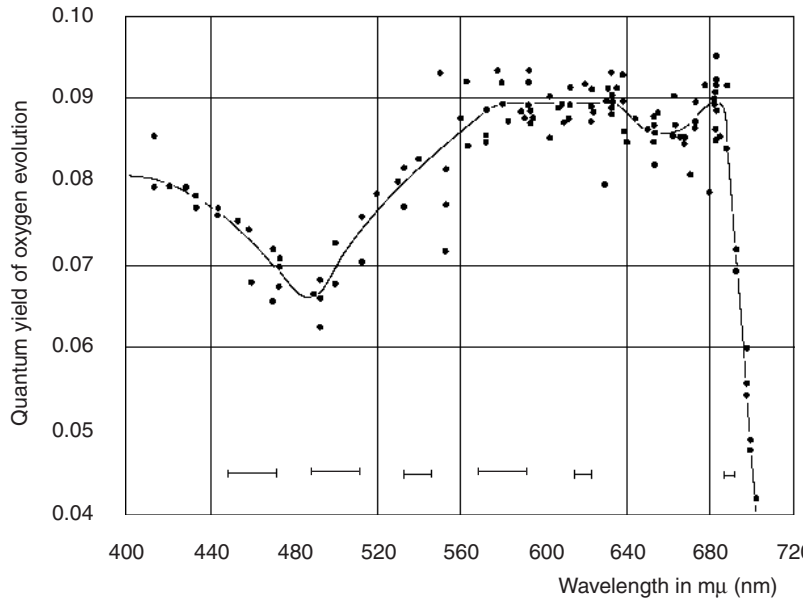


Fig. 1.2 The Red Drop in Photosynthesis in the green alga *Chlorella* (1943). By dividing the number of oxygen molecules formed in weak light by the number of photons absorbed, Emerson and Lewis (1943) determined the maximum quantum yield for the green alga *Chlorella*. It was highest (0.09; corresponding to a minimum of ~ 11 photons per oxygen) in the wavelength range 580 to 680 nm, with a little dip around 660 nm. The lower yield in the violet-green range can be ascribed to carotenoids that do not efficiently transfer absorbed energy to chlorophyll *a*. For wavelengths above ~682 nm, the quantum yield drops steeply, despite the fact that the only substance absorbing this radiation is chlorophyll *a*. This steep decline is called the "Red Drop". It is now known to be due to only Photosystem I absorbing energy; photosynthesis requires another Photosystem (II) as well. Modified from Emerson and Lewis (1943).

In other words, the quantum yield action spectrum declined more with increasing wavelength than the absorption spectrum did. This was the discovery of "Red Drop" in oxygenic photosynthesis (Fig. 1.2). Although chlorophyll *a* still absorbed light beyond 680 nm, the quantum yield of photosynthesis declined (cf. Levring 1947). Emerson and Lewis (1943) discussed many possibilities, but none seemed to make much sense. Perhaps, there existed two forms of chlorophyll *a*: an active (that absorbed up to 680 nm) and an inactive chlorophyll *a* (that absorbed beyond 680 nm)! The concept of active chlorophyll *a* (to later become chlorophyll *a* of Photosystem II, PSII) and inactive chlorophyll *a* (to later become active chlorophyll *a* of Photosystem I, PSI) was clearly described in the doctoral thesis of Duysens (1952) when he observed that absorption in phycobilins, but not in chlorophyll *a*, led efficiently to chlorophyll *a* fluorescence. Duysens (1952) suggested that in red algae, chlorophyll *a* existed in two forms, one was fluorescent (to later become chlorophyll *a* of PSII), and another non-or weakly fluorescent (to later become chlorophyll *a* of PSI).

In the first volume of his monumental work on photosynthesis, Rabinowitch (1945) devised a number of reaction schemes compatible with either a 4 quanta or 8 quanta requirement per CO_2 uptake or O_2 released. One of his 8 quanta reaction schemes was based on some of the ideas of Franck and Herzfeld (1941), but presented in a refined and clear manner in scheme #7.V on p. 162 p.162 in Rabinowitch (1945); see Fig. 1.3 below).

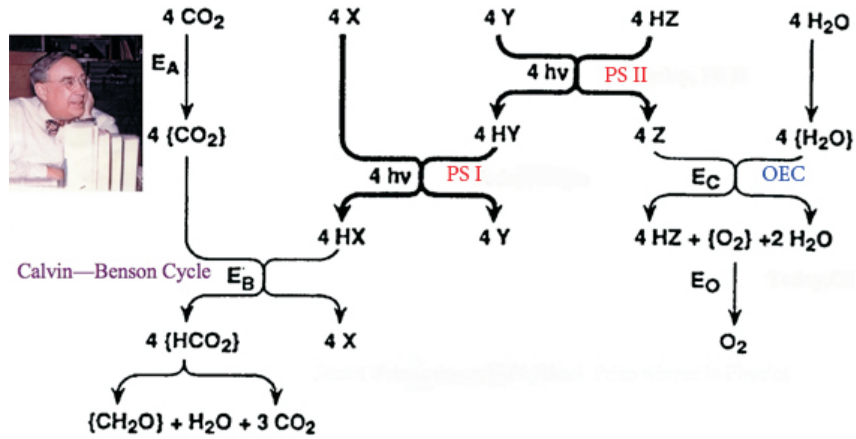


Fig. 1.3 One of the Rabinowitch's Schemes of Photosynthesis (1945) with additions, in color, by the authors. In his 1945 monograph on photosynthesis, Rabinowitch explained the minimum quantum requirement of 8-10 per oxygen evolved by postulating that photosynthesis is driven by two different photochemical redox reactions: 4Y + 4HZ + light ⇒ 4HY + 4Z followed by 4X + 4HY + light ⇒ 4HX + 4Y. In this scheme X, Y, and Z were hypothetical substances; the chemical nature of these intermediates has subsequently been revealed. This scheme was based on the ideas of J. Franck and G. Herzfeld (1941). On the left corner of the diagram, we show a photograph of Eugene Rabinowitch. We show in the diagram the reactions that we now call Calvin-Benson cycle; Photosystem I (PS I), and Photosystem II (PS II). OEC stands for 'Oxygen Evolving Complex'. Modified from Rabinowitch (1945).

In equation form (retaining Rabinowitch's original numbers), it is as follows:

(7.11a) $4\,Y + 4\,HZ + 4\text{ photons} \Rightarrow 4\,HY + 4\,Z$

(7.11d) $4\,Z + 4\,\{H_2O\} + 4\text{ photons} \Rightarrow 4\,HZ + O_2 + 2H_2O$

(7.11b) $4\,HY + 4\,X \Rightarrow 4\,HX + 4\,Y$

(7.11c) $4\,\{CO_2\} + 4\,HX \Rightarrow \{CH_2O\} + 3CO_2 + H_2O + 4\,X$

The first three reactions in this scheme are, in principle, in agreement with the present-day view, if we replace 4 HY with 2 PQH_2 and 4 HX with 2 NADPH. The scheme correctly assumes two different light reactions, of which the first one (7.11a, equivalent to today's PSII) generates a reductant that is used to deliver reduction equivalents (electrons) to the second one (7.11b, equivalent to today's PSI), and also an oxidant powerful enough to oxidize water (7.11d; equivalent to OEC, oxygen evolving complex). The fourth equation (7.11c; equivalent to Calvin-Benson cycle) is too simple since there are many intermediates in between, and more importantly, the essential role of high-energy phosphate was not included in this scheme.

Action spectra of photosynthesis, per incident light intensity, instead of per absorbed light intensity, and using polarography, instead of manometry, were measured by Francis Haxo and Lawrence Blinks. Following a preliminary congress communication (Haxo and Blinks, 1946), Haxo and Blinks (1950) published very detailed action spectra for photosynthesis of the green alga *Ulva taeniata*, the brown algae *Laminaria* sp. and *Coilodesme californica*, and for many species of red algae, including several species of the genus *Porphyra*, and compared them to the absorption spectra of the same organisms. In all cases the action spectra, measured per incident light intensity, dropped at wavelengths shorter than the absorption spectra did. Even more surprising, in the red algae the green region of the spectrum, absorbed mainly by phycoerythrin, was much more effective than the blue and red lights, even at the chlorophyll *a* absorption peaks. Chlorophyll *a*, when directly absorbed, was less active in photosynthesis than when it was excited via the phycobilins, suggesting the presence of inactive and active forms of chlorophyll *a*. Haxo and Blinks (1950) ruled out any artifacts in their results, but concluded their discussion in an air of bewilderment.

After studying all the available experiments on the oxidation-reduction of cytochromes, including that of Louis N.M. Duysens, Rabinowitch (1956; p. 1862, para 2, lines 15-19) made one of the most significant statements on the possibility of two-light reactions in oxygenic photosynthesis before the two-light effect was experimentally discovered:

> "*….two quanta will be needed to transfer each of the four required H atoms (or electrons), first from water to the cytochrome, and then from the cytochrome to the final acceptor*."

What a prophecy, it was! Clearly, it explained the question we posed above "Why should one need 2 or more photons to move one electron from water to CO_2?" The answer is that electrons from water do not go directly to CO_2, and thus, Einstein's law of photochemical equivalency would require a minimum of 8 photons to evolve one molecule of oxygen. In hindsight, if Otto Warburg had thought of this, perhaps, we would not have had the long-drawn controversy between Warburg and the others, especially his own student Robert Emerson.

THE EMERSON ENHANCEMENT EFFECT, AND THE BLINKS'S CHROMATIC TRANSIENT EFFECT; CHLOROPHYLL *a* IN BOTH PHOTOSYSTEMS

Emerson *et al.* (1957) discovered that the low quantum yield of photosynthesis in the long-wavelength light could be improved by adding supplementary light of shorter wavelengths. In other words, the effect of both lights exceeds the sum of the effect of each light separately. This phenomenon was then called the "Second Emerson effect", because the CO_2 burst, mentioned earlier, had already been labelled as the "Emerson Effect". However, today, the enhancement effect, discovered in 1957, is simply known as the Emerson Enhancement Effect. Emerson and Chalmers (1958) and Emerson and Rabinowitch

(1960) reported that the action spectrum of this Emerson Effect follows the percent absorption spectrum of various accessory pigments (e.g., chlorophyll *b* in green algae). Thus, it was assumed that one light reaction was sensitized by chlorophyll *a* and the other by chlorophyll *b*, or other accessory pigments (Fig. 1.4) (Emerson and Chalmers 1958; Myers and French 1960). However, Govindjee and Rabinowitch (1960) and R. Govindjee *et al.* (1960) showed that a short wavelength absorbing form of chlorophyll *a* was present in the same system that used chlorophyll *b* (or other accessory pigments) (Figs. 1.5 and 1.6). This agreed with the earlier observations of Duysens (1952) that all energy absorbed by chlorophyll *b* is transferred to chlorophyll *a*. Further, Govindjee *et al.* (1960) discovered a corresponding effect on chlorophyll *a* fluorescence from *Chlorella* cells: light absorbed in the "red drop" region quenched chlorophyll *a* fluorescence excited by high intensity short wavelength light. This phenomenon was thoroughly explored by Duysens and Sweers (1963) leading to an establishment of the relationship of two light reactions and two pigment systems with chlorophyll fluorescence (Govindjee 2004a). It is important to state that the Emerson Enhancement Effect is not in respiration, but in photosynthesis, since it was observed in the Hill Reaction (e.g., R. Govindjee *et al.* 1960, 1962, 1964; and Govindjee and Bazzaz 1967) and shown by ^{18}O experiments by mass spectroscopy (e.g., Govindjee *et al.* 1963). For further information on Emerson Enhancement Effect, see Govindjee and Govindjee (1965) and a review by Myers (1971).

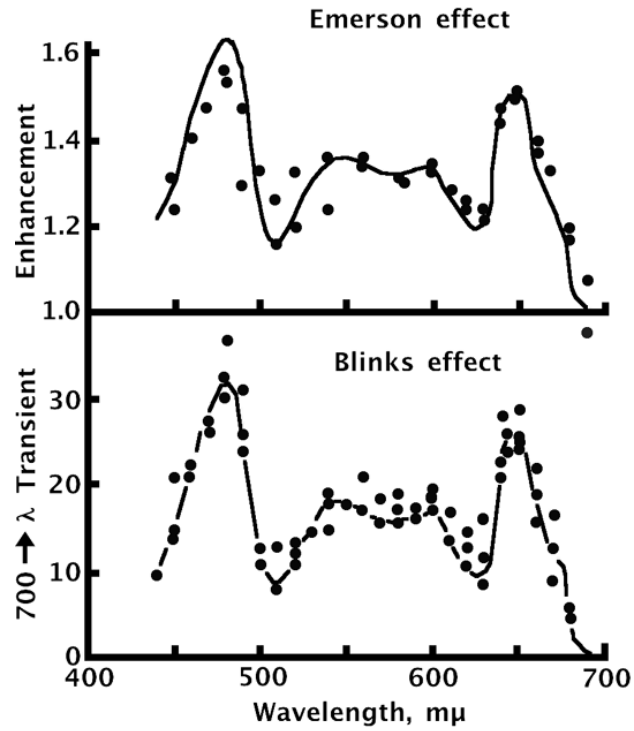


Fig. 1.4 The Emerson Enhancement Effect and the Blinks's Chromatic Transient Effect in Chlorella (1960). (Top): Action spectra of the Enhancement in the rate of photosynthesis (oxygen evolution) by different wavelengths of light added to far-red light (Emerson Effect). (Bottom): Action spectra of the chromatic transient (Blinks effect) in oxygen exchange when 700 nm light was changed to different wavelengths of light (see abscissa). Myers and French suggested that chlorophyll *b* was involved in one of the reactions as Robert Emerson had suggested earlier. Redrawn from Myers and French (1960).

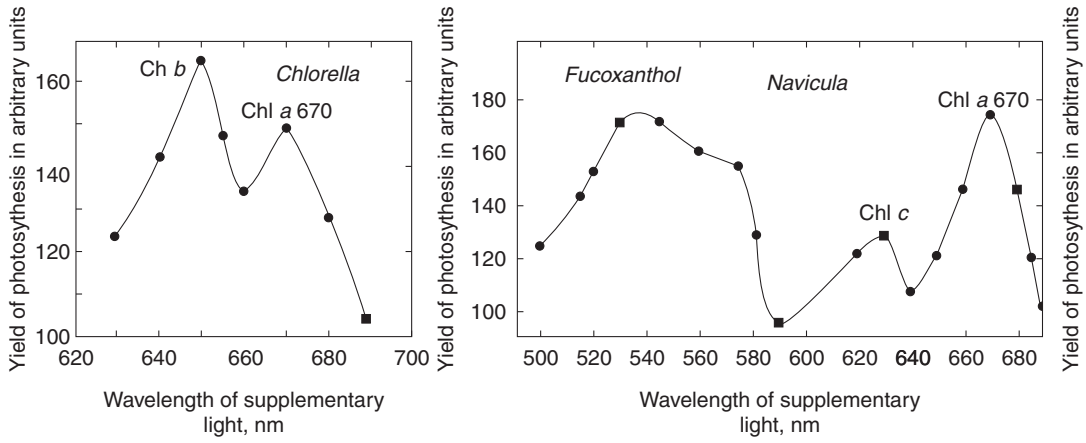


Fig. 1.5 Action Spectra of the Emerson Enhancement Effect in the green alga *Chlorella* (left) and the diatom Navicula (1960). Chl *b* stands for chlorophyll *b* peak around 650 nm; Chl *a* 670 stands for a short wavelength absorbing form of chlorophyll *a;* Chl *c* stands for chlorophyll *c*. This experiment was the first one to show that the two light reactions of photosynthesis were sensitized by different spectral forms of Chl *a*, not Chl *b* and Chl *a*. Redrawn from Govindjee and Rabinowitch (1960).

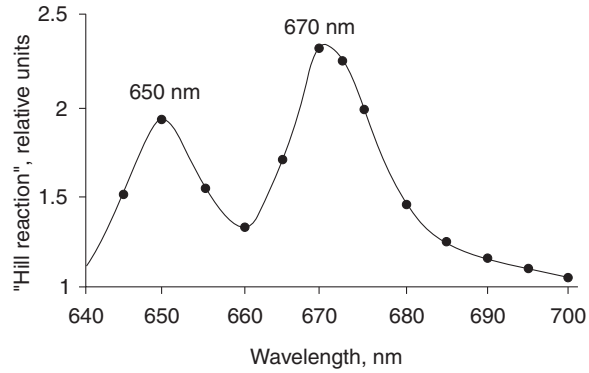


Fig. 1.6 Action Spectrum of the Emerson Enhancement in the Hill Reaction with Quinone as an oxidant in Chlorella (1960). This experiment not only confirmed the role of Chl a 670 in the same system as Chl b, but also showed clearly that the two-light effect is not in respiration. Redrawn from Rajni Govindjee *et al.* (1960).

At about the time of the discovery of Emerson Enhancement Effect, Blinks (1957, 1959) described another puzzling phenomenon that he named chromatic transients. He adjusted two lights of different wavelength so that they resulted in the same photosynthetic rates. When he switched from one kind of light to the other, the expectation was that photosynthesis would continue at a steady rate, but he found that this was not the case. In green algae, he found that in going from red light (absorbed mainly in chlorophyll *b*) to far-red light (absorbed mainly in chlorophyll *a*), photosynthesis rate first decreased rapidly, and then went back to the steady state. However, if the red light was given after the far-red light, the rate of photosynthesis increased before falling back to the steady state. Blinks was unable to explain these results, and interpreted this phenomenon, later known as the Blinks effect, to be in respiration (see Thorhaug and Berlyn, 2009, for a tribute to Blinks). Experiments of R. Govindjee *et al*. (1960) showed that the Emerson Effect was not in respiration. Myers and French (1960) showed that the action

spectra of the Blinks effect and the Emerson Enhancement effect were identical, thus connecting the two phenomena to the emerging two-photosystem two-light reaction concept that was now in the air in many laboratories (Fig. 1.4).

THE TWO PHOTOSYSTEMS

In addition to the discovery of the Emerson Enhancement, and the far-sighted ideas at Urbana, Illinois, which clearly led to the concept of the two light reactions and two photosystems, the 1959 work of Bessel Kok and soon thereafter that of Louis N.M. Duysens deserves a special mention. Kok (1959), in the Robert Emerson Memorial issue of Plant Physiology, showed a two-light effect in a cyanobacterium *Anacystis nidulans*, on the redox state of the reaction centre chlorophyll "P700" (Kok 1956, 1957). He showed that a long wavelength light (far-red) oxidized "P700", and when a short wavelength light (orange-red) was added, the oxidized P700 became reduced. In this paper, Kok related these findings to the Emerson Enhancement Effect and two pigment system concept (Fig. 1.7). This was presented in details in March, 1960, at John Hopkins University (see Kok and Hoch 1961).

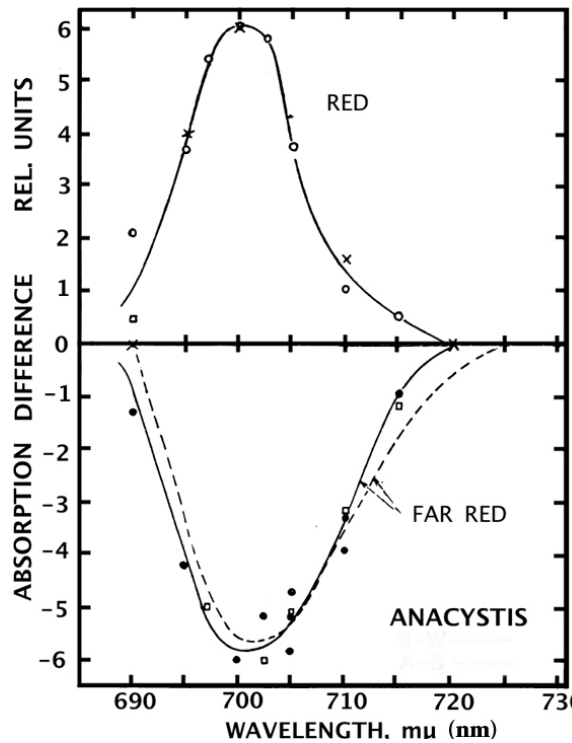


Fig. 1.7 Antagonistic Effect of Two Light Beams of Two Different Colors on the the Reaction Center P700 in a cyanobacterium (1959). Reversible absorbance changes in *Anacystis* caused by orange-red light (referred to as red light) and darker red light (referred to as far-red light). Far-red light oxidized (bleached), whereas orange red light reduced (restored) what we now call the Photosystem I reaction center pigment P700. From Kok (1959).

Duysens (1989) has presented his own story about his discoveries. After the discovery of the Emerson Enhancement Effect, Duysens examined the action spectra for cytochrome oxidation and

$NADP^+$ reduction in different wavelengths of light. Duysens (in August1960) presented his results at the 3rd International Congress on Photobiology at Copenhagen, Denmark, which showed that in red alga *Porphyridium*, green light, absorbed by phycoerythrin, gave a low yield of cytochrome oxidation when photosynthetic yield was high, but a high yield of cytochrome oxidation in red light, absorbed by chlorophyll a, where photosynthetic yield was low. Duysens (1989), who was unaware of the paper of Hill and Bendall (1960) at that time, wrote about his presentation in 1960:

> "I postulated the existence of t*wo major photosystems 1 and 2. System 1 contained the weakly fluorescent chlorophyll a, formerly said to be inactive, and oxidized cytochrome; system 2 contained the fluorescent chlorophyll a. An interaction between the two systems was shown by the different kinetics of cytochrome oxidation at different actini*c wavelengths."

On the other hand, in March, 1960, Rabinowitch and Govindjee stated (see Rabinowitch and Govindjee, 1961)

> *" ..the primary photochemical process in photosynthesis might consist of two steps: whereas one type of chlorophyll a was able to bring about both, the other type was restricted to one of these* steps."

The key experiment for the two light reactions was published by Duysens *et al.* (1961) (also see Duysens and Amesz, 1962): System 1 light oxidized a cytochrome, and system 2 light reduced it (Fig. 1.8). This was the discovery of the antagonistic effect of light 1 and 2 on an intermediate that was predicted by Rabinowitch (1956) and is still the best experimental evidence for the series scheme of photosynthesis. Further, Duysens *et al.* (1961) showed that in the presence of diuron (DCMU), cytochrome can be oxidized, but not reduced.

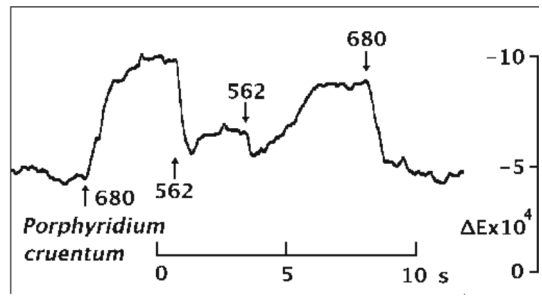


Fig. 1.8 Antagonistic Effect of Light 1 and 2 on the redox state of a cytochrome in a red alga (1961). Red light (680 nm; light 1, absorbed in chlorophyll *a* in Photosystem I) decreased absorbance at 420 nm (oxidation of cytochrome *f*) in the red alga *Porphyridium cruentum;* superimposition of green light (562 nm, absorbed in phycoerythrin in Photosystem II) caused reversal of absorbance decrease (reduction of cytochrome *f*). This is the best proof for the current series scheme of photosynthesis. Redrawn after Duysens *et al.* (1961).

The paper of Hill and Bendall (1960) is a landmark in the field of 'light reactions of photosynthesis', or let us say, the "Hill reaction" (see Walker 2002). They proposed the so-called "Z-scheme" on a totally theoretical ground. Cytochromes had been found in chloroplasts earlier, and the possibility that they could link the two photosystems (two light reactions) and provide energy for the formation of ATP through a downhill step between the two cytochromes was clearly a novel concept in the Robert (Robin) Hill and Fay Bendall scheme; the idea that there are two light reactions and two pigment systems was already there, although Hill and Bendall missed, *unintentionally*, we believe, citing the pioneering work of Robert Emerson. Instead of presenting the various versions of the Z-Scheme over the years (see e.g.,

earlier schemes by one of us (G) in Govindjee and R. Govindjee (1975), and in Demeter and Govindjee 1989), we show here one of the current versions of the Z-scheme (cf. Allen 2003; Govindjee *et al.* 2010; Fig. 1.9 and its detailed legend).

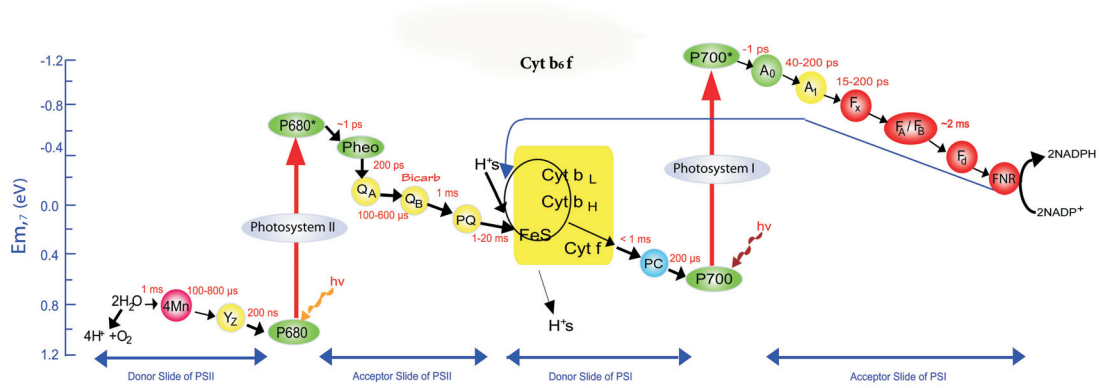


Fig. 1.9 One of the Versions of the Z-Scheme for Electron Transport from Water to the Pyridine Nucleotide, $NADP^+$ (2004). There are two Photosystems --I (PS I) and II (PSII)--connected in series, with a cytochrome *b6 f* complex in between; a cyclic electron transport around PSI also occurs (see below). In PSII, there is a tetranuclear manganese–oxygen–calcium cluster (Mn_4O_xCa, represented by 4Mn in the diagram; see Fig. 1.12 for further information, x implies unknown number); Yz, tyrosine-161 on the D1 protein; P680, primary electron donor of photosystem II; P680*, excited electronic state of P680; Pheo, pheophytin; Q_A, a tightly bound plastoquinone; Q_B, a plastoquinone that binds and unbinds from photosystem II; PQ, a pool of mobile plastoquinone molecules; Cyt b_6, cytochrome b_6; FeS, an iron–sulfur protein known as Rieske FeS protein, Cyt *f*, cytochrome *f*; PC, plastocyanin (cyanobacteria often employ Cytc6 instead); P700, primary electron donor of photosystem I; P700*, excited electronic state of P700; A_0, a special chlorophyll *a* molecule; A_1, vitamin K1; F_X, F_A, F_B, iron–sulfur centers ; F_d, ferredoxin; FNR, ferredoxin–NADP reductase; $NADP^+$, nicotinamide– adenine dinucleotide phosphate. The cyclic electron flow, that is usually a small fraction of non-cyclic electron flow, begins at the iron sulfur centers, not at FNR. In this diagram, light-harvesting complexes of photosystems I and II, and the ATP synthase are not shown. Estimated (or measured) times for electron transport between intermediates are also shown. A unique role of bicarbonate/carbonate (labeled as Bicarb) in PSII is to play a role in protonation at the Q_B site (see e.g., a review in Van Rensen *et al.*, 1999; McConnell *et al.*, 2010); another role of bicarbonate has been suggested to act on the electron donor side of PSII (not shown in this diagram). Also not shown in the diagram is chloride that plays an important role on the electron donor side of PSII (see e.g., Coleman and Goovindjee, 1987; and Homann, 2005). Modified from Govindjee (2004a; color version was reproduced earlier in Satoh *et al.*, 2005)

Witt *et al.* (1961) provided the most detailed biophysical measurements on the two-light reaction scheme. By 1963, the major concepts and experiments on the two light reaction and two pigment scheme had been settled (see Kok and Jagendorf 1963). The research group of Horst T. Witt provided new information on the participation of plastoquinone in the "Z-Scheme", detailed information on the kinetics of most of the reaction steps, and they discovered in 1969, the second reaction center "P680" of what we now call "Photosystem II", "P700" being the reaction center chlorophyll a of Photosystem I (see a review in Witt 2004, and references cited therein). Losada *et al.* (1961) from Daniel Arnon's research group provided one of the first biochemical measurements supporting the "Z-Scheme". However, Daniel Arnon soon abandoned this scheme in favor of a 3-light reaction scheme, or a 2-light light scheme, both run by two types of Photosystem II and even a one light reaction scheme. We shall not discuss them here.

The two-light reaction two-pigment system scheme was soon supported by the physical separation of two photosystems (see e.g., Boardman and Anderson 1964; Anderson 2005), chemical surgery of the entire scheme and by the use of specific inhibitors, artificial donors and acceptors of partial reactions (see e.g., reviews by Vernon and Avron (1965) and Trebst (1974), and through the use of mutants that lacked

specific intermediates in the electron transport chain (see e.g., Gorman and Levine 1966; see Levine 1969, for a review). The best evidence for the existence and operation of the two-light reactions and two-pigment systems is the isolation, characterization, and crystallization of the reaction center complexes I (see e.g., Jordan *et al.* 2001; Ben-Shem *et al.* 2003; for a complete review of PSI, see chapters in Golbeck 2006) and PSII (see e.g., Zouni *et al.* 2001; Ferreira *et al.* 2004; Loll *et al.*, 2005; Guskov *et al.* 2009; for a complete review of PS II, see chapters in Wydrzynski and Satoh 2005, and for a basic review of PSII, see Govindjee *et al.* 2010). We expect to see changes in the future in our detailed description of PSII because J-R. Shen (from Japan) has now obtained a 1.9 angstrom resolution structure (paper presented in 2010 at the 15th International Photosynthesis Congress, held in Beijing, China).

We do not discuss here the details concerning the primary photochemistry of PSI and PSII, but rather refer the readers to a review by Renger (2010), a book by Ke (2001) and an edited book by Renger (2008). However, for the story of the personal participation of one of us (G), with Michael Wasielewski, in PSII photochemistry, see Govindjee and Seibert (2010). Further, we have not discussed in this chapter the role of bicarbonate in PSII, a topic very dear to one of us (G) (see e.g., Van Rensen *et al.* 1999; McConnell *et al.* 2011).

THE OXYGEN CLOCK AND THE OXYGEN-EVOLVING CENTER

Pierre Joliot (1956) developed a highly sensitive technique in which he used a bare platinum electrode to measure the oxygen produced by *Chlorella* cells or spinach chloroplasts in response to short, saturating flashes (see further development in Joliot 1960, 1965a,b, 1968; Joliot *et al.*, 1969). Surprisingly, Joliot *et al.* (1969) found that if the flashes were short enough, no oxygen at all was observed after the first flash, no matter how strong the flash was. A little oxygen came out after the second flash, but the

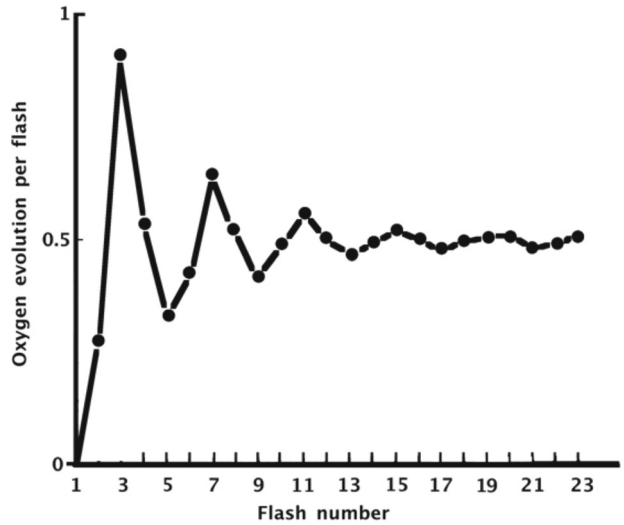


Fig. 1.10 Flash Number Dependence of Oxygen Release (1969). Amount of oxygen produced by spinach chloroplasts in response to short, single and intense flashes, was plotted against flash number. This diagram has been redrawn from Joliot *et al.* (1969). The periodicity of 4 in this plot led Bessel Kok and coworkers (Kok *et al.*, 1970) to propose their famous "S-states" scheme, in which the evolution of oxygen from water required four steps (see Mar and Govindjee, 1972, for all the schemes debated at that time): S_0 to S_1; S_1 to S_2 to S_3 and finally S_3 to S_4, where oxygen is evolved. Since the first maximum in the data was on the third flash, Kok suggested that the system started mostly in the S_1 state in darkness. The damping in the flash pattern was explained to be due to what was called "misses" and "double-hits".

third flash produced a maximum amount, and the fourth flash less again (Fig. 1.10). This "4-stroke" oscillatory pattern was repeated during the following flashes, but with decreasing amplitudes, until a steady level was reached.

Kok *et al.* (1970) repeated the measurements of Joliot *et al.* (1969) with various variations. This was a landmark paper, as it was here that the four-step charge accumulation model of oxygen evolution was first presented. The system works like a clock (see Govindjee and Coleman (1990) for a general description). According to this model, photochemical process progresses through a series of states of the oxygen-evolving complex, called the S states, one step for each quantum absorbed by the system (Fig. 1.11).

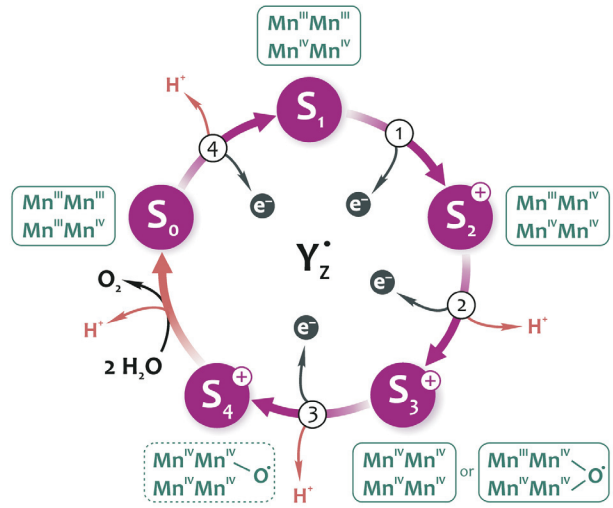


Fig. 1.11 A current view of the Oxygen Clock of Photosynthesis (2010). It shows Kok's S-state cycle, the oxidation states of the 4 manganese atoms in the oxygen evolving complex as well as where the oxygen molecule, the protons and the electrons come out. Except for the transition from S_4 to S_0, in which the water is oxidized, and the oxygen molecule is released, the other S-state transitions are driven by positive charges arriving from the oxidized tyrosine (Yz) that receives its positive charge from the oxidized P680, which is formed in a light reaction in PSII. The redox states of the 4 Mn atoms in S_0, S_1 and S_2 are generally agreed upon by most investigators. However for S_3 it may be either a manganese cluster with all Mn atoms in oxidation state IV, or one where there are 3 Mn^{IV}, one Mn^{III} and an oxygen radical. The configuration indicated for S_4 is hypothetical and is based on theoretical considerations. The figure is based on research of many researchers (see Govindjee *et al.*, 2010; Sproviero *et al.*, 2008a, b; Siegbahn, 2008, 2009; Dau and Haumann, 2008; Haumann *et al.*, 2005, among others). For a further detailed view on the release of protons, see Suzuki *et al.*, 2009. We are thankful to Gary Brudvig, Holgar Dau, Johannes Messinger, Gernot Renger and Junko Yano for discussions. The figure has been drawn for the authors by Dimitry Shevala in Johannes Messinger's group in Sweden.

The photochemical reaction, the primary charge separation, occurs at the reaction center chlorophyll complex, the P680. The negative charge (the electron) moves ultimately towards Photosystem I, whereas it is the positive charge that moves towards the oxygen-evolving complex via a tyrosine, labeled as Yz. In each step, the system gains a positive charge, or, in other words, it is oxidized. As a consequence of four photochemical steps, in series, S_0 becomes S_1; S_1 becomes S_2; S_2 becomes S_3; and finally S_3 becomes S_4 and the 2 molecules of water are oxidized by the four positive charges on S_4 releasing an oxygen molecule. It was apparent from the very beginning that after a prolonged dark period, an equilibrium between S_0 and S_1 states is established: 25% S_0 and 75% S_1. To get an agreement with the experimental

results, one must also assume a certain frequency of "double hits", i.e., a certain percentage of the photosynthetic units advance two steps during one flash, and a certain number of "misses" i.e., some units do not advance (for earlier reviews, see Mar and Govindjee, 1972; Joliot and Kok, 1975).

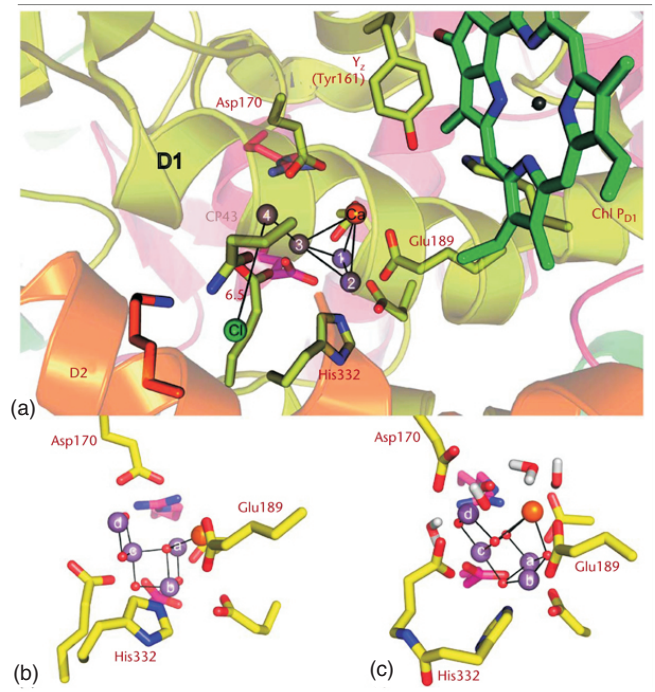


Fig. 1.12 A Representation of the water oxidizing complex of photosystem II (PSII) (2010). This figure is based on the work of Yano *et al.* (2006), Siegbahn (2008), and Guskov *et al.* (2009), and was drawn by Johannes Messinger (see Govindjee *et al.*, 2010). Here, the amino acids are shown with their 3-letter codes. (a: top figure) Structural model for the metal ions and amino acid ligands of the Mn_4O_xCa cluster (x implies unknown number), the redox active tyrosine Yz (Tyr161) and the chlorophyll PD1(a component ofthe reaction center complex of Photosystem II), as derived from the 2.9 Å resolution crystal structure (Guskov *et al.*, 2009); the view is along the membrane with lumen at the bottom and cytoplasm at the top. The protein surrounding is shown in cartoon mode in light yellow (D1), orange (D2) and magenta (CP43). Mn (purple), Ca^{2+}(orange) and Cl (green) ions are shown as spheres, ligating amino acids as sticks. The nitrogen and oxygen atoms of the amino acid ligands are colored in blue and red, respectively; the carbon atoms are colored depending on the subunit the amino acid belongs to: yellow for D1, orange for D2 and magenta for CP43. (b: bottom left) Model for the Mn_4O_xCa cluster in the dark stable S_1 state of the water oxidizing complex, obtained from orientation dependent X-ray spectroscopy on PSII single crystals (Yano *et al.*, 2006) embedded in the ligand environment derived from the crystal structure. The coloring and the view direction is as in panel (a), bridging oxygens are shown as small red spheres. (c: bottom right) Theoretical model for the Mn_4O_xCa cluster and its first ligand sphere in the S_1 state were derived from density functional calculations (Siegbahn, 2008); the coloring and the view direction is as in panel (a); the bridging oxygens are shown as small red spheres. This model also includes some water/hydroxide groups (hydrogens shown in grey) as ligands to the manganese and calcium ions. [Unfortunately, chloride that is known to be involved in these reactions was not modeled; see Coleman and Govindjee (1987) and Homann *et al.*, (2005) .]This figure is a courtesy of Johannes Messinger of Sweden.

For the current detailed view of the oxygen clock cycle, including the suggested redox states of the 4 Mn, see Fig. 1.11 and its legend (that includes references). Mn is successively oxidized in going from S_0 to S_1 and from S_1 to S_2; however, in S_3 and S_4, oxyl radicals may be involved; no experimental data is available for the S_4 state, but theoretical consideration is included in the scheme shown (cf. Dau and

Haumann 2008; Haumann *et al.* 2005; Sproviero *et al.* 2008a, 2008b; Siegbahn, 2008, 2009; among others; see Govindjee *et al.* 2010 for a review). A consensus view is that in the S_0 state three of the Mn atoms are in the trivalent and one in tetravalent state; in S_1 state two of the Mn are in the trivalent and two in tetravalent state; in the S_2 state one is in the trivalent state and three are in the tetravalent state. A progressive oxidation of Mn cluster is suggested. However, the Mn oxidation levels in the S_3 and S_4 states are less certain (see Fig. 1.11). The oxygen release pattern is suggested to be 1, 0, 1, 1, 1 during the S-state conversions; for a detailed view on the release of protons, see Suzuki *et al.* (2009).

Manganese has long been known to be an essential nutrient for plants. Pirson (1937) suspected a role for Mn in oxygen evolution. Spencer and Possingham (1961) found that manganese deficiency in spinach impairs oxygen evolution by chloroplasts prepared from it. The role of manganese in oxygen evolution was later studied in a long series of investigations (see e.g., Cheniae and Martin 1967). Gradually, the connection between the S states and oxidation states of manganese became established (see chapters in Wydrzynski and Satoh 2005).

A view of manganese, as part of the water oxidizing enzyme connected to specific amino acids on the PSII, is described in its essentials (and in details) in Fig. 1.12 (Johannes Messinger, personal communication). It is based on the extensive work of Yano *et al.* (2006), Siegbahn (2008) and Guskov *et al.* (2009).

The progression through the S states, and changes of redox states of the chemical components of the system, has been monitored by means other than by recording the bursts of oxygen. Thus oxidation changes of manganese can be monitored by absorption at 292 nm (Fig. 1.13, Sugiura *et al.* 2009), EPR, infrared spectroscopy or X-ray spectroscopy (Ono *et al.* 1992). Further, chlorophyll fluorescence from PSII (Shinkarev *et al.* 1997; Srivastava *et al.* 1999; for historical reviews, see Delosme and Joliot (2002), and Govindjee 2004a) exhibit corresponding oscillating patterns in response to short flashes.

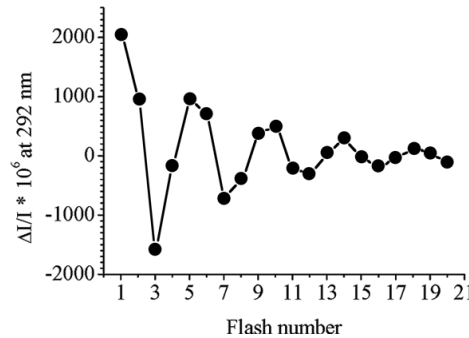


Fig. 1.13 Absorption changes in manganese ions in photosystem II from Thermosynechococcus elongatus (2009). Absorption changes at 292 nm as a function of flash number shows a periodicity of 4 with maxima at flash 1 and 5 supporting the relationship between redox changes in Mn with the S-states. Mutant studies revealed that D1His-252 is more involved with S_3 than with the S_2, supporting the concept of conformational changes in the process. Redrawn from Sugiura *et al.* (2009).

We estimate that in full sunlight a molecule of chlorophyll *a* which is not screened by other pigments, will absorb, on an average, ~2 photons per second. With an antenna size of 300 chlorophyll *a* molecules, we will have 600 excitations per second in the reaction center. In other words, the reaction center must transfer the energy within 2 ms. The rate-limiting step on the oxidizing side of PSII is thought to be due to the reduction of oxidized tyrosine residue Yz^+ by manganese in the oxygen evolving center, precisely during the steps from the S_2 to S_3 and S_3 via S_4 to S_0 states (Shinkarev and Wraight 1993; Grabolle and Dau 2005), while the S_0 to S_1 and S_1 to S_2 steps seem to proceed faster. It takes even more time for the

plastoquinone to deliver charges from PSII to PSI. In fact, this is the bottle-neck reaction of electron transport in photosynthesis (see Discussion in Ke 2001).

We have not discussed in this chapter the role of chloride on the electron donor side of PSII (see Coleman and Govindjee 1987; and Homann 2005).

PHOTOPHOSPHORYLATION

The first person to suggest that phosphorylation steps may be involved in photosynthesis was Ruben (1943). He realized that the reaction RH + CO_2 ⇒ RCOOH is energetically unfavorable, and instead proposed the following enzymatically catalyzed reaction sequence:

(1) energy rich phosphate donor + RH ⇒ "free donor" + phosphorylated RH

(2) phosphorylated RH + CO_2 ⇒ RCOOH + inorganic phosphate.

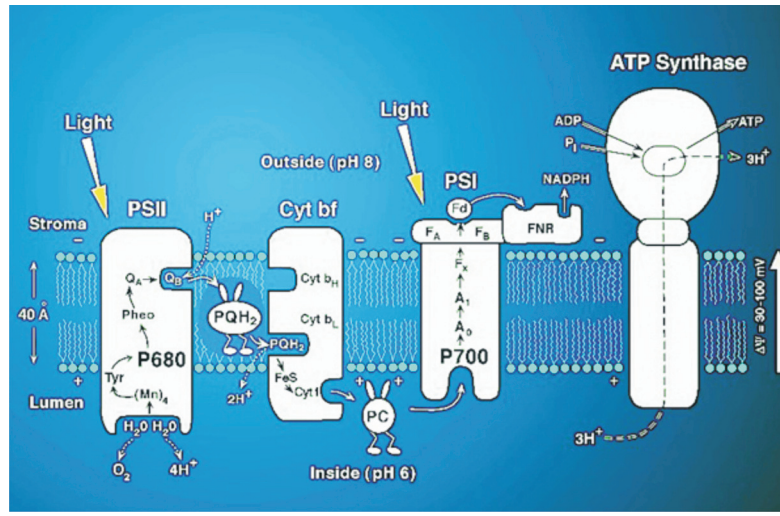
Further, Emerson *et al.* (1944) asked if photosynthetic organisms might use energy from ATP [hydrolysis] for the endergonic process of CO_2 reduction. However, Rabinowitch (1945) wrote:

> *"Until more positive evidence is provided, we are inclined to consider as more convincing a general argument against this hypothesis, which can be derived from energy considerations. Photosynthesis is eminently a problem of energy accumulation. What good can be served, then, by converting light quanta (even those of red light which amount to about 43 kcal per einstein) into "phosphate quanta" of only 10 kcal per mole?".*

We now know that light quanta are not only used for making ATP, but also for oxidizing water to oxygen and reducing NADP. ATP is produced due to energy drop between PS II and PS I, as Hill and Bendall suggested, but also due to a cyclic electron flow around PSI. Thus, Rabinowitch's concern is well taken. In an early scheme of the path of carbon in photosynthesis, Calvin and Benson (1948) had assigned an essential role to phosphorylated intermediates. But it took several years more before photosynthetic phosphorylation could be experimentally demonstrated. Strehler (1952, 1953) presented, using the luciferin-luciferase assay, the very first evidence for ATP formation upon illumination of plants, and that this took place in the chloroplast. However, photophosphorylation was clearly demonstrated by Frenkel (1954) in isolated bacterial chromatophores, and by Arnon *et al.* (1954a, 1954 b) in isolated spinach chloroplast fragments. The use of isolated photosynthetic membranes rather than the whole cells was important for proving that high-energy phosphate generated in respiration was not involved. (To make sure that no oxidative phosphorylation could take place, sodium chloride instead of sugar was used as osmoticum in the medium for chloroplast isolation.)

Peter Mitchell (1961a, 1961b) published his chemiosmotic theory, according to which there is no fixed stoichometric relation between the electron flow and phosphorylation. According to this theory, the electron flow (in the case of chloroplasts) drives a flow of protons into the thylakoid, and this concentration gradient (electrochemical gradient) is converted to phosphate energy when the protons flow back through the ATP-synthase complex (Fig. 1.14, top). Mitchell received the 1978 Nobel Prize in Chemistry for this theory. It was not until 1997 that Paul Boyer and John Walker shared a Nobel Prize, again in Chemistry, to show how precisely the enzyme ATP synthase makes ATP from the proton and membrane potential gradient. Fig. 1.14 (bottom) shows the current concepts as presented by Wolfgang Junge. The electrochemical gradient drives ATPase's Fo, which is the rotary nanomotor of the enzyme. This then drives F_1, the chemical nanomotor by 'elastic mechanical-power' transmission, as Junge *et al.* (2009) have explained. This then produces ATP, the energy currency of life (for the earlier pioneering ideas of how ATP-synthase functions, see Boyer *et al.* 1973 and Abrahams *et al.* 1994).

(a)



(b)

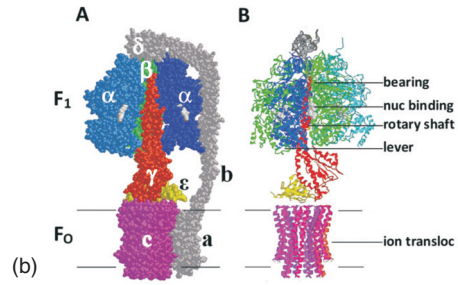


Fig. 1.14 (Top) A Cartoon of a thylakoid membrane that produces electrochemical potential difference of protons (redrawn from Govindjee, 2000). (Bottom) The ATP synthase that uses this power to make ATP (source: W. Junge, personal communication; see Junge *et al.*, 2009 for details). (Top): By a non-cyclic electron flow from water, via Photosystems II (PSII) and I (PSI) (with the Cyt bf, cytochrome b_6f complex in between) to nicotinamide adenine dinucleotide phosphate ($NADP^+$), protons are pumped into the thylakoid lumen. It is during water oxidation (on the lumen side of PS II) and when plastoquinol (PQH2) delivers its electrons to Cyt b6f complex (on the lumen side of Cyt b_6f), protons are translocated from the outer side of the membrane to the lumen. When protons flow out through the ATP synthase, ATP is generated from ADP and inorganic phosphate. Electrons can also circulate through PS I, and the Cyt b_6f complex, pumping protons without the involvement of PS II, driving a cyclic reaction producing more protons. This electrochemical potential difference of protons drives ATP synthesis (Mitchell, 1961a, b). (Bottom): A is the spacefilling model; and B is the ribbon model; nuc= nucleotide; transloc=translocator): Adenosine triphosphate (ATP) is synthesized from adenosine diphosphate (ADP) and inorganic phosphate (Pi) by 'ATP synthase' (FOF1-ATPase) as depicted by Junge *et al.* (2009). The electrochemical gradient drives ATPase's Fo, which is the rotary nanomotor of the enzyme. This then drives F1, the chemical nanomotor by 'elastic mechanicalpower' transmission, as Junge *et al.* (2009) have explained. This then produces ATP, the energy currency of life (for the earlier pioneering ideas of how ATP synthase functions, see Boyer *et al.* 1973 and Abrahams *et al.* 1994).

THYLAKOID MEMBRANE

In parallel with the above-mentioned biophysical investigations there has been enormous development on the biochemical side. The first ones to have been able to separate fractions exhibiting mainly PSI or PSII activity were Boardman and Anderson (1964); further, the phase partition method pioneered by Per-Åke Albertsson'sgroup (Åkerlund *et al.* (1976) merits special mention (see Anderson 2005 for an overview). There are four main protein complexes in the thylakoid membrane: PSI, cytochrome b_6f, PSII,

and the ATP synthase (see Fig. 1.14). Each one of these consists of many polypeptides, and in addition there are proteins that can move around by themselves and associate with one or two of these large complexes, such as ferredoxin, ferredoxin NADPH-oxidoreductase (FNR), plastocyanin, and several pigment-carrying proteins. These protein complexes have been isolated in higher and higher purity, and after some obstacles, due to their hydrophobic character, had been overcome, they could also be crystallized and their three-dimensional structures determined. We shall not be discussing this aspect in this chapter any further. However, readers can refer to Fig. 1.15 for a cartoon of the thylakoid membrane (Jon Nield's cartoon, available free at: http://www.queenmaryphotosynthesis.org/nield/psIIimages/oxygenicphotosynthmodel.ht ml).

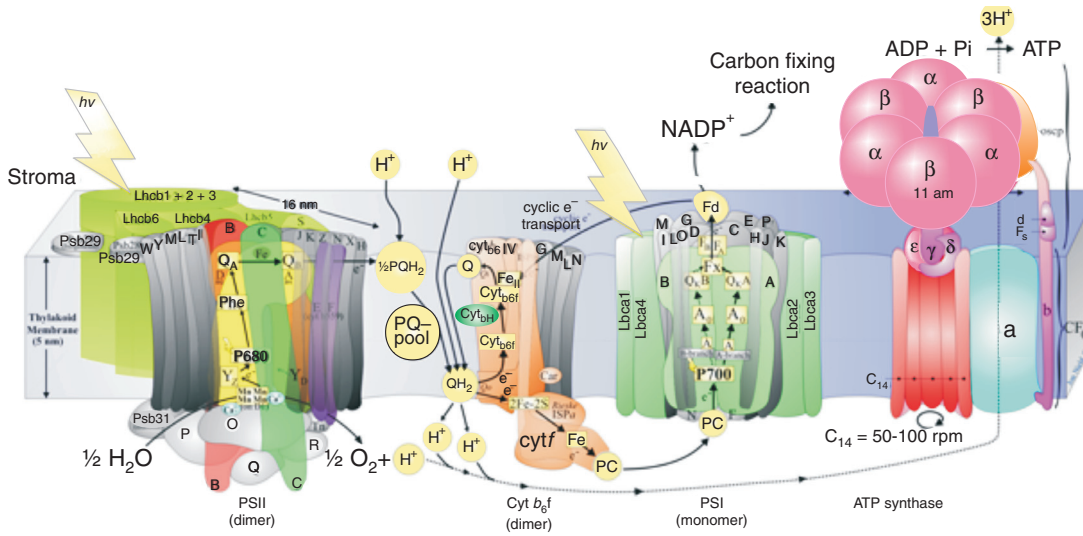


Fig. 1.15 A detailed cartoon model of the thylakoid membrane by Jon Nield (2010). It includes structural information on the organization of the protein complexes involved in electron (e^-) and proton (H^+) transport within the thylakoid membrane of plants and green algae. Relevant 3D information has been represented in 2D as reasonably as possible i.e., subunit-subunit positioning, relative spatial positioning of cofactors. (For simplicity, only the major symbols in the diagram are defined here.) Structural information is from e.g., Guskov *et al.* (2009) for Photosystem II, PSII; Stroebel *et al.* (2003) and Zhang *et al.* (2003) for cytochrome b6f, Cyt b6f; Jordan *et al.* (2001) for Photosystem I, PSI; *Amunts et al.* (2007) for Light Harvesting Complex I (LHC I). Lhca/b proteins: Light (hv) is captured and the energy channeled to the P680 reaction center complex of PSII, or the P700 reaction center of PSI. **PSII**: In caps are psb gene products; P680, primary electron donor; Phe, pheophytin, primary electron acceptor; Q_A, primary plastoquinone electron acceptor; Q_B, secondary plastoquinone acceptor; PQ, plastoquinone. **Cyt *b*$_6$*f***: In caps are pet gene products; Cyt bH, high potential cytochrome *b*; Cyt bL, low potential cytochrome; ISP, a 'mobile' Rieske Iron-Sulfur binding protein, able to share its intrinsic/extrinsic domains across each monomer in the dimer, hence ISPa/b; Note: there are a total of 4 haem centers per monomer (Fe), including a new 'haem ci', a possible link for cyclic e- transport; Q, quinone, Car, carotenoid, Chl *a*, Chlorophyll *a* molecule. **PSI**: In caps are psa gene products; P700, primary electron donor; PC, plastocyanin; A0 (primary electron acceptor Chl*a*), A_1 (vitamin K_1, a phylloquinone, secondary electron acceptor); F_A, F_B and F_X, bound FeS centers; Fd, ferredoxin. Accessory Chls are not shown. ATP synthase: oscp (oligomycin sensitivity conferring polypeptide). Courtesy of Jon Nield, Queen Mary, University of London, UK; freely available at: http://www.queenmaryphotosynthesis.org/nield/psIIimages/oxygenicphotosynthmodel.html

The model for thylakoid domain organization, based on the work of several authors, as summarized by Dekker and Boekema (2005), is shown in Fig. 1.16. In higher plants, having grana in their chloroplasts, the different parts of the thylakoid membrane do not contain the same proteins, nor do they have the same function. Most of the PSII is separated from PS I.

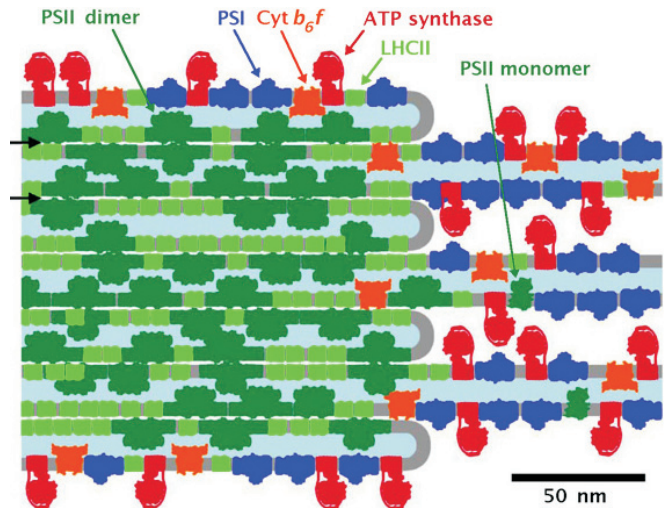


Fig. 1.16 Domain organization of thylakoid membranes in a higher plant (2005). Grana lamellae are shown in the left part of the diagram, and stroma lamellae are on the right. Cyclic electron transport, usually a small fraction of electron flow, which is accompanied by phosphorylation, takes place in the stroma region. Non-cyclic electron transport takes place in the grana region. PS II, Photosystem II; PS I, Photosystem I; Cyt b6f, cytochrome b6f; LHCII, Light harvesting complex II. We thank John Allen and Jan Anderson for discussions (see Allen and Forsberg, 2001, and Albertsson, 1995, 2001, for further information). Redrawn from Dekker and Boekema (2005).

CONCLUDING REMARKS

We have given a brief outline of what is known. The question we ask today is: Where do we go from here? The world is facing a shortage of energy to support its population. Photosynthesis provides hope for the future provided we learn, in depth, the intricate physics and chemistry of the process, its regulation mechanisms, and channel our energies to improve the yield of the overall process in plants, algae as well cyanobacteria. We know that each system has its niche and we must exploit all the systems, not one or the other.

ACKNOWLEDGEMENTS

We thank Prasanna Mohanty, Shigeru Itoh and K.N. Guruprasad for their invitation and for their patience with us. We thank Dimitrty Shevela, Johannes Messinger and Jon Nield for Figures 1.11, 1.12 and 1.15, respectively. We are grateful to Gary Brudvig, Holgar Dau, Johannes Messinger, Gernot Renger, Junko Yano, John Allen and Jan Anderson for discussions on specific aspects of this overview, also cited in the figure legends. All errors and mistakes in ideas, concepts and descriptions are of the authors.

Govindjee is ever grateful to his mentors Robert Emerson (1903—1959) and Eugene Rabinowitch (1898-1973) for everything they taught him (Govindjee 2004b).

REFERENCES

Abrahams JP, Leslie AGW, Lutter R, Walker JE (1994) The structure of F1ATPase from bovine heart mitochondria determined at 2.8 Å resolution. Nature 370: 621-628

Åkerlund HE, Andersson B, Albertsson PA (1976) Isolation of photosystem-II enriched membrane-vesicles from spinach-chloroplasts by phase partition. Biochim Biophys Acta 449: 525-535

Albertson P-A (1995) The structure and function of the chloroplast photosynthetic membrane: a model for the domain organization. Photosynth Res 46: 141-149

Albertsson P-A (2001) A quantitative model of the domain structure of the photosynthetic membrane. Trends Plant Sci (TIPS) 6: 349-353

Allen JF (2003) Cyclic, pseudocyclic and non-cyclic photophosphorylation: new links in the chain. Trends Plant Sci (TIPS) 8: 15-19

Allen JF, Forsberg J (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci (TIPS) 6: 317-326

Amunts A, Drory O, Nelson N (2007) The structure of Plant Photosystem I supercomplex at 3.4 A resolution. Nature 447: 58-63

Anderson J (2005) Changing concepts about the distribution of Photosystems I and II between grana-appressed and stroma-exposed thylakoid membranes. In: Govindjee, Beatty JT, Gest H, Allen JF (eds) Discoveries in Photosynthesis. Springer, Dordrecht, pp 729-736

Arnold W (1935) Photosynthesis. Doctoral thesis, Harvard University, Cambridge, MA, see pp 35-38 and Table ix.

Arnold W (1949) A calorimetric determination of the quantum yield in photosynthesis. In: J Franck and WE Loomis (eds) Photosynthesis in Plants, Iowa State College Press, Ames, Iowa, pp 273-276

Arnold W, Kohn H I (1934) The chlorophyll unit in photosynthesis. J Gen Physiol 18: 109-112

Arnon DI, Allen MB, Whatley FR (1954a) Photosynthesis in isolated chloroplasts. Nature 174: 394-396

Arnon DI, Allen MB, Whatley FR (1954b) Photosynthesis in isolated chloroplasts. II. Photosynthetic phosphorylation, the conversion of light into phosphate bond energy. Nature 76: 6324-6329

Ben-Shem A, Frolow F, Nelson N (2003) Crystal structure of plant Photosystem I. Nature 426: 630-635

Björn LO (1965) Chlorophyll formation in excised wheat roots. Physiol Plantarum 18: 1130-1142

Blackman FF (1905) Optima and limiting factors. Ann Bot 19: 281-296

Blankenship RE (2002) Molecular Mechanisms of Photosynthesis. Blackwell Science, Oxford. UK.

Blinks LR (1957) Chromatic transient in photosynthesis of red algae. In: Gaffron H, Brown AH, French CS, Livingston R, Rabinowitch EI, Strehler B, Tolbert NE (eds) Research in Photosynthesis. Interscience Publishers, New York, pp 444-449

Blinks LR (1959) Chromatic transients in the photosynthesis of a green alga. Plant Physiol 34: 200-203

Boardman NK, Anderson JM (1964) Isolation from spinach chloroplasts of particles containing different proportions of chlorophyll *a* and chlorophyll *b* and their possible role in light reactions of photosynthesis. Nature 203: 166-167

Boyer PD, Cross RL, Momsen W (1973) A new concept for energy coupling in oxidative phosphorylation based on the molecular explanation of the oxygen exchange reactions. Proc Natl Acad Sci USA 70: 2837-2839

Brody M, Emerson R (1959) Quantum yield of photosynthesis in *Porphyridium* cruentum and the role of chlorophyll *a* in photosynthesis of red algae. J Gen Physiol 43: 251-264

Calvin M, Benson AA (1948) The path of carbon in photosynthesis. Science 107: 476-480

Cheniae GM, Martin IF (1967) Photoreactivation of manganese catalyst in photosynthetic oxygen evolution. Biochem Biophys Res Comm 28: 89-95

Clegg RM, Sener M, Govindjee (2010) From Förster resonance energy transfer to coherent resonance energy transfer and back. In: R Alfano (ed) Optical Biopsy VII, Proceedings of SPIE, Vol. 7561 (SPIE, Bellingham, WA, 2010), paper number: 7561-12; article CID Number 75610C, 21 pages (See http://www.life.illinois.edu/govindjee/recent_papers.html)

Coleman WJ, Govindjee (1987) A model for the mechanism of chloride activation of oxygen evolution in Photosystem II. Photosynth Res 13: 199-223

Damaschke K, Tödt F, Burk D, Warburg O (1953) An electrochemical demonstration of the energy cycle and the maximum quantum yield in photosynthesis. Biochim Biophys Acta 12: 347-355

Dau H, Haumann M (2008) The manganese complex of photosystem II in its reaction cycle—basic framework and possible realization at the atomic level. Coord Chem Rev 252: 273-293

Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta 1706: 12–39

Delosme R, Joliot P (2002) Period four oscillations in chlorophyll *a* fluorescence. In : Govindjee, JT Beatty, H Gest and JF Allen, (eds), Discoveries in Photosynthesis pp 379-382, Springer, Dordrecht

Delosme R , Joliot P (2005) Period four oscillations in chlorophyll *a* fluorescence. In : Govindjee, JT Beatty, H Gest and JF Allen, (eds) Discoveries in Photosynthesis, pp 379—382, Springer, Dordrecht

Demeter S and Govindjee (1989) Thermoluminescence from plants. Physiol Plantarum 75: 121-130

Duysens LNM (1952) Transfer of excitation energy in photosynthesis. Doctoral Thesis. State University, Utrecht, The Netherlands

Duysens LNM (1989) The discovery of the two photosynthetic systems: a personal account. Photosynth Res 21: 61-80

Duysens LNM, Amesz J, Kamp BM (1961) Two photochemical systems in photosynthesis. Nature 190: 510-511

Duysens LNM, Amesz J (1962) Function and identification of two photochemical systems in photosynthesis. Biochim Biophys Acta 64: 243-260

Duysens LNM, Sweers HE (1963) Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: Japanese Society of Plant Physiologists (eds) Studies on Microalgae and Photosynthetic Bacteria, pp. 353-372. University of Tokyo Press, Tokyo

Einstein A (1912) Thermodynamische Begründung des photochemischen Äquivalentgesetzes. Ann Phys 14 (Suppl): 437-443

Emerson R (1958) The quantum yield of photosynthesis. Annu Rev Plant Physiol 9: 124

Emerson R, Arnold W (1932a) A separation of the reactions in photosynthesis by means of intermittent light. J Gen Physiol 15: 391-420

Emerson R, Arnold W (1932b) The photochemical reaction in photosynthesis. J Gen Physiol 16: 191-205

Emerson R, Chalmers RV (1955) Transient changes in cellular gas exchange and the problem of maximum efficiency of photosynthesis. Plant Physiol 30: 504-529

Emerson R, Chalmers RV, Cederstrand CN (1957) Some factors influencing the long wave limit of photosynthesis. Proc Natl Acad Sci USA 43: 133-143

Emerson R, Chalmers RV(1958) Speculations concerning the function and phylogenetic significance of the accessory pigments of algae. Phycol Soc News Bull 11: 51-56

Emerson R, Lewis CM (1941) Carbon dioxide exchange and measurement of the quantum yield of photosynthesis. Am J Bot 28: 789-804

Emerson R, Lewis CM (1943) The dependence of the quantum yield of *Chlorella* photosynthesis on wavelength of light. Am J Bot 30: 165-178

Emerson R, Rabinowitch E (1960) Red drop and role of auxiliary pigments in photosynthesis. Plant Physiol 35: 477-485

Emerson R, Stauffer JF, Umbreit WW (1944) Relationships between phosphorylation and photosynthesis. Amer J Bot 31: 107-120

Engelmann TW (1884) Untersuchungen über die quantitativen Beziehungen zwischen Absorption des Lichtes und Assimilation in Pflanzenzellen. Bot Z 42: 81-94 and 97106

Ferreira KN, Iverson TM, Maghlaoui, Barber J, Iwata S (2004) Architecture of the photosynthetic oxygen evolving center. Science 303: 1831-1837

Franck J, Herzfeld KF (1941) Contributions to a theory of photosynthesis. J Phys Chem 45: 978-1025

Frenkel A (1954) Light-induced phosphorylation in cell-free preparations of photosynthetic bacteria. 76: 5568-5569

Gaffron H, Wohl K (1936) Zur Theorie der Assimilation. Naturwissenschaften 24: 81-90; 103-107

Golbeck JH (ed) (2006) Photosystem I: The light-driven plastocyanin: ferredoxin oxidoreductase. Springer, Dordrecht

Gorman DS, Levine RP (1966) Photosynthetic electron transport chain of *Chlamydomonas reinhardtii*.VI. Electron transport in mutant strains lacking either Cyt 559 or plastocyanin. Plant Physiol 41: 1648: 1656

Govindjee (1999a) On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis. Photosynth Res 59: 249-254

Govindjee (1999b) In: HA Frank, AJ Young, G Britton, RJ Cogdell (eds) Carotenoids in photosynthesis: An historical perspective. In: The Photochemistry of Carotenoids, Kluwer Academic Publishers (now Springer), Dordrecht, Netherlands pp 1-19

Govindjee (2000) Milestones in Photosynthesis Research. In: Probing Photosynthesis. Edited by M.Younis, U. Pathre and P. Mohanty. Taylor & Francis, UK, pp. 9-39 [An electronic file is available at: <http://www.life.illinois.edu/govindjee/papers/milestones.html>]

Govindjee (2004a) Chlorophyll a Fluorescence: A Bit of Basics and History. In G Papageorgiou and Govindjee (eds) Chlorophyll a Fluorescence: A Probe of Photosynthesis. pp. 2-42, Kluwer Academic (now Springer), Dordrecht, The Netherlands

Govindjee (2004b) Robert Emerson, Eugene Rabinowitch: Understanding Photosynthesis. In: Lillian Hoddeson (editor). "No Boundaries: University of Illinois Vignettes", Chapter 12, pp. 181-194. University of Illinois Press, Urbana and Chicago. ISBN: 0-252-0703-0 (paperback)

Govindjee, Bazzaz M (1967) On the Emerson enhancement effect in the ferricyanide Hill reaction in chloroplast fragments. Photochem Photobiol 6: 885-894

Govindjee, Coleman WJ (1990) How plants make oxygen. Scientific American 262: 50-58

Govindjee, Govindjee R (1965) Two different manifestations of enhancement in the photosynthesis of Porphyridium cruentum in flashing monochromatic light. Photochem Photobiol 4: 401-415

Govindjee and Govindjee R (1975) In : Govindjee (ed) Introduction to Photosynthesis, In: Bioenergetics of Photosynthesis, Academic Press, NY, pp 1-50

Govindjee, Ichimura S, Cederstrand C, Rabinowitch E (1960) Effect of combining far-red light with shorter wave light in the excitation of fluorescence in *Chlorella*. Arch Biochem Biophys 89: 322-323

Govindjee and Krogmann D (2004) Discoveries in oxygenic photosynthesis (1727– 2003): a perspective: Dedicated to the memories of Martin Kamen (1920–2002) and William A Arnold (1904–2001). Photosynth Res 80: 15–57

Govindjee, Rabinowitch E (1960) Two forms of chlorophyll *a* in vivo with distinct photochemical functions. Science 132: 159-160

Govindjee, Seibert M (2010) Picosecond spectroscopy of the isolated reaction centers from the photosystems of oxygenic photosynthesis-ten years (1987-1997) fun. A tribute to Michael R Waisielewski on his 60th birthday. Photosynth Res 103: 1-6

Govindjee, Kern JF, Messinger J, Whitmarsh J (2010) Photosystem II. In Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0000669.pub2 (See http://www.life.illinois.edu/govindjee/recent_papers.html)

Govindjee, Owens O v H, Hoch G (1963) A mass spectroscopic study of the Emerson enhancement effect. Biochim Biophys Acta75: 281-284

Govindjee R, Govindjee, Hoch G (1962) The Emerson enhancement effect in TPNphotoreduction by spinach chloroplasts. Biochem Biophys Res Comm 9: 222-225

Govindjee R, Govindjee, Hoch G (1964) Emerson enhancement effect in chloroplast reactions. Plant Physiol 39: 10-14

Govindjee R, Rabinowitch E, Govindjee (1968) Maximum quantum yield and action spectrum of photosynthesis and fluorescence in *Chlorella*. Biochim Biophys Acta 162: 539-544

Govindjee R, Thomas JB, Rabinowitch E (1960) Second Emerson effect in the Hill reaction of *Chlorella* cells with quinone as oxidant. Science 132: 421

Grabolle M, Dau H (2005) Energetics of primary and secondary electron transfer in photosystem II membrane particles of spinach revisited on basis of recombination-fluorescence measurements. Biochim Biophys Acta 1708: 209–218

Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W (2009) Cyanobacterial photosystem II at 2.9 Å resolution and the role of quinones, lipids, channels and chloride. Nature Structural and Molecular Biology 16: 334-342

Homann M , Liebisch P, Muller C, Barra M , Grabolle M, Dau H (2005) Photosynthetic O_2 formation tracked by time-resolved X-ray experiments. Science 310: 1019-1021

Haxo FT, Blinks LR (1946) Photosynthetic action spectra in red algae. Am J Bot 33: 836-837

Haxo FT, Blinks LR (1950) Photosynthetic action spectra of marine algae. J Gen Physiol 33: 389-422

Hill R, Bendall F (1960) Function of the cytochrome components in chloroplasts: a working hypothesis. Nature 186: 136-137

Homann PH (2005) Chloride and calcium in Photosystem II: from effects to enigma. In: Govindjee, JT Beatty, H Gest and JF Allen (eds) Discoveries in Photosynthesis, pp 383-389, Springer: Dordrecht

Joliot P (1956) Dispositif ampérometrique de measure de la photosnthèse. CR Acad Sci Paris 243: 677-680

Joliot P (1960) Contribution à l'étude des phénomènes d'induction de la photosynthèse. Thèse de Doctorat es-Sciences, Faculté de Sciences de Paris

Joliot P (1965a) Cinetiques des reactions liees a l'emission d'oxygene photosynthetique. Biochim Biophys Acta 102: 116-134

Joliot P (1965b) Etudes simultanees des cinetiques de fluorescence et d'emission d'oxygene photosynthetique. Biochim Biophys Acta 102: 135-148

Joliot P (1968) Kinetic studies of photosystem 2 in photosynthesis. Photochem. photobiology 8: 461-463

Joliot P, Barbieri G and Chabaud R (1969) Un nouveau modele des centres photochimiques du systeme II. Photochem Photobiol 10: 309-329

Joliot P, Kok B (1975) Oxygen evolution in photosaynthesis. In: Govindjee (ed), Bioenergetics of Photosynthesis pp 387-412. Academic Press, New York

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three dimensional structure of cyanobacterial Photosystem I at 2.5 Å resolution. Nature 411: 909-917

Junge W, Sielaff H, Engelbrecht S (2009) Torque generation and elastic power transmission in the rotary FoF1-ATPase. Nature 459: 364-370

Ke B (2001) Photosynthesis:Photobiochemistry and Photobiophysics. In Govindjee (Series ed) Advances in Photosynthesis, volume 10, Springer, Dordrecht

Kohn HI (1936) Number of chlorophyll molecules acting as an absorbing unit in photosynthesis. Nature 37: 706-706

Kok B, Forbush M, McGloin M (1970) Cooperation of charges in photosynthetic O_2 evolution 1. Photochem Photobiol 11: 457-475

Kok B, Hoch G (1961) Spectral changes in photosynthesis. In : WD McElroy and B Glass (eds.) Light and Life, pp 397-423. The Johns Hopkins Press, Baltimore, Maryland

Kok B (Chairman), Jagendorf AT (Organizer) (1963) Photosynthetic Mechanisms of Green Plants. Publication #1145. National Academy of Sciences—National Research Council, Washington, DC, see 'Foreword', p. ix; papers from many groups were presented and there was a general agreement on the basic scheme(s)

Kok B (1956) On the reversible absorption change at 705 mμ in photosynthetic organisms. Biochim Biophys Acta 22: 399-401

Kok B (1957) Absorption changes induced by the photochemical reaction of photosynthesis. Nature 179: 583-584

Kok B (1959) Light-induced absorption changes in photosynthetic organisms. II. A split-beam difference spectrophotometer. Plant Physiol 34: 184-192

Levine RP (1969) The analysis of photosynthesis using mutant strains of algae and higher plants. Annu Rev Plant Physiol 20: 523-540

Levring T (1947) Submarine daylight and the photosynthesis of marine algae. Göteborgs K Vetenskaps-och Vitterhets-samhällets Handl 5(6): 1-90

Loll B, Kern J, Saenger W, Zouni A, Biesiadka J (2005) Towards complete cofactor arrangement in the 3.0 A resolution structure of Photosystem II. Nature 438: 1040-1044

Losada M, Whatley FR, Arnon DI (1961) Separation of two light reactions in non-cyclic photophosphorylation of green plants. Nature 190: 606-610

Magee JL, de Witt TW, Smith EC, Daniels F (1939) A photocalorimeter.The quantum efficiency of photosynthesis in algae. J Amer Chem Soc 61: 3529-3533

Mar T and Govindjee (1972) Kinetic models of oxygen evolution in photosynthesis. J Theoret Biol 36: 427-446

McConnell IL, Eaton-Rye JJ and van Rensen JJS (2011) Regulation of Photosystem II electron transport by bicarbonate. In: Eaton-Rye JJS, Tripathy BC, Sharkey TD (eds) Photosynthesis: Plastic Biology, Energy Conversion and Carbon Assimilation, Volume 34, Advances in Photosynthesis and Respiration, Springer, Dordrecht, in the press

Mitchell P (1961a) Coupling of photophosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism. Nature 191: 144-148

Mitchell P (1961b) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biochem J 79: P23-P24

Myers J (1971) Enhancement studies in photosynthesis. Annu Rev Plant Physiol 22: 289-312

Myers J (1994) The 1932 experiments. Photosynth Res 40: 303-310

Myers J, French CS (1960) Evidences from action spectra for a specific participation of chlorophyll *b* in photosynthesis. J Gen Physiol 43: 723-736

Nickelsen K (2010) Of Light and Darkness—Modelling Photosynthesis 1840–1960.Habilitationschrift-eingereicht der Phil.-nat.Fakultat der Universutat Bern, Switzerland, Kluwer Academic Press, in the press

Nield J (2010) See Web page accessed August 8, 2010: http://www.queenmaryphotosynthesis.org/nield/psIIimages/oxygenicphotosynthmodel.html

Ono T, Noguchi T, Inoue Y, Kusunoki M, Matsushita T, Oyanagi H (1992) X-Ray detection of the period-four cycling of the manganese cluster in photosynthetic water oxidizing enzyme. Science 258: 1335-1337

Orr L, Govindjee (2010) Photosynthesis Online. Photosynth Res DOI 10.1007/s11120-010-9570-8, 34 pages (See http://www.life.illinois.edu/govindjee/recent_papers.

Pirson A (1937) Ernährungs- und stoffwechselphysiologische Untersuchungen an Fontinalis und Chlorella. Z Botan 31: 193-267

Rabinowitch E (1945) Photosynthesis and Related Processes. Volume I. Interscience Publishers, Inc., New York, NY, 599 pp, see p 162

Rabinowitch EI (1956) Photosynthesis and Related Processes, Vol II (Part 2). Kinetics of Photosynthesis (continued); Addenda to Vol I and Vol II, Part 1, pp 1211–2088. Interscience Publishers, New York, see p 1862

Rabinowitch E (1961) Robert Emerson (1903–1959) Biographical Memoirs of the National Academy of Sciences, USA 25: 112-131

Rabinowitch EI, Govindjee (1961) Different forms of chlorophyll *a* in vivo and their photochemical function. In: McElroy WD, Glass B (eds) A Symposium on Light and Life, pp 378-391. The Johns Hopkins Press, Baltimore, Maryland

Rabinowitch E, Govindjee (1969) Photosynthesis. John Wiley & Sons, New York. See Chapters 11; 13; 14; and 16. The book is available free on the internet at http://www.life.illinois.edu/govindjee/photosynBook.html

Renger, G. (ed) (2008) Primary Processes of Photosynthesis: Principles and Apparatus, Part I, Photophysical Principles, Pigments and Light Harvesting/Adaptation/Stress, Royal Society of Chemistry, Cambridge

Renger G (2010) The light reactions of photosynthesis. Current Science 98: 1305-1319

Ruben S (1943) Photosynthesis and phosphorylation. J Amer Chem Soc 65: 279-282

Satoh K, Wydrzynski T, Govindjee (2005) Introduction to Photosynthesis. In: Wydrzynski T and Satoh (eds) Photosystem II: The light-driven water-plastoquinone oxidoreductase, pp 11-22, Springer, Dordecht

Schmid G H, Gaffron, H (1968) Photosynthetic units. J Gen Physiol 52: 212-239

Shinkarev VP, Wraight CA (1993) Kinetic factors in the bicycle model of oxygen evolution by Photosystem II. Photosynth Res 38: 315-321

Shinkarev V P, Xu C, Govindjee, Wraight CA(1997) Kinetics of the oxygen evolution step in plants determined from flash-induced chlorophyll *a* fluorescence. Photosynth Res 51: 43-49

Siegbahn PEM (2008) A structure-consistent mechanism for dioxygen formation in Photosystem II. Chem Eur J 27: 8290-8302

Siegbahn PEM (2009) Structures and energetics for oxygen formation in Photosystem II. Accnts Chem Res 42: 1871-1880

Skillman JB (2008) Quantum yield variation across the three pathways of photosynthesis: not yet out of the dark. J Exp Bot 59: 1647-1661

Spencer D, Possingham JV (1961) The effect of manganese deficiency on photophosphorylation and the oxygen-evolving sequence in spinach chloroplasts. Biochim Biophys Acta 52: 379-38

Sproviero EM, Gascon JA McEvoy JP, Brudvig GW, Batista VS (2008a) Quantum mechanics/molecular mechanics study of the catalytic cycle of water splitting in Photosystem II. J Amer Chem Soc 130: 3428-3442

Sproviero EM, Gascon JA, McEvoy JP, Brudvig GW, Batista VS (2008b) A model of the oxygen-evolving center of Photosystem II predicted by structural refinement based on EXAFS simulations. J Am Chem Soc 130: 6728-6730

Srivastava A, Strasser RJ and Govindjee (1999) Greening of peas: parallel measurements of 77K emission spectra, OJIP chlorophyll a fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700. Photosynthetica 37: 365-392

Stark J (1908) The atomising effect of light and optical sensitising. Physikal Z 9:894900

Strehler BL (1952) Photosynthesis-energetics and phosphate metabolism. In: McElroy WD, Glass HB (eds) Phosphorous Metabolism II. Johns Hopkins Press, Baltimore, Md, pp 491-502

Strehler BL (1953) Firefly luminescence in the study of energy transfer mechanisms II: Adenosine triphosphate and photosynthesis. Arch Biochem Biophys 43: 67-79

Stroebel D, Choquet Y, Popot J-L, Picot D (2003) An atypical haem in the cytochrome b_6f complex. Nature 426: 413-418

Sugiura M, Rappaport F, Hillier W, Dorlet P, Ohno Y, Hayashi H, Boussac A (2009) Evidence that D1-His332 in Photosystem II from *Thermosynechococcus elongatus* interacts with the S3-state and not with the S2-state. Biochemistry 48: 7856-7866

Sundqvist C (1969) Transformation of protochlorophyllide, formed from exogenous δ aminolevulinic acid, in continuous light and in flashlight. Physiol Plantarum 22: 147-156

Suzuki H, Suggiura M, Noguchi T (2009) Monitoring proton release during photosynthetic water oxidation in Photosystem II by means of isotope-edited infrared spectroscopy. J Am Chem Soc 131: 7849-7857

Tanada T (1951), The photosynthetic efficiency of carotenoid pigments in *Navicula minima*. Am J Bot 38: 276-283

Thorhaug A, Berlyn G (2009) A tribute to Lawrence Rogers Blinks (1900-1989): light and algae. Photosynth Res 100: 129-140

Trebst A (1974) Energy conservation in photosynthetic electron transport of chloroplasts. Annu Rev Plant Physiol 25: 423-458

Van Niel CB (1941) The bacterial photosynthesis and their importance for the general problem of photosynthesis. Adv Enzymol 1: 263-328

Van Rensen JJS, Xu C, Govindjee (1999) Role of bicarbonate in the photosystem II, the water-plastoquinone oxido-reductase of plant photosynthesis. Physiol Plantarum 105: 585-592

Vernon LP, Avron M (1965) Photosynth. Annu Rev Biochem 34: 269-296

Walker DA (2002) The Z-scheme-down hill all the way. Trends Plant Sci 7: 183-185

Warburg O (1958) Photosynthesis. Science 128: 68-73

Warburg O, Burk D, Schocken V, Hendricks SB (1950) The quantum efficiency of photosynthesis. Biochim Biophys Acta 4:335-348

Warburg O, Krippahl G, Lehman A (1969) Chlorophyll catalysis and Einstein's law of photochemical equivalence in photosynthesis. Am J Bot 56: 961-971

Witt HT (2004) Steps on the way to building blocks, topologies, crystals and X-ray structural analysis of Photosystem I and II of water oxidizing photosynthesis. Photosynth Res. 80: 86-107

Witt HT, Muller A, Rumberg B (1961) Experimental evidence for the mechanism of photosynthesis. Nature 191: 194-195

Wydrzynski TJ, Satoh K (eds) (2005) Photosystem II: the light-driven water: plastoquinone oxidoreductase. Springer, Dordrecht

Yano J, Kern J, Sauer K, Latimer MJ, Pushkar Y, Biesiadka J, Loll B, Saenger W, Messinger J, Zouni A and Yachandra, VK (2006) Where water is oxidized to dioxygen: Structure of the photosynthetic Mn_4Ca cluster. Science 314: 821-825

Zhang H, Kurisu G, Smith JL, Cramer WA (2003) A defined protein-detergent-lipid complex for crystallization of integral membrane proteins: The cytochrome $b_6 f$ complex of oxygenic photosynthesis. Proc Natl Acad Sci USA 100: 5160-5163

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W, Orth P (2001) Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8Å resolution. Nature 409: 739-743