REGULATION OF PHOTOSYNTHETIC PHOSPHORYLATION

Author: **J. F. Allen**
Department of Biological Sciences
University of Warwick
Coventry, England

Referee: F. R. Whatley
Botany School
Oxford University
Oxford, England

I. INTRODUCTION

The term "photosynthetic phosphorylation" (or "photophosphorylation") is used to describe the processes by which radiant energy (sunlight) is converted to the biologically useful, chemical potential energy of ATP.

The "regulation" with which this article is concerned refers to two important and interdependent control mechanisms that would appear to allow high efficiency of photosynthetic energy conversion despite otherwise unfavorable environmental changes. The first is the mechanism by which relative rates of synthesis of ATP and NADPH may be adjusted to meet the requirements of CO_2 fixation and other assimilatory reactions of plants. This is the problem of regulation of the overall stoicheiometry of photosynthetic phosphorylation. The second mechanism controls the distribution of absorbed excitation energy between the two photosystems of the photosynthetic electron transport chain. This regulation brings about an optimization of the efficiency of photosynthetic phosphorylation.

The review is restricted arbitrarily to plant-type photosynthesis, with emphasis on studies of isolated chloroplasts. Regulation of bacterial photosynthetic phosphorylation is an important but relatively uncharted area, despite the considerable characterization of bacterial primary photochemical reactions. In any event, the aspects of regulation discussed here depend on the serial operation of two photosystems.

Though the mechanism of photophosphorylation also lies outside the scope of this article, it is difficult to see how much of the work discussed could be interpreted if electron transport were not coupled to ATP synthesis by means of a transmembrane electrochemical potential gradient of protons.[1] A general account of the chemiosmotic theory has recently been published.[2]

Recent reviews on the enzymology of chloroplast ATP synthesis include those of Shavit[3] and Nelson,[4] while the topic of the stoicheiometry and regulation of coupling ATPase activity is reviewed for chloroplasts by Reeves and Hall.[5] Earlier reviews of relevance are those of Simonis and Urbach,[6] Avron,[7] and Heber,[8] while the clearest account of the vectorial arrangement of the electron transport chain into protein-translocating loops is perhaps still that of Trebst.[9] Recent work on the modulation of coupling factor ATPase activity[10] is concerned with inhibition of ATP hydrolysis in the dark. Despite the relevance of this work to the present topic, light activation does not constitute a regulation of photosynthetic phosphorylation per se, and is not discussed.

II. REGULATION OF CYCLIC PHOSPHORYLATION

A. Is Cyclic Necessary?

Cyclic phosphorylation is easily demonstrated in vitro, and was in fact the first form of plant photophosphorylation to be discovered.[11] It is cyclic only in that it is coupled to cyclic electron transport, which is driven by photosystem I. Net oxidation-reduction does not occur, and so ATP synthesis is not coupled stoicheiometrically to reduction of $NADP^+$. In contrast, noncyclic photophosphorylation (which accompanies electron transfer to a terminal acceptor such as $NADP^+$) is likely to have a fixed stoicheiometry of ATP and NADPH synthesis. It has been held for some time that the cyclic and noncyclic reactions operate in parallel, cyclic making good the inability of noncyclic to provide sufficient ATP for CO_2 fixation and other assimilatory processes.[11]

The sufficiency of noncyclic phosphorylation and the consequent redundancy of cyclic has nevertheless been argued by certain authors, largely on the basis of estimated values of two for the ATP:$2e^-$ ratio of purely noncyclic phosphorylation driven by both photosystems,[12,13] and of one for that of each photosystem working alone.[14-16]

The possibility, raised by the chemiosmotic theory, of predicting the ATP:$2e^-$ ratio of noncyclic phosphorylation from the stoicheiometries of proton translocation has also been considered.[17] Thus, if ATP synthesis is perfectly coupled to electron transport with ΔpH as the only intermediate, then the ATP:$2e^-$ ratio will be equal to twice the value for H^+:e^- divided by the value for H^+:ATP. Reeves and Hall[5] have tabulated various authors' estimates of these values, from which some degree of concensus emerges that the H^+:e^- value is 2.0 for the whole noncyclic chain (this is consistent with the idea of two proton-translocating loops, each with an H^+:e^- of 1.0),[9] while the H^+:ATP ratio may be either 2.0 or 3.0. This would lead one to expect the maximum ATP:$2e^-$ ratio to be either 2.0 or 1.33. Since the standard error of corporate estimates[5] for H^+:ATP is no smaller than that for ATP:$2e^-$, one can have no more confidence in the derived stoicheiometry of ATP:$2e^-$ than one has in the value obtained by "direct" measurement. It is likely that only a new technique will allow H^+:ATP and therefore ATP:$2e^-$ to be estimated with greater confidence; for example, measurements of phase shift in the response of the proton gradient to modulated excitation[18] may prove to be applicable to problems of stoicheiometry.

In the meantime, estimated ATP:$2e^-$ values of 2.0 and 1.33 mean that noncyclic phosphorylation either does or does not satisfy the theoretical requirements of the reductive pentose phosphate pathway. These are ATP and NADPH in the ratio 3:2, that is, an ATP:$2e^-$ of 1.5. There is in the author's view ample evidence of the occurrence of cyclic phosphorylation under what appear to be physiological conditions (see the following sections), but this does not in turn require that the ATP:$2e^-$ of noncyclic phosphorylation is less than 1.5. It is possible that the theoretical requirements of the Calvin cycle take practical effect comparatively rarely even in C_3 plants. Other sinks for ATP exist within the chloroplast (synthesis and phosphorylation of chloroplast proteins, for example) and outside it (nitrate reductase, for example). It has been mentioned before in this context that the additional CO_2-concentrating steps of C4 photosynthesis result in an ATP:NADPH requirement of 5:2,[19] which even an ATP:$2e^-$ of 2.0 would not satisfy. The diversity of cyanobacterial bioenergetics provides two relevant examples: aerobic nitrogen fixation in cyanobacteria is thought to be driven by purely cyclic photophosphorylation in the heterocyst,[20] while CO_2 assimilation in sulfide-oxidizing *Oscillatorea limnetica* has been shown to be driven by photosystem I alone,[21] with what can only be assumed to be an inadequate maximum ATP:$2e^-$ for noncyclic phosphorylation of 1.0.

Thus whatever may be the absolute value for the stoicheiometry of purely noncyclic

photophosphorylation, it is possible to think of circumstances such as bundle sheath chloroplasts of C4 plants and cyanobacterial heterocysts where noncyclic photophosphorylation has been "avoided" because it produces oxygen, and where cyclic photophosphorylation must occur instead. Even in C3 higher plant chloroplasts, a convincing demonstration of an ATP:$2e^-$ ratio of 1.5 or greater will not constitute *a priori* grounds for regarding cyclic (or indeed pseudocyclic) photophosphorylation as a physiologically unimportant reaction.

B. Measurement of Cyclic Electron Transport

1. The Cyclic Chain

The cyclic and noncyclic electron transport chains have common electron carriers, and the proton motive force that they produce is delocalized. It is therefore impossible to distinguish a cyclic or noncyclic site of ATP synthesis. These factors make it experimentally difficult to analyze photophosphorylation into cyclic and noncyclic components. Light-driven ATP synthesis in the presence of an inhibitor of noncyclic electron transport such as DCMU must be a result of cyclic, but, by definition, is also nonphysiological. Although direct measurement of cyclic electron transport is impossible, numerous indirect and independent measurements of variables closely dependent on cyclic electron flow have now been made, and are discussed in this and the following sections. These variables include turnover of electron transport components measured by optical absorption spectrometry and EPR spectrometry; room-temperature chlorophyll fluorescence yield; "P518", that is, the electrochromic pigment bandshift; quenching of the pH-dependent fluorescence of 9-aminoacridine; and light scattering.

The electron carriers shared by cyclic and noncyclic electron transport include ferredoxin,[22] plastoquinone, plastocyanin and cytochrome f,[23,23a] and the Rieske iron-sulfur protein (see next section). The role of plastoquinone (a hydrogen rather than electron carrier) in cyclic is clearly essential if the reaction is to produce a proton gradient; indeed, artificial cofactors of cyclic electron transport must mimic plastoquinone in this respect if coupled phosphorylation is to occur.[9,23] Antibodies to ferredoxin-$NADP^+$ reductase have no effect on cyclic electron flow, while those against ferredoxin are inhibitory.[24] This indicates that one branch point of cyclic and noncyclic electron flow is at ferredoxin and that the reductase is not directly involved in the former. The unique component of the cyclic chain appears to be cytochrome b_{563} (cytochrome b_6),[25] the sensitivity of which to antimycin A accounts for the specific effect of this compound as an inhibitor of cyclic phosphorylation.[22] Cytochrome b_{563} may therefore be regarded as a necessary (and sufficient?) endogenous link between ferredoxin and plastoquinone, the two branch points in the chain.

2. Electron Flow from Ferredoxin to Intersystem Carriers

An additional component of the intersystem electron transport chain is the high-potential Rieske iron-sulfur protein.[26] The EPR studies of Malkin and Chain indicate that it may be involved in both cyclic and noncyclic electron transport.[27] The evidence for its involvement in noncyclic electron transport is based on its photooxidation in the presence of DCMU,[26] and on its photoreduction in the absence of DCMU.[27] The Rieske iron-sulfur center has a midpoint redox potential of around +300 mV[26] and is found in its reduced form in the dark. It is therefore necessary to prepare ferricyanide-washed membranes in order to demonstrate its reduction by photosystem II.[27] Similar results are obtained for plastocyanin.[26,27] The participation of the Rieske center in the cyclic chain is inferred from the ability of reduced ferredoxin to reduce it in the dark following its photooxidation in the presence of DCMU. This dark reduction of the Rieske center by ferredoxin can be inhibited by antimycin A.[27] Again, similar results were obtained for the

plastocyanin EPR signal, which indicates that an antimycin A-sensitive pathway of electron transport runs from the reducing side of photosystem I to the intermediary electron transport carriers. Although the EPR signals of the Rieske iron-sulfur protein and of plastocyanin are measurable only at liquid nitrogen temperature, the signal from the oxidized form of P700 (the reaction center chlorophyll of photosystem I) can be measured at room temperature, making kinetic studies possible. This allowed Malkin and Chain to demonstrate a double effect of reduced ferredoxin in introducing a lag phase in P700 oxidation by 740 nm light (absorbed primarily by photosystem I) and in increasing the rate of rereduction of P700 in a subsequent dark period. Again, both effects were found to be sensitive to antimycin A.[27]

A study of electron flow from ferredoxin via cytochrome b_{563} in the dark has also been carried out by Mills et al.[28] In this case the electron acceptor for the reaction was the primary acceptor of photosystem II, "Q", and its redox state was measured as the yield of variable chlorophyll fluorescence. This measurement depends on the fact that oxidized Q accepts electrons in the primary photochemical reaction of photosystem II, thereby quenching room-temperature chlorophyll fluorescence (emitted from antenna chlorophylls of photosystem II). Thus fluorescence yield increases as Q is reduced, that is, as the traps for excitation energy in photosystem II become closed. Mills et al.[28] estimated that about half the traps were closed in the presence of ferredoxin, NADPH, and magnesium ions, with a much lower proportion of traps being closed (11% or less) if any of these cofactors had been omitted. The pathway of electron flow from NADPH to Q was investigated further by the use of inhibitors. Thus the fluorescence increase was partially inhibited by antibodies to ferredoxin-$NADP^+$ reductase and by N-ethylmaleimide, a sulfhydryl inhibitor which inhibits the reductase in the presence of NADPH. Besides the requirement for ferredoxin, its involvement was indicated by inhibition with disulfodisalicylidenepropanediamine. The fluorescence increase was not affected by uncouplers, but was sensitive to antimycin A, and by implication therefore involves cytochrome b_{563} It is unlikely that the proton motive force exercised an effect on a rate-limiting step in this reaction and so the lack of effect of uncouplers does not mean that the pathway is not coupled to ATP synthesis in vivo. In fact the results of Mills et al.,[28] taken together with those of Malkin and Chain,[27] demonstrate that the cyclic chain is likely to involve plastoquinone (the link between Q and the cytochrome f-Rieske iron-sulfur part of the chain) and hence to be proton translocating.

3. Effects of Antimycin A in Intact Chloroplasts

Crowther et al.[29] and Slovacek et al.[30] have carried out direct measurement of factors dependent on the proton motive force that is generated by cyclic electron flow, under the relatively physiological conditions prevailing in intact chloroplasts.

The experiments of Crowther et al.[29] were carried out on intact chloroplasts in the presence of DCMU, and to which dithionite had been added to donate electrons to the otherwise fully oxidized cyclic chain. Under these conditions they were able to demonstrate light-induced quenching of the fluorescence of 9-aminoacridine, an effect that was absent in the presence of DCMU alone. Quenching of 9-aminoacridine fluorescence indicates a transmembrane pH gradient, since the fluorescent 9-aminoacridine is then taken up by the thylakoid and trapped there in its protonated, nonfluorescent form. Similar results were obtained for the flashed-induced change in A_{518}, a measure of the membrane potential which causes an absorption bandshift in carotenoid pigments at about 518 nm. Confirmation of these measurements was obtained by use of the uncoupling ionophores nigercin and valinomycin. Nigercin, which facilitates exchange of K^+ and H^+, eliminated the 9-aminoacridine fluorescence quenching but not the ΔA_{518}. Valinomycin, which facilitates transport of cations other

than H^+, eliminated ΔA_{518} but not the 9-aminoacridine fluorescence change. Again, both effects of cyclic electron flow were inhibited by antimycin A, and DBMIB (an inhibitor of plastoquinone oxidation) was also found to be inhibitory. Although the conditions employed (with DCMU and dithionite) were unphysiological, the results of Crowther et al.[29] certainly provide a demonstration of the generation by cyclic electron transport of both components of the proton motive force in intact chloroplasts in the absence of any artificial cyclic cofactor.

In intact chloroplasts at low oxygen concentration and without an added electron acceptor, Slovacek et al.[30] found 9-aminoacridine fluorescence quenching and ΔA_{518} to be maximal in the presence of low concentrations ($0.1\ \mu M$) of DCMU. This phenomenon is easily explicable in terms of "poising" (see Section II.D). Antimycin A was then inhibitory, which indicates a cyclic electron flow. Though its effect was proportionately greater in the presence of $0.1\ \mu M$ DCMU, antimycin A showed some effect even under aerobic conditions and in the absence of DCMU, which suggests that cyclic electron flow makes some contribution to the proton motive force in intact chloroplasts when oxygen is the only plausible electron acceptor.

Using intact chloroplasts supplied with CO_2 as electron acceptor, Slovacek and Hind[31] had previously shown that antimycin A actually stimulates photosynthetic oxygen evolution under conditions of low oxygen concentration. The greater stimulatory effect of antimycin A with electron acceptors (phosphoglycerate, oxaloacetate) that require less ATP than does CO_2 suggested that the effect of antimycin was to inhibit cyclic phosphorylation in this system, thereby preventing the inhibition of electron transport that would otherwise be caused by the back-pressure of a high ATP:ADP ratio. This conclusion was strengthened by the observed stimulation of oxygen evolution by low uncoupler concentrations and by ribose-5-phosphate (an "ATP-sink").[31] However, Mills et al.[32] have effectively withdrawn this suggestion, and have reported instead an inhibition of CO_2 fixation by antimycin A under otherwise optimal conditions. They suggest[32] that the stimulation reported in the previous paper[31] had nothing to do with the back-pressure of the high energy state, but instead was caused by the action of antimycin A in inhibiting cyclic electron flow as a route of ferredoxin oxidation. The more reduced state of ferredoxin that antimycin A produced would then reduce thioredoxin and thereby activate the rate-limiting steps of the Calvin cycle that are under thioredoxin control.[33]

Rosa and Whatley have presented evidence for such a mechanism being responsible for the stimulatory effect of uncouplers on CO_2 fixation.[34] The particular suboptimal condition required for the observation of stimulation by uncouplers is likely to be production of hydrogen peroxide by a Mehler reaction (that is, photosynthetic oxygen reduction) in the broken chloroplasts that are always present as contaminants of intact chloroplast preparations. The hydrogen peroxide tends to inactivate fructose bisphosphatase and glyceraldehyde phosphate dehydrogenase whose reactions then become rate limiting, such that further activation of the enzymes by increased reduction of ferredoxin and thioredoxin has a stimulatory effect on CO_2-dependent oxygen evolution.[34] Activation by reduced ferredoxin had previously been offered by Walker et al.[35] as an explanation of stimulation by antimycin A of fructose bisphosphatase and thereby CO_2 fixation. The importance both of hydrogen peroxide production and of regulation by thioredoxin in this system is indicated by the further experiments of Slovacek and Hind.[36]

From the point of view of a study of possible contributions of cyclic phosphorylation under physiological conditions, the redundancy of the "back-pressure" hypothesis of stimulation by uncouplers and by antimycin removes the need for the assumption that noncyclic itself produces more ATP than can be used by CO_2 fixation.[37] Correspond-

ingly, the inhibitory effects of uncouplers and of antimycin[32,36] can be viewed as evidence that noncyclic phosphorylation itself cannot meet total assimilatory requirements for ATP, and that antimycin-sensitive cyclic phosphorylation occurs under physiological circumstances.

4. Turnover of Cytochromes f and b_{563}

The characteristic α bands of cytochromes f and b_{563}, with absorbance maxima at 554 and 563 nm, respectively, have made these components particularly useful in the study of contributions of cyclic electron flow in intact chloroplasts. Slovacek et al.[38] observed a light-minus-dark steady-state difference spectrum for intact chloroplasts that was dominated by a peak at 518 nm, corresponding to the electrochromic change associated with the membrane potential. The portion of the difference spectrum relating to cytochromes f and b_{563} was isolated by subtraction of an extrapolated tail of the 518-nm peak and from the isosbestic points of the cytochromes. It was found that the cytochrome absorbance peaks decayed much more rapidly after a single flash of light than did the 518-nm peak,[38] and averaging the individual absorbance changes after a sequence of flashes at 1.5 Hz gave traces of the turnover of the cytochromes. Each flash caused oxidation of cytochrome f and reduction of cytochrome b_{563}. The rates of the subsequent dark reduction of cytochrome f and oxidation of cytochrome b_{563} were dependent on the conditions employed. For cytochrome f, the half-time of the dark reduction was decreased by uncouplers from 17 to 2 msec. For cytochrome b_{563}, the half-time of dark oxidation was decreased from 15 to 4 msec. DBMIB increased the half-times of the reactions (to 107 msec for cytochrome f and 47 msec for cytochrome b_{563}), as did antimycin A (to 166 msec for f and 48 msec for b_{563}). Slovacek et al.[38] conclude that cytochrome b_{563} is a donor and cytochrome f an acceptor for a coupling site which involves plastoquinone, and that electron transfer from b_{563} to f through the coupling site is sensitive antimycin A. They suggest on this basis that cyclic phosphorylation can supply ATP at a rate of 40 μmol (mg chl)$^{-1}$hr^{-1}, a figure derived from the observed turnover time (40 msec) of cytochrome f, an H^+:e^- value of 1 for the coupling site, and H^+:ATP value of 3.

Crowther and Hind[39] made similar observations with DCMU-inhibited intact chloroplasts supplemented with dithionite as a source of electrons for the cyclic chain, while Slovacek et al.[40] measured turnover of cytochromes f and b_{563} during CO_2- and phosphoglycerate-dependent electron transport in intact chloroplasts. In the latter study, the flash yield of cytochrome f oxidation was approximately equal to the sum of the yield of cytochrome b_{563} reduction and electron transfer through photosystem II. The flash yield of cytochrome b_{563} was 29% of that of cytochrome f during CO_2 fixation,[40] which represents strong evidence for a similar contribution of cyclic electron transport to total electron transfer through photosystem I under physiological conditions. The cyclic contribution to total photosynthetic phosphorylation would then be between 14 and 15%. If the ATP:$2e^-$ ratio for noncyclic is 1.33, then this amount of cyclic phosphorylation would increase the ATP:NADPH ratio from 1.33 to 1.52, that is, to a value sufficient for the steady-state fixation of CO_2 in the Calvin cycle.

C. Cyclic or Pseudocyclic?

Pseudocyclic photophosphorylation resembles truly cyclic photophosphorylation in that ATP synthesis appears in both cases to proceed independently of oxidation and reduction of substrate amounts of an electron donor or acceptor. In pseudocyclic phosphorylation this is no more than appearance, since dissolved oxygen from the air is in fact the terminal electron acceptor for a strictly noncyclic sequence of electron transfers. If the hydrogen peroxide so produced is allowed to accumulate, net oxygen

uptake occurs and can be measured. In the presence of catalase, however, the reduced oxygen is liberated again, and so net oxygen exchange does not occur.

Ferredoxin will act as a cofactor for pseudocyclic as well as for cyclic phosphorylation,[41,42] and so pseudocyclic phosphorylation is a plausible alternative candidate for the reaction that supplements any deficiency in the stoicheiometry of noncyclic phosphorylation.[43] This suggestion has been supported by an ability of ferredoxin to reduce oxygen and $NADP^+$ simultaneously in broken, washed chloroplasts,[44] and by an observed reduction of oxygen to hydrogen peroxide in intact chloroplast preparations fixing CO_2.[45]

Production of the toxic superoxide anion radical by pseudocyclic electron flow may not occur at significant rates provided ferredoxin is the cofactor.[46,47] Equally, the potentially deleterious effects of hydrogen peroxide on CO_2 fixation[48] can in principle be overcome by the operation of ascorbate-dependent peroxidation reactions.[49-51] Although production of hydrogen peroxide in intact chloroplast preparations has been repeatedly demonstrated,[45,52,53] it has not yet been shown that this is a physiological occurrence. By virtue of their inhibitory effects on CO_2 fixation, addition of nonspecific catalase inhibitors such as azide[52] and cyanide[53] would be expected to induce, rather than merely reveal, electron flow to oxygen. A more selective inhibitor, aminotriazole, has been used to inhibit catalase but not CO_2 fixation,[54] a possibility which demonstrates that autoinhibitory concentrations of hydrogen peroxide are not necessarily produced during CO_2-dependent electron transport. Endogenous catalase activity of chloroplast preparations can be removed by washing, and this also fails to reveal the sensitivity to added catalase that would result from endogenous production of hydrogen peroxide.[55] It therefore seems likely that the now routine addition of catalase to intact chloroplast preparations is a precaution taken against production of hydrogen peroxide in the broken chloroplasts that are present as contaminants.[56]

For certain algae and cyanobacteria evidence for physiological and continuous photosynthetic reduction of oxygen is stronger than it is for isolated chloroplasts. Thus mass spectrometry with ^{18}O-oxygen has been used to show appreciable rates of oxygen reduction in *Scenedesmus, Chlorella,* and *Anacystis*,[57-59] while rapid production of hydrogen peroxide has been detected by a fluorescence technique applied to a strain of *Anacystis nidulans*.[60]

In the present context it appears that photosynthetic reduction of oxygen is important physiologically and under optimal conditions only as a transient phenomenon by which the carriers of the cyclic chain may be poised (see the next section). This does not preclude the continuous coupled flow of electrons to oxygen under certain circumstances, such as when CO_2 is unavailable. Although it is possible to visualize extreme circumstances in which poising may be impossible and the regulatory flow of electrons to oxygen may be permanently switched on, it is not obvious from the evidence now available that pseudocyclic phosphorylation by itself supplies ATP in steady-state photosynthesis under optimal conditions.

D. Redox Poising

It is axiomatic that electron transfer depends on a reduced donor and an oxidized acceptor, and that the rate of the reaction will be a function of the activity of each. In the cyclic chain, however, all carriers are effectively both donors and acceptors, and so the overall rate of electron flow will depend on each carrier being present in both the oxidized and the reduced state. If the entire cyclic chain were fully reduced, no electron acceptors would be available and the rate of electron flow (and of coupled phosphorylation) would be zero. Such a chain can be described as "overreduced". Conversely, if all the carriers were completely oxidized, then the rate of electron transfer

and coupled phosphorylation would also be zero; such a chain can be said to be "overoxidized". Between these extremes lies an optimum steady redox state of the carriers at which the rate of electron flow would be maximal, and at which the chain could be described as completely "poised".

If the cyclic chain has carriers in common with the noncyclic chain, then even a slow net influx of electrons will lead quickly to inhibition of cyclic electron flow by overreduction of its components. Poising here will depend on an electron acceptor being available to counteract the net influx of electrons. A net efflux of electrons would, on the other hand, produce inhibition by overoxidation, and poising will then depend on the presence of a suitable electron donor.

Under experimental conditions, a poising agent may be as permanent a requirement as the imbalance in influx and efflux that would otherwise cause inhibition. Under physiological circumstances, one can visualize poising as a transient phenomenon. If the cyclic chain were for some reason overreduced and ATP synthesis thereby insufficient for the operation of the Calvin cycle, NADPH would not be oxidized by the triose phosphate dehydrogenase reaction. The high NADPH:$NADP^+$ ratio would keep the cyclic chain overreduced and the inhibition would be permanent. A brief "bleeding-off" of electrons (a plausible oxidant is, of course, oxygen) would poise the cycle chain and hence allow some synthesis of ATP. This in turn would allow oxidation of NADPH by the reactions of CO_2 fixation, whereupon the lower NADPH: $NADP^+$ ratio would keep the cyclic chain poised without further need for an additional poising oxidant.

The concept of redox poising was introduced by Whatley[61] and by Tagawa et al.[62] in order to explain a requirement for oxygen of truly cyclic phosphorylation catalyzed by phenazinemethosulfate and by ferredoxin. Further studies on broken, washed chloroplasts were carried out by Grant and Whatley,[63] and interest in poising appears to have been revived by work of Arnon and Chain,[64,65] who demonstrated a partial requirement for oxygen in ferredoxin-catalyzed cyclic phosphorylation and suggested that oxygen serves as a poising agent both in vitro and in vivo.

In vitro, cyclic phosphorylation with ferredoxin and under 554 nm light was found to be stimulated by DCMU,[64] despite the fact that DCMU is known to have a direct (inhibitory) effect only on noncyclic electron flow. The explanation offered for this by Arnon and Chain is that the operation of photosystem II will tend to overreduce the cyclic chain, and that DCMU prevents this overreduction. In 715 nm light, however, DCMU is inhibitory to cyclic phosphorylation under aerobic conditions. This indicates that a slow turnover of photosystem II is required to replenish the supply of electrons otherwise lost to oxygen. Arnon and Chain[64] also show an inhibitory effect of antimycin A on the observed P:$2e^-$ ratio with added ferredoxin and $NADP^+$; a value of 1.55 declined with increasing antimycin concentration to a value of 1.0 and was then relatively independent of further increase in antimycin concentration. They further demonstrated a poising effect of NADPH, which they attributed to diminution of electron flow from water, though it also appears that NADPH will poise an otherwise overoxidized cyclic chain in the presence of concentrations of DCMU that are partially inhibitory to electron flow from water.[65,66]

Redox poising of cyclic phosphorylation has also been demonstrated under the more physiological conditions prevailing in isolated intact chloroplasts. Heber et al.[67] have even provided evidence for its occurrence in vivo, using light scattering as a measure of the proton gradient across the thylakoid membrane.[68] Using intact chloroplasts in vitro, Heber et al.[67] found that the presence of nitrite (as a terminal electron acceptor) increased both 9-aminoacridine fluorescence quenching and light scattering (measured at 535 nm) in red light (651 nm). Conversely, nitrite had an inhibitory effect on both variables if the chloroplasts were illuminated instead with far-red light (710 nm). These observations

suggest that the proton gradient is established predominantly by noncyclic electron flow in red light and by cyclic electron flow in far-red light, the cyclic flow being susceptible to overoxidation in the presence of nitrite.

Under anaerobic conditions, red light causes little quenching of 9-aminoacridine fluorescence in intact chloroplasts, and a continuous fluorescence trace shows an appreciable effect of red light only when oxygen has been introduced.[67] In comparison, far-red light quenches 9-aminoacridine fluorescence under anaerobic conditions, and the degree of quenching is decreased when oxygen is introduced. Thus evidence is presented again that cyclic phosphorylation is susceptible to overreduction by red light under anaerobic conditions and to overoxidation under far-red light in the presence of oxygen. Heber et al.[67] go on to demonstrate that, in addition to nitrite and oxygen, oxaloacetate will also have effects on cyclic electron flow and its attendant proton gradient in intact chloroplasts. In the absence of oxygen and oxaloacetate, far-red light is much more effective than red light in causing 9-aminoacridine fluorescence quenching, while after addition of oxaloacetate, red light becomes more effective than far-red light.

Spinach leaves illuminated with far-red light in the absence of CO_2 scatter light to a greater extent in nitrogen than in air; in red light they scatter light more in air than in nitrogen.[67] In the absence of oxygen and CO_2, scattering that is induced by far-red light is inhibited by a supplementary beam of red light, and the degree of inhibition is dependent on the intensity of the supplementary beam.

In isolated intact chloroplasts in red light and provided with CO_2 as the electron acceptor, the transition from anaerobic to aerobic conditions is accompanied by an increase in the transthylakoid proton gradient.[69] Here oxygen is clearly necessary for the establishment of the high energy state upon which phosphorylation depends. In this experiment of Ziem-Hanck and Heber[69] the proton gradient was shown not to be oxygen dependent where phosphoglycerate (with a low ATP requirement) or nitrite (with none at all) served as electron acceptors. This suggests that the cyclic chain is overreduced if oxygen is absent even where CO_2 is available for photosynthesis. The implications are that insufficient ATP for CO_2 fixation is produced by noncyclic phosphorylation acting alone, and that without oxygen cyclic cannot function to provide the missing ATP because the chain is then overreduced. Oxygen can then correct the overreduction and thereby allow the establishment of a proton gradient and ATP synthesis. The onset of CO_2 fixation is then accompanied by oxidation of NADPH at rates sufficient to prevent further overreduction of the cyclic chain without the participation of oxygen. Having served to unlock photophosphorylation by these means, further reduction of oxygen becomes unnecessary.

This interpretation is strengthened by Ziem-Hanck and Heber's observation[69] that CO_2 fixation in intact chloroplasts is absent under totally anaerobic conditions, these being obtained enzymically with glucose, glucose oxidase, and catalase. Addition of a small quantity of hydrogen peroxide causes a transient increase in oxygen concentration which is sufficient to initiate CO_2 fixation. CO_2 fixation then proceeds at the same rate as in an aerobic control, despite the return of the oxygen concentration to zero by the action of the glucose-glucose oxidase trap. These elegant experiments are, of course, entirely consistent with the hypothesis that oxygen serves as a poising agent under physiological conditions, and that poised cyclic photophosphorylation is a necessary condition for the onset of photosynthesis. Additional evidence for this hypothesis has been provided by Steiger and Beck.[70]

It is also clear that the relative electron flux through the cyclic chain will depend in part on the $NADP^+$:NADPH ratio[71] There is evidence that NADPH may act as an electron donor and so poise cyclic phosphorylation in the bundle sheath chloroplasts of C_4 plants.[72] Here cyclic electron flow is particularly susceptible to overoxidation because of

the absence of photosystem II. The NADPH may be provided by oxidation of malate.[72] In isolated *mesophyll* chloroplasts of C_4 plants, oxygen has been implicated as a poising agent for cyclic phosphorylation when it drives pyruvate-dependent CO_2 fixation.[73] The existence of the ancillary C_4 pathway (which requires only ATP) and the Calvin cycle in separate compartments makes flexibility of the ATP:NADPH ratio an absolute requirement in C_4 plants. The absence of photosystem II activity from the bundle sheath[74] is further evidence that photosystem I-driven cyclic phosphorylation is a physiological occurrence, while the effect of malate in this system[72] shows that redox poising is a plausible mechanism of control.

III. REGULATION OF NONCYCLIC PHOSPHORYLATION

A. Thylakoid Protein Phosphorylation and Distribution of Excitation Energy

Protein phosphorylation is a widespread and biochemically important example of regulation of enzyme activity by covalent modification,[75] and usually takes the form of transfer of the terminal phosphate group of ATP to the hydroxyl groups of seryl or threonyl residues of the protein substrate. The transfer is catalyzed by a protein kinase, and the reverse reaction (of dephosphorylation) by a protein phosphatase.

Protein phosphorylation is conveniently measured using $\gamma[^{32}P]$ATP; phosphorylation then leaves the relevant protein covalently labeled. The labeled phosphoprotein can easily be detected by autoradiography of a polyacrylamide gel after electrophoresis; only the labeled bands on the gel will expose an X-ray film. Comparison of the film with the gel allows immediate identification of the participants in the phosphorylation reaction, and scintillation counting of the cut-out bands gives a measure of the degree of their phosphorylation.

Chloroplast protein phosphorylation was first demonstrated in this way by Bennett,[76] who subsequently identified the most conspicuously phosphorylated thylakoid protein as light-harvesting chlorophyll a/b protein.[77] Bennett also showed that both the kinase and the phosphatase are themselves thylakoid bound.[78] Thus the reaction may be studied not only in intact chloroplasts supplied with ^{32}P-orthophosphate, but also in thylakoids supplied with $\gamma[^{32}P]$ATP. Protein phosphorylation driven by photophosphorylation should in principle be measureable in coupled thylakoids supplied with ^{32}P-orthophosphate and ADP, though this experiment seems not to have been tried.

The major substrate, light-harvesting chlorophyll a/b protein (LHCP), is one of a small class of chlorophyll-binding proteins.[79] It has a molecular weight of 26,000, and despite being predominantly hydrophobic, it has a terminal sequence of hydrophilic amino acid residues which is exposed at the surface of thylakoid; one of these residues is the threonine phosphorylation site.[78] LHCP binds chlorophylls *a* and *b* in equal amounts.[79] These chlorophylls function in light harvesting, that is, in absorption of light and not in the transfer of excitation energy that is absorbed elsewhere. The destination of excitation from light-harvesting chlorophyll is largely (and variably, as discussed later) the reaction center of photosystem II. Both photosystems also have distinct antenna and reaction center chlorophylls.[80,81]

Bennett's conclusions[76–78] have been substantiated by work both on chloroplasts[82] and on *Euglena* cells.[83] A number of other thylakoid phosphoproteins have been assigned identities by Süss.[84] The principle that a complex and expensive reaction must occur for some reason is only superficially teleological, and, as this article attempts to show, has been fruitfully applied in the case phosphorylation of LHCP. As yet, however, the "function" of the numerous other protein phosphorylations of chloroplasts is unknown. The suggested[82] phosphorylation of a subunit of the proton channel (CF_o) of the coupling ATPase is clearly of potential relevance to the subject of this review, but is at present contested[84] and unexplained.

The practical effect of phosphorylation of LHCP is now thought to be diversion of excitation from photosystem II to photosystem I. This conclusion has followed from earlier demonstrations that modification of LHCP affects excitation distribution. Burke et al.[85] showed that antibodies to purified LHCP decrease the effects of magnesium ions on chlorophyll fluorescence in chloroplasts, while Steinback et al.[86] showed that the surface-exposed segment of LHCP was essential for cation-mediated thylakoid stacking and the attendant regulation of excitation distribution. The suggestion that phosphorylation of LHCP alters its functional properties was made by Bennett et al.[87]

In support of this conclusion, Bennett et al.[87] report fluorescence changes in chloroplasts incubated under the conditions that are known to support protein phosphorylation. Their evidence that excitation is redistributed to photosystem I on phosphorylation is twofold; when ATP is present, room temperature chlorophyll fluorescence is decreased and liquid nitrogen-temperature chlorophyll fluorescence undergoes a spectral change indicating increased relative fluorescence emission from photosystem I.

Chlorophyll fluorescence at room temperature arises predominantly from chlorophylls of photosystem II. The fluorescence intensity is dependent both on the amount of excitation absorbed and on the competence of photosystem II to use the excitation for electron transport. Where these factors are kept constant, one or more further factors must be responsible for any fluorescence change observed. The additional factor responsible for the room-temperature fluorescence decrease observed in the presence of ATP is held to be increased transfer of excitation to the nonfluorescent chlorophylls of photosystem I. ATP has an additional effect on room-temperature fluorescence—known as "high energy-state quenching"—and can be produced either by ATP or by electron transport. In both cases it is inhibited by uncouplers. Uncouplers have no effect on protein phosphorylation or on the protein phosphorylation-dependent fluorescence quenching.[87]

At 77K two peaks are apparent in the chlorophyll fluorescence emission spectrum of chloroplasts. The peak at 685 nm is fluorescence from chlorophylls of photosystem II; that at 735 nm is from chlorophylls of photosystem I.[80] Bennett et al.[87] showed that incubation of chloroplasts at room temperature under protein phosphorylation conditions caused an increase in emission at 735 nm relative to that at 685 nm in spectra obtained subsequently at 77K. In other words, the presence of light and ATP causes changes in low-temperature fluorescence that may indicate increased excitation transfer to photosystem I, at the expense of photosystem II.

The same conclusion was reached by Horton and Black[88] who went on to show that the rate of quenching of room-temperature chlorophyll fluorescence was increased by fluoride, which is consistent with its action as an inhibitor of the phosphatase.[78] Horton and Black[88] found that protein phosphorylation conditions decreased both F_m (the maximum fluorescence seen when all photosystem II traps are closed) and F_o (the fluorescence at the moment when illumination begins). In contrast Bennett et al.[87] report an effect only on F_m.

B. Regulation by Plastoquinone

1. Control by Oxidation Reduction

Thylakoid protein kinase is dependent on light and sensitive to inhibition by the electron transport inhibitor DCMU,[89] as is the ATP-induced fluorescence quenching[88] that it is thought to produce. The possibility that the reaction is dependent on a component of the proton motive force can be discounted immediately on the grounds of its insensitivity to uncouplers.[87-89]

An alternative explanation of light dependence and DCMU sensitivity is that the kinase is controlled in some way by the redox state of an electron carrier either in or in

contact with the noncyclic chain. Thus the kinase could be activated either by the oxidized form of a carrier situated before the site of inhibition by DCMU or by the reduced form of a carrier situated after it. The results of Allen et al.[90] indicate that it is a photoreduction that activates the kinase, and the regulatory component must therefore come after the site of action of DCMU.

The initial basis for this assertion was the inhibitory effect of the oxidant ferricyanide and the ability of the strong reductant dithionite to activate the protein kinase both in darkness and in the light in the presence of DCMU.[90] In fact dithionite gave a greater degree of activation of the kinase than light. The proposed relationship between protein phosphorylation and distribution of excitation energy was borne out by the corresponding effects of ferricyanide and dithionite on fluorescence emission spectra at 77K.[90] Thus the light-dependent increase in 735 nm emission relative to that at 685 nm was suppressed by the presence of ferricyanide in the room-temperature preillumination, while a similar increase could be seen in darkness provided dithionite was present instead.[90] The changes in low-temperature fluorescence were at least partly dependent on the presence of ATP during the room-temperature incubation, in agreement with the idea that they depend on the activity of the protein kinase.[90]

2. Donors and Inhibitors

Ferredoxin was initially a plausible candidate for the component that controls protein kinase activity. In support of this Bennett[89] found a doubling of activity on addition of ferredoxin (to 10 μM) to illuminated, washed thylakoids. He also saw an increase in kinase activity in the dark when NADPH and ferredoxin were present. The view that ferredoxin was the controlling element first became untenable when it was found that dichlorophenolindophenol (DCPIP) with ascorbate would not restore protein phosphorylation that had been inhibited by DCMU.[88,90] The ineffectiveness of the donor couple ascorbate-DCPIP was the first indication that a component of the interphotosystem chain might be involved.[88,90] The observation that reduced ferredoxin activates the kinase somewhat in the dark[89] can be accommodated by this alternative hypothesis, since it is known that ferredoxin will reduce such a component via the cyclic chain.[27,28,64] If this explanation is correct then dark activation of the kinase by reduced ferredoxin should be inhibited by antimycin A.

Unlike DCPIP-ascorbate, the electron donor tetramethyl-*p*-hydroquinone ("$TMQH_2$", also known as duroquinol) has been found to activate protein phosphorylation in the dark.[91] Also unlike that of DCPIP-ascorbate, donation by $TMQH_2$ (which is prepared by reduction of TMQ with borohydride) supports a photosystem I electron transport that is sensitive to inhibition by DBMIB, an inhibitor of plastoquinone oxidation.[91-93] A useful control is provided by the DBMIB insensitivity of photosystem I electron transport where TMQ with ascorbate functions as the donor; TMQ with ascorbate does not activate protein phosphorylation in the dark.[91] As expected, quenching of room-temperature chlorophyll fluorescence is obtained in the presence of $TMQH_2$, but not in the presence of TMQ with ascorbate. The association between fluorescence quenching and protein phosphorylation is maintained: both are activated by $TMQH_2$, a donor to the chain at some point between the sites of inhibition by DCMU and by DBMIB; neither is activated by DCPIP or by TMQ with ascorbate, which donate at sites unaffected by DBMIB and therefore closer to photosystem I.[91]

DBMIB itself actually has no inhibitory effect on activation of the protein kinase, at least at concentrations (e.g., 0.2 μM) at which it inhibits oxidation of plastoquinone.[90] In fact it may give slight stimulation of protein phosphorylation at these concentrations.[90] At 10- to 20-fold higher concentrations DBMIB begins to inhibit protein phosphorylation. This secondary, low-affinity site precedes plastoquinone in the chain, as indicated by the similar effect of DBMIB on electron transport from water to the

photosystem II acceptor, diaminodurene.[90] The conclusion of studies with donors and inhibitors is that the protein kinase is active only in the presence of the reduced form of an electron carrier situated immediately before the primary site of action of DBMIB.[90,91]

3. Fluorescence Induction and Redox Titration

The redox state of the plastoquinone pool can be controlled by varying the number of saturating, single-turnover flashes of light given to dark-adapted chloroplasts; the greater the number of flashes the more reduced the pool becomes. Plastoquinone redox state was controlled in this way by Allen et al.[90] and was measured as the area above the induction curve of chlorophyll fluorescence at room temperature. After 16 flashes, the area above the curve was found to have been decreased by 35% and the degree of protein phosphorylation that the flashes had induced in the presence of ATP was of a similar magnitude. Protein phosphorylation showed a dependency on the number of flashes which was similar to that shown by reduction of plastoquinone.

The most direct way of controlling redox state is by potentiometric redox titration. This was applied to ATP-dependent chlorophyll fluorescence quenching by Horton and Black[94] and to fluorescence quenching and protein phosphorylation, measured in the same samples, by Horton et al.[95] Horton et al.[95] also report results of a titration of the area above the fluorescence induction curve, effectively a titration of plastoquinone. Horton and Black[94] found a midpoint potential at pH 7.8 of +55 mV for the fluorescence quenching, while Horton et al.[95] found a slightly lower value of about 0 mV. Horton et al.[95] found precisely the same value (0 mV) for the titrations both of phosphorylation and of the area above the induction curve. The correlation between the degree of dark protein phosphorylation (as a proportion of that seen after 10 min light) and chlorophyll fluorescence quenching was extremely close. Since the fluorescence measurements were made on radioactive chloroplast suspensions from which samples were drawn for subsequent measurement of ^{32}P incorporation, the connection between fluorescence quenching and protein phosphorylation is particularly well established by these results. All the titration curves obtained[94,95] fit a Nernst plot for a two-equivalent carrier.

In summary, redox titration shows that both thylakoid protein phosphorylation and chlorophyll fluorescence quenching are activated by a two-equivalent carrier with a midpoint potential of between 0 and 50 mV. The carrier is indistinguishable by these means from plastoquinone.

C. Self-Adjusting Distribution of Excitation Energy: Protein Phosphorylation and State 1-State 2 Transitions

Before describing the central hypothesis of this section a preliminary qualification is warranted: Arnon et al.[96,97] have recently called into question the chief paradigm of photosynthesis research, the "Z-scheme". The Z-scheme is at present indispensable for the intelligibility of most of the work carried out in this area since 1960; Emerson enhancement, state 1-state 2 transitions, and light-driven oxidation reduction of inter-photosystem components are all simply inconsistent with the idea[97] that the two photosystems operate other than in series. The theory cannot therefore be lightly cast aside, though its proponents perhaps have some obligation to show how it may accommodate the heterodox results.

In the present context, plastoquinone-mediated protein phosphorylation is held to enable the Z-scheme to accommodate adaptations such as state 1-state 2 transitions. It is not the author's intention to exclude the possibility that the whole theory is false; any impression of conviction is an accidental product of his attempts to explain certain experimental results and to avoid overuse of the conditional. Myers[98] has pointed out that in photosynthesis (as elsewhere) a tacit assumption may be a dangerous assumption.

If the photosystems operate in series then their rates must be equal for any *constant*

rate of purely noncyclic electron transport; electrons are neither created nor destroyed. Any transient disparity in the relative rates of the photosystems will depend on the plastoquinone pool, and will have an immediate effect on its redox state. For example, plastoquinone will become reduced if photosystem II runs momentarily faster than photosystem I. If the protein kinase is then activated because plastoquinone is reduced, and if excitation is diverted to the rate-limiting photosystem I because LHCP is phosphorylated, then photosystem I will increase in rate until its equality with photosystem II is restored. In short, an imbalance in excitation distribution will have been self correcting. Furthermore, excess excitation energy previously wasted on the essentially light-saturated photosystem II will have been usefully diverted to the light-limited photosystem I, with a corresponding increase in overall quantum efficiency. Consider an imbalance in the opposite direction: if for any reason photosystem I starts to run faster than photosystem II, plastoquinone will become oxidized and the kinase switched off. The phosphatase will then dephosphorylate LHCP, and a greater proportion of absorbed excitation energy will become available to the previously rate-limiting photosystem II.

This negative-feedback loop will make excitation distribution self regulating within certain limits, and is a solution to the "carburetor" problem described by Myers;[99] quantum yield is known to be high and constant over a range of wavelengths which would otherwise be expected to favor one or other of the photosystems and so give limited efficiency. The hypothesis is depicted here in Figure 1, and in slightly different form elsewhere.[90,95]

The operation of this regulatory mechanism is offered as an explanation of the experimental phenomenon of state I-state 2 transitions,[99] described first by Bonaventura and Myers[100] and by Murata.[101] Bonaventura and Myers[100] measured the rate of oxygen evolution of a *Chlorella* suspension illuminated by a modulated beam of 645 nm light. In previously dark-adapted cells, the rate of oxygen evolution increased slowly (half-time about 2 min) with time of illumination. On addition of continuous 710 nm light the rate of oxygen evolution increased quickly, as the increased turnover of photosystem I made the acceptor of photosystem II more oxidized. There followed, however, an additional, slow increase in rate. This increase represents an adaptation to increased efficiency of use of the 710 nm light; no direct effect of the 710 nm light could be seen since measurement was made of only the oxygen evolution that was modulated at the frequency of the 645 nm light. On switching off the continuous 710 nm light, the rate decreased to its initial, low level whereupon it increased again slowly as before. Since 645 nm light is absorbed preferentially by photosystem II, it was termed "light 2" and the slow adaptation which it produced was termed a transition to "state 2". 710 nm light is partially selective for photosystem I and was termed "light 1"; the second adaptation (in the presence of continuous light 1) was therefore a transition from state 2 to state 1. Simultaneous measurement of modulated chlorophyll fluorescence[100] suggested that the transition to state 2 could be explained as increased excitation transfer to the otherwise rate-limiting photosystem I; fluorescence decreased as the rate of oxygen evolution increased. Similarly, the transition to state 1 (in continuous light 1) involved redistribution of excitation to photosystem II; modulated fluorescence increased as the rate of oxygen evolution also increased.

The explanation of these adaptations in terms of plastoquinone-regulated protein phosphorylation is attractive and economical (Figure 1). In light 2, excitation distribution favors photosystem II, and plastoquinone becomes reduced. The protein kinase is activated, and LHCP is phosphorylated. Excitation is then distributed more equitably to photosystem I, and less light energy is wasted on photosystem II. In consequence, quantum yield increases and fluorescence drops. Thus reduction of plastoquinone and consequent phosphorylation of LHCP form the basis of the transition to state 2. On

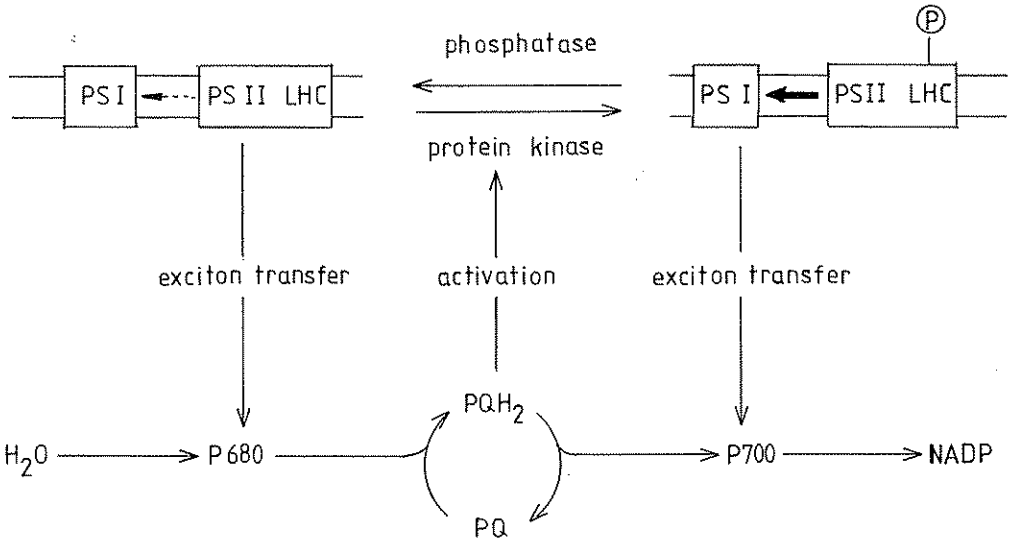

FIGURE 1. Regulation of distribution of excitation energy by redox state of plastoquinone, as proposed by Allen et al.[90] and Horton and Black.[94] Surplus exciton transfer to P680 causes reduction of plastoquinone, activation of protein kinase, phosphorylation of LHC protein, and increased exciton transfer to P700. Surplus exciton transfer to P700 causes oxidation of plastoquinone, inactivation of the kinase, dephosphorylation of LHC protein, and increases exciton transfer to P680. By means of this negative feedback control, distribution of excitation energy (excitons) between the photosystems will be self adjusting within certain limits. PQ, plastoquinone; PQH_2, plastoquinol; PSI, photosystem I; PSII, photosystem II; P680, reaction center chlorophyll of PSII; P700, reaction center chlorophyll of PSI; LHC(P), light-harvesting chlorophyll (protein); P, phosphate group.

addition of light 1, photosystem I goes momentarily faster than photosystem II, and plastoquinone is oxidized. The protein kinase is switched off, and the phosphatase catalyzes dephosphorylation of LHCP. Excitation from light-harvesting chlorophyll is then directed back to the rate-limiting photosystem II, and both quantum and fluorescence yield increase. The transition to state 1 has been initiated by oxidation of plastoquinone and carried out by dephosphorylation of LHCP.

The idea of the redox state of an interphotosystem electron carrier being involved in state 1-state 2 transitions was first put forward by Duysens,[102] and evidence for it has more recently been obtained by Ried and Reinhardt.[103,104] Reid and Reinhardt[104] discuss previous proposals that state 1-state 2 is in some way a result of efflux of cations from the thylakoid,[105] and point out that these are not easily compatible with their observation that both transitions (to state 1 *and* to state 2) have a quantum requirement. One state should be low energy, and the transition to it a passive process if concentration gradients are involved. In addition, the antagonistic effects of lights 1 and 2 are evidence for control by redox state and against control by ion fluxes. The hypothesis depicted in Figure 1 is, of course, a more detailed restatement of Duysens' original proposal.[102]

While a number of aspects of the hypothesis remains unexplained, it has been suggested that phosphorylation of LHCP causes increased excitation transfer to photosystem I by affecting the electrical charge on the thylakoid surface.[106] If this is correct, phosphorylation and magnesium ions should have antagonistic effects. Magnesium ions are thought to cause thylakoid stacking and minimize excitation transfer to photosystem I by electrostatic screening of fixed negative charges, and the number of these charges should be increased by phosphorylation of LHCP.[106] Phosphorylation-induced changes in linear dichroism spectra indicate a 10% unstacking of phosphorylated

thylakoids.[106a] As far as the mechanism of activation of the protein kinase is concerned, it has been found that lipophilic sulfydryl reagents inhibit kinase activity, and the suggestion has been made that the kinase is sulfydryl activated.[107]

Further evidence for the role of protein phosphorylation in state 1-state 2 transitions has been obtained by Telfer and Barber[108] and by Chow et al.,[109] who demonstrated a requirement for ATP in state 1-state 2 transitions in isolated chloroplasts when measured as changes in chlorophyll fluorescence. The measurement of state 1-state 2 transitions in broken chloroplasts depends on the presence of ferredoxin or ferredoxin with $NADP^+$ at suitable concentrations; artificial Hill oxidants will tend to keep the plastoquinone pool fully oxidized and suppress kinase activity. This work has been extended by simultaneous measurement of LHCP phosphorylation; the transition to state 2 in chloroplasts is accompanied by a rise in LHCP phosphorylation, and the transition to state 1 with a fall (Telfer and Allen, unpublished results). In the same experiments, the transition to state 1 and its attendant dephosphorylation were found to be inhibited by fluoride, which is consistent with the proposed role of the protein phosphatase.

ATP dependency of the fluorescence changes associated with state 1-state 2 in chloroplasts has also been demonstrated by Sinclair and Cousineau,[110] who also measured an ATP-dependent change in Emerson enhancement.[99] In this case[110] enhancement was measured as the effect of supplementary light 1 (700 nm) on oxygen evolution driven by modulated light 2 (640 nm). Enhancement caused by supplementary light 1 indicates that photosystem II receives more of the excitation derived from light 2 than does photosystem I; the system is in state 1. In the experiment of Sinclair and Cousineau,[110] the presence of ATP permitted a redistribution of excitation such that the enhancing effect of supplementary light 1 decreased. Surprisingly, the rate of oxygen evolution in light 2 was itself unaffected by ATP. Under appropriate conditions, of course, the transition to state 2 should give an increased quantum yield in light 2, and therefore an increase in rate of the kind first reported by Bonaventura and Myers.[100] The enhancing effect of light 1 will then be small or absent.

Perhaps the least substantiated assumption of the "phosphorylation and excitation distribution" hypothesis (Figure 1) is that phosphorylation causes redirection of excitation to photosystem I. The experiments on room-temperature chlorophyll fluorescence[87,88,91,94,108-110] merely indicate a decreased fluorescence yield from chlorophyll of photosystem I. Equally, the corresponding changes in 77K fluorescence spectra[87,88,90,109] merely indicate a decrease in emission from photosystem II relative to that from photosystem I. Direct effects of phosphorylation on photosystem I electron transport are required; the only effects of this kind published so far have been somewhat unconvincing.[111]

Horton and Black[112] have actually suggested that phosphorylation of LHCP leads merely to a decrease in the rate of delivery of excitation to the reaction center of photosystem II. Their conclusion was reached on the basis of experiments on redox changes of cytochrome f in chloroplasts. In the presence of methyl viologen and oxygen as electron acceptors, light 1 caused an oxidation of cytochrome f while light 2 reversed the effect.[112] In phosphorylated membranes, light 1 caused a similar oxidation but the amplitude of the re-reduction by light 2 was greatly decreased. This suggests that phosphorylation attenuates photosystem II without amplifying photosystem I. In addition, oxidation of cytochrome f by light 2 in the presence of DCMU was unaffected by phosphorylation. If excitation from light 2 is transferred to photosystem I by phosphorylated LHCP, then phosphorylation should have increased the amplitude of cytochrome f oxidation by light 2.

The results of Horton and Black[112] are a challenge to the hypothesis depicted in *Figure* 1; if excess excitation is not transferred to photosystem I, then phosphorylation can have no direct effect on quantum efficiency. Sinclair and Cousineau[110] saw no effect of ATP on oxygen yield in chloroplasts, which is not to say that such an effect does not

exist — merely that it has yet to be demonstrated. The hypothesis would certainly be more securely based if the original detailed studies of oxygen and fluorescence yields by Bonaventura and Myers were to be repeated and found to be dependent on changes in phosphorylation of LHCP.

IV. DISTRIBUTION OF EXCITATION ENERGY AND THE EFFICIENCY OF PHOTOPHOSPHORYLATION

The selective advantage of the adaptations that may be demonstrated experimentally as state 1-state 2 transitions is not immediately clear; monochromatic lights 1 and 2 do not occur in nature. Changes in the spectral composition of daylight are usually slow, being diurnal or seasonal. For terrestrial plants at least, adaptations to being shaded by other plants may be important but need not be rapid. It is known that plants grown under far red-enriched light develop a higher ratio of photosystem II to photosystem I, and show associated changes in chloroplast morphology.[113] For pelagic algae rapid response to changing wavelength may be more important; besides shading effects of other algal cells, the absorption spectrum of water itself makes spectral composition dependent on depth.

It is possible, however, that adaptive regulation of excitation distribution also has to do with adjustment of the relative rates of the two photosystems even under illumination of constant wavelength. In the foregoing discussion of the role of plastoquinone in correcting imbalances in the rates of the photosystems (Section III.C), emphasis was placed on the photosystems having equal rates of turnover. Clearly this does not apply if cyclic phosphorylation occurs at appreciable rates. Thus a balanced distribution of excitation for noncyclic electron transport would be an imbalance favoring photosystem II if cyclic electron transport were introduced.

Consider a hypothetical transition from purely noncyclic photophosphorylation to a mixed photophosphorylation with a 15% higher overall production of ATP (the feasibility of such a contribution was discussed in Section II.B.4). If pseudocyclic phosphorylation were the ancillary reaction, a 15% contribution of electron flow to oxygen would suffice; the quantum yields of non- and pseudocyclic phosphorylation are equal, and so the photosynthetic quantum requirement would also increase by 15%. However, if cyclic phosphorylation (using only one of the two coupling sites) were the ancillary reaction, then a cyclic electron flow of 30% of that through photosystem I would be required. If distribution of excitation were fixed, then relative excitation delivery to both photosystems would have to be increased by 30%; the 15% increase in ATP synthesis would increase the overall quantum requirement by 30%, and cyclic would effectively have only half the efficiency of non- or pseudocyclic phosphorylation. If, however, the excess excitation otherwise delivered to photosystem II could be diverted to photosystem I, the quantum yield of cyclic phosphorylation would remain high and equal to that of noncyclic phosphorylation, despite the required change in the relative turnover of the photosystems. Thus without a mechanism for redistribution of excitation a change in the contribution of cyclic phosphorylation could be achieved only with an impairment of quantum yield.

During the lag phase of CO_2 fixation in intact chloroplasts, phosphorylation of LHCP is rapid, and phosphorylation slows when CO_2 fixation begins.[114] The mechanism of this effect is presumably that plastoquinone is reduced and the protein kinase activated when the NADPH:$NADP^+$ ratio is high. The significance of the effect may be that diversion of excitation energy to photosystem I makes cyclic phosphorylation more efficient than it could otherwise be.

Plastoquinone-controlled phosphorylation of LHCP allows redistribution of excitation energy between the photosystems in proportion to their relative rates. It is conceiv-

able that the chief selective advantage of this system is the maintenance of high efficiency during changing contributions of cyclic to total photosynthetic phosphorylation. Without it the efficiency of cyclic photophosphorylation would certainly be impaired.

ACKNOWLEDGMENTS

I thank Professor R. J. Ellis and other members of the Warwick Chloroplast Research Group for their interest and encouragement, and Drs. A. Telfer, P. Horton, and J. Bennett for helpful discussions. I also acknowledge the financial support of the U.K. Science and Engineering Research Council in the form of a research grant to Dr. Bennett.

REFERENCES

1. **Mitchell, P.,** Coupling of phosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism, *Nature (London),* 191, 144, 1961.
2. **Nicholls, D. G.,** *Bioenergetics — An Introduction to the Chemiosmotic Theory,* Academic Press, London, 1982.
3. **Shavit, N.,** Energy transduction in chloroplasts: structure and function of the ATPase complex, *Annu. Rev. Biochem.,* 49, 111, 1980.
4. **Nelson, N.,** Proton ATPase of chloroplasts, *Curr. Top. Bioenerg.,* 11, 1, 1981.
5. **Reeves, S. G. and Hall, D. O.,** Photophosphorylation in chloroplasts, *Biochim. Biophys. Acta,* 463, 275, 1978.
6. **Simonis, W. and Urbach, W.,** Photophosphorylation *in vivo, Annu. Rev. Plant Physiol.,* 24, 89, 1973.
7. **Avron, M.,** Energy transduction in chloroplasts, *Annu. Rev. Biochem.,* 46, 143, 1977.
8. **Heber, U.,** Energy coupling in chloroplasts, *J. Bioenerg. Biomembranes,* 8, 157, 1976.
9. **Trebst, A.,** Energy conservation in photosynthetic electron transport of chloroplasts, *Annu. Rev. Plant Physiol.,* 25, 423, 1974.
10. **Mills, J. D. and Mitchell, P.,** Modulation of coupling factor ATPase activity in intact chloroplasts. Reversal of thiol modulation in the dark, *Biochim. Biophys. Acta,* 679, 75, 1982.
11. **Arnon, D. I., Whatley, F. R., and Allen, M. B.,** Assimilatory power in photosynthesis. Photosynthetic phosphorylation by isolated chloroplasts is coupled with TPN reduction, *Science,* 127, 1026, 1958.
12. **Izawa, S. and Good, N.,** The stoichiometric relation of phosphorylation to electron transport in isolated chloroplasts, *Biochim. Biophys. Acta,* 162, 380, 1968.
13. **Hall, D. O., Reeves, S. G., and Baltscheffsky, H.,** Photosynthetic control in isolated spinach chloroplasts with artificial electron acceptors, *Biochem. Biophys. Res. Commun.,* 43, 359, 1971.
14. **Heathcote, P. and Hall, D. O.,** Photosynthetic control and photophosphorylation in photosystem II of isolated spinach chloroplasts, *Biochem. Biophys. Res. Commun.,* 56, 767, 1974.
15. **Ort, D. R. and Izawa, S.,** Studies on the energy-coupling sites of photophosphorylation. V. Phosphorylation efficiencies (P/e_2) associated with aerobic photooxidation of artificial electron donors. *Plant Physiol.,* 53, 370., 1974.
16. **Allen, J. F. and Hall, D. O.,** The relationship of oxygen uptake to electron transport in photosystem I of isolated chloroplasts: the role of superoxide and ascorbate, *Biochem. Biophys. Res. Commun.,* 58, 579, 1974.
17. **Hall, D. O. and Evans, M. C. W.,** Photosynthetic phosphorylation in chloroplasts, *Sub-Cell Biochem.,* 1, 197, 1972.
18. **Dewey, T. G. and Hammes, G. G.,** Method for studying kinetics of light-induced transport across membranes, *Proc. Natl. Acad. Sci. U.S.A.,* 78, 7422, 1981.
19. **Black, C. C.,** Photosynthetic carbon fixation in reltion to net CO_2 uptake, *Annu. Rev. Plant Physiol.,* 24, 253, 1973.
20. **Tel-Or, E. and Stewart, W. D. P.,** Photosynthetic components and activities of nitrogen-fixing isolated heterocysts of *Anabaena cylindrica, Proc. R. Soc. Lond. (Biol).,* 198, 61, 1977.
21. **Cohen, Y., Padan, E., and Shilo, M.,** Facultative anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica, J. Bacteriol.,* 123, 855, 1975.
22. **Arnon, D. I., Tsujimoto, H. Y., and McSwain, B. D.,** Ferredoxin and photosynthetic phosphorylation, *Nature (London),* 214, 562, 1967.
23. **Hauska, G., Reimer, S., and Trebst, A.,** Native and artificial energy-conserving sites in cyclic photophosphorylation systems, *Biochim. Biophys. Acta,* 357, 1, 1974.

23a. **Chain, R. K.,** The role of cytochrome f in ferredoxin-dependent electron-transport reactions in spinach chloroplasts, *FEBS Lett.,* 105, 365, 1979.

24. **Bohme, H.,** On the role of ferredoxin and ferredoxin-NADP$^+$ reductase in cyclic electron transport of spinach chloroplasts, *Eur. J. Biochem.,* 72, 283, 1977.
25. **Bohme, J. and Cramer, W. A.,** The role of cytochrome b_6 in cyclic electron transport: evidence for an energy coupling site in the pathway of cytochrome b_6 oxidation in spinach chloroplasts, *Biochim. Biophys. Acta,* 283, 302, 1972.
26. **Whitmarsh, J. and Cramer, W. A.,** Photoxidation of the high-potential iron-sulphur center in chloroplasts, *Proc. Natl. Acad. Sci. U.S.A.,* 76, 4417, 1979.
27. **Malkin, R. and Chain, R. K.,** The relationship of the cyclic and non-cyclic electron transport pathways in chloroplasts, *Biochim. Biophys. Acta,* 591, 381, 1980.
28. **Mills, J. D., Crowther, D., Slovacek, R. E., Hind, G., and McCarty, R. E.,** Electron transport pathways in spinach chloroplasts. Reduction of the primary acceptor of photosystem II by reduced nicotinamide adenine dinucleotide phosphate in the dark, *Biochim. Biophys Acta,* 547, 127, 1979.
29. **Crowther, D., Mills, J. D., and Hind, G.,** Proton motive cyclic electron flow around photosystem I in intact chloroplasts, *FEBS Lett.,* 98, 386, 1979.
30. **Slovacek, R. E., Mills, J. D., and Hind, G.,** The function of cyclic electron transport in photosynthesis, *FEBS Lett.,* 87, 73, 1978.
31. **Slovacek, R. E. and Hind, G. E.,** Influence of antimycin A and uncouplers on anaerobic photosynthesis in isolated chloroplasts, *Plant Physiol.,* 60, 538, 1977.
32. **Mills, J. D., Slovacek, R. E., and Hind, G.,** Cyclic electron transport in isolated intact chloroplasts. Further studies with antimycin, *Biochim. Biophys. Acta,* 504, 298, 1978.
33. **Wolosiuk, R. A. and Buchanan, B. B.,** Thioredoxin and glutathione regulate photosynthesis in chloroplasts, *Nature (London),* 266, 565, 1977.
34. **Rosa, L. and Whatley, F. R.,** Uncouplers stimulate photosynthesis in intact chloroplasts by enhancing light-activation of enzymes regulated by the ferredoxin-thioredoxin system, *Plant Physiol.,* 68, 364, 1981.
35. **Walker, D. A., Slabas, A. R., and Fitzgerald, M. P.,** Photosynthesis in a reconstituted chloroplast system from spinach. Some factors affecting CO_2-dependent oxygen evolution with fructose-1,6-bisphosphate as substrate, *Biochim. Biophys. Acta,* 440, 147, 1976.
36. **Slovacek, R. E. and Hind, G.,** Energetic factors affecting carbon dioxide fixation in isolated chloroplasts, *Plant Physiol.,* 65, 526, 1980.
37. **Rosa, L.,** The ATP/2e ratio during photosynthesis in intact spinach chloroplasts, *Biochem. Biophys. Res. Commun.,* 88, 154, 1979.
38. **Slovacek, R. E., Crowther, D., and Hind, G.,** Cytochrome function in the cyclic electron transport pathway of chloroplasts, *Biochim. Biophys. Acta,* 547, 138, 1979.
39. **Crowther, D. and Hind, G.,** Partial characterization of cyclic electron transport in intact chloroplasts, *Arch. Biochem. Biophys.,* 204, 568, 1980.
40. **Slovacek, R. E., Crowther, D., and Hind, G.,** Relative activities of linear and cyclic electron flows during chloroplast CO_2-fixation, *Biochim. Biophys. Acta,* 592, 495, 1980.
41. **Arnon, D. I., Losada, M., Whatley, F. R., Tsujimoto, H. Y., Hall, D. O., and Horton, A. A.,** Photosynthetic phosphorylation and molecular oxygen. *Proc. Natl. Acad. Sci. U.S.A.,* 47, 1314, 1961.
42. **Telfer, A., Cammack, R., and Evans, M. C. W.,** Hydrogen peroxide as the product of antioxidation of ferredoxin reduced either chemically or by illuminated chloroplasts, *FEBS Lett.,* 10, 21, 1970.
43. **Allen, J. F.,** Oxygen — a physiological electron acceptor in photosynthesis? *Curr. Adv. Plant Sci.,* 9, 459, 1977.
44. **Allen, J. F.,** Oxygen reduction and optimum production of ATP in photosynthesis, *Nature (London),* 256, 599, 1975.
45. **Egneus, H., Heber, U., Matthiesen, U., and Kirk, M.,** Reduction of oxygen by the electron transport chain of chloroplasts during assimilation of carbon dioxide, *Biochim. Biophys. Acta,* 408, 252, 1975.
46. **Allen, J. F.,** A two-step mechanism for the photosynthetic reduction of oxygen by ferredoxin, *Biochem. Biophys. Res. Commun.,* 66, 36, 1975.
47. **Allen, J. F.,** Superoxide and photosynthetic reduction of oxygen, in *Superoxide and Superoxide Dismutases,* Michelson, A. M., McCord, J. M., and Fridovich, I., Eds., Academic Press, London, 1977, 417.
48. **Kaiser, W. M.,** Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplasts by hydrogen peroxide, *Planta,* 145, 377, 1979.
49. **Foyer, C. H. and Halliwell, B.,** The presence of glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism, *Planta,* 133, 21, 1976.
50. **Groden, D. and Beck, E.,** H_2O_2 destruction by ascorbate-dependent and systems from chloroplasts, *Biochim. Biophys. Acta,* 546, 426, 1979.
51. **Nakano, Y. and Asada, K.,** Spinach chloroplasts scavenge hydrogen peroxide on illumination, *Plant Cell Physiol.,* 21, 1295, 1980.

52. **Forti, G. and Gerola, P.,** Inhibition of photosynthesis by azide and cyanide and the role of oxygen in photosynthesis, *Plant Physiol.*, 59, 859, 1977.
53. **Kaiser, W.,** The effect of hydrogen peroxide on CO_2 fixation of isolated intact chloroplasts, *Biochim. Biophys. Acta,* 440, 476, 1976.
54. **Allen, J. F. and Whatley, F. R.,** Effects of inhibitors of catalase on photosynthesis and on catalase activity in unwashed preparations of intact chloroplasts, *Plant Physiol.*, 61, 957, 1978.
55. **Allen, J. F.,** Effects of washing and osmotic shock on catalase activity of intact chloroplast preparations, *FEBS Lett.*, 84, 221, 1977.
56. **Allen, J. F.,** Induction of a Mehler reaction in chloroplast preparations by flavin mononucleotide: effects on photosynthesis by intact chloroplasts, *Plant Sci. Lett.*, 12, 151, 1978.
57. **Radmer, R. J. and Kok, B.,** Photoreduction of O_2 primes and replaces CO_2-assimilation, *Plant Physiol.*, 58, 336, 1976.
58. **Radmer, R., Kok, B., and Ollinger, O.,** Kinetics and apparent Km of oxygen cycle under conditions of limiting carbon dioxide fixation, *Plant Physiol.*, 61, 915, 1978.
59. **Radmer, R. and Ollinger, O.,** Light-driven uptake of oxygen, carbon dioxide, and bicarbonate by the green algae *Scenedesmus, Plant Physiol.*, 65, 723, 1980.
60. **Patterson, C. O. P. and Myers, J.,** Photosynthetic production of hydrogen peroxide by *Anacystis nidulans, Plant Physiol.*, 51, 104, 1973.
61. **Whatley, F. R.,** Some effects of oxygen in photosynthesis by chloroplast preparations, in *Photosynthetic Mechanisms of Green Plants,* National Academy of Sciences-National Research Council, Washington, D.C., 1963, 243.
62. **Tagawa, K., Tsujimoto, H. Y., and Arnon, D. I.,** Analysis of photosynthetic reactions by the use of monochromatic light, *Nature (London),* 199, 1247, 1963.
63. **Grant, B. R. and Whatley, F. R.,** Some factors affecting the onset of cyclic photophosphorylation, in *Biochemistry of Chloroplasts,* Vol. 2, Goodwin, T. W., Ed., Academic Press, New York, 1967, 505.
64. **Arnon, D. I. and Chain, R. K.,** Regulation of ferredoxin-catalyzed photosynthetic phosphorylations, *Proc. Natl. Acad. Sci., U.S.A.*, 72, 4961, 1975.
65. **Arnon, D. I. and Chain, R. K.,** Role of oxygen in ferredoxin-catalyzed cyclic photophosphorylations, *FEBS Lett.*, 82, 297, 1977.
66. **Tsujimoto, H. Y., Hiyama, T., and Arnon, D. I.,** Affinity of ferredoxin for electrons from water and the regulation of cyclic photophosphorylation, *Biochem. Biophys. Res. Commun.*, 93, 215, 1980.
67. **Heber, U., Egneus, H., Hanck, U., Jensen, M., and Köster, S.,** Regulation of photosynthetic electron transport and photophosphorylation in intact chloroplasts and leaves of *Spinacia oleracea* L., *Planta,* 143, 41, 1978.
68. **Krause, G. H.,** High-energy state of the thylakoid system as indicated by chlorophyll fluorescence and chloroplast shrinkage, *Biochim. Biophys. Acta,* 292, 715, 1973.
69. **Ziem-Hanck, U. and Heber, U.,** Oxygen requirement of photosynthetic CO_2 assimilation, *Biochim. Biophys. Acta,* 591, 266, 1980.
70. **Steiger, H.-M. and Beck, E.,** Formation of hydrogen peroxide and oxygen dependence of photosynthetic CO_2 assimilation by intact chloroplasts, *Plant Cell Physiol.*, 22, 561, 1981.
71. **Takahama, U., Shimizu-Takahama, M., and Heber, U.,** The redox state of the NADP system in illuminated chloroplasts, *Biochim. Biophys. Acta,* 637, 530, 1981.
72. **Leegood, R. C., Crowther, D., Walker, D. A., and Hind, G.,** Photosynthetic electron transport in the bundle sheath of maize, *FEBS Lett.*, 126, 89, 1981.
73. **Huber, S. C., and Edwards, G. E.,** Studies on the pathway of cyclic electron flow in mesophyll chloroplasts of a C4 plant, *Biochim. Biophys. Acta,* 449, 420, 1976.
74. **Woo, K. C., Anderson, J. M., Boardman, N. K., Downton, W. J. S., Osmond, C. B., and Thorne, S. W.,** Deficient photosystem II in agranal bundle sheath chloroplasts of C4 plants, *Proc. Natl. Acad. Sci. U.S.A.*, 67, 18, 1971.
75. **Krebs, E. G. and Beavo, J. A.,** Phosphorylation-dephosphorylation of enzymes, *Ann. Rev. Biochem.*, 48, 923, 1979.
76. **Bennett, J.,** Phosphorylation of chloroplast membrane polypeptides, *Nature (London),* 269, 344, 1977.
77. **Bennett, J.,** Chloroplast phosphoproteins: phosphorylation of polypeptides of the light-harvesting chlorophyll protein complex, *Eur. J. Biochem.*, 99, 133, 1979.
78. **Bennett, J.,** Chloroplast phosphoproteins: evidence for a thylakoid-bound phosphoprotein phosphatase, *Eur. J. Biochem.*, 104, 85, 1980.
79. **Thornber, J. P.,** Chlorophyll-proteins: light-harvesting and reaction center components of plants, *Ann. Rev. Plant Physiol.*, 26, 127, 1975.
80. **Butler, W. L.,** Energy distribution in the photochemical apparatus of photosynthesis, *Ann. Rev. Plant Physiol.*, 29, 345, 1978.
81. **Arntzen, C. J.,** Dynamic structural features of chloroplast lamellae, *Curr. Top. Bioenerg.*, 8, 111, 1978.

82. **Alfonzo, R., Nelson, N., and Racker, E.,** A light-dependent protein kinase activity of chloroplasts, *Plant Physiol.*, 65, 730, 1980.
83. **Beliveau, R. and Bellemare, G.,** Light-dependent phosphorylation of thylakoid membrane polypeptides, *Biochem. Biophys. Res. Commun.*, 88, 797, 1979.
84. **Süss, K.-H.,** Identification of chloroplast thylakoid phosphoproteins. Evidence for the absence of phosphoryl-polypeptide intermediates in the ATPase complex, *Biochem. Biophys. Res. Commun.*, 102, 724, 1981.
85. **Burke, J. J., Ditto, C. L., and Arntzen, C. J.,** Involvement of the light-harvesting complex in cation regulation of excitation energy distribution in chloroplasts, *Arch. Biochem. Biophys.*, 187, 252, 1978.
86. **Steinback, K. E., Burke, J. J., and Arntzen, C. J.,** Evidence for the role of surface-exposed segments of the light-harvesting complex in cation mediated control of chloroplast structure and function, *Arch. Biochem. Biophys.*, 195, 546, 1979.
87. **Bennett, J., Steinback, K. E., and Arntzen, C. J.,** Chloroplast phosphoproteins: regulation of excitation energy transfer by phosphorylation of thylakoid membrane polypeptides, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 5253, 1980.
88. **Horton, P. and Black, M. T.,** Light-dependent quenching of chlorophyll fluorescence in pea chloroplasts induced by adenosine-5′-triphosphate, *Biochim. Biophys. Acta,* 635, 53, 1981.
89. **Bennett, J.,** Chloroplast phosphoproteins. The protein kinase of thylakoid membranes is light-dependent, *FEBS Lett.*, 103, 342, 1979.
90. **Allen, J. F., Bennett, J., Steinback, K. E., and Arntzen, C. J.,** Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems, *Nature (London),* 291, 25, 1981.
91. **Allen, J. F. and Horton, P.,** Chloroplast protein phosphorylation and chlorophyll fluorescence quenching. Activation by tetramethyl-*p*-hydroquinone, an electron donor to plastoquinone, *Biochim. Biophys. Acta,* 638, 290, 1981.
92. **White, C. C., Chain, R. K., and Malkin, R.,** Duroquinol as an electron donor for chloroplast electron transfer reactions, *Biochim. Biophys. Acta,* 502, 127, 1978.
93. **Izawa, S. and Pan, R. L.,** Photosystem I electron transport and phosphorylation supported by electron donation to the plastoquinone region, *Biochem. Biophys. Res. Commun.*, 83, 1171, 1978.
94. **Horton, P. and Black, M. T.,** Activation of adenosine-5′-triphosphate-induced quenching of chlorophyll fluorescence by reduced plastoquinone. The basis of state I-state II transitions in chloroplasts. *FEBS Lett.*, 119, 141, 1980.
95. **Horton, P., Allen, J. F., Black, M. T., and Bennett, J.,** Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of plastoquinone, *FEBS Lett.*, 125, 193, 1981.
96. **Arnon, D. I., Tsujimoto, H. Y., and Tang, G. M.-S.,** Contrasts between oxygenic and anoxygenic photoreduction of ferredoxin: incompatibilities with prevailing concepts of photosynthetic electron transport, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 2676, 1980.
97. **Arnon, D. I., Tsujimoto, H. Y., and Tang, G. M.-S.,** Proton transport in photooxidation of water: a new perspective on photosynthesis, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 2942, 1981.
98. **Myers, J.,** Conceptual developments in photosynthesis, 1924-1974, *Plant Physiol.*, 54, 420, 1974.
99. **Myers, J.,** Enhancement studies in photosynthesis, *Ann. Rev. Plant Physiol.*, 22, 289, 1971.
100. **Bonaventura, C. and Myers, J.,** Fluorescence and oxygen evolution from *Chlorella pyrenoidosa, Biochim. Biophys. Acta,* 189, 366, 1969.
101. **Murata, N.,** Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum, Biochim. Biophys. Acta,* 172, 242, 1969.
102. **Duysens, L. N. M.,** 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) inhibition of system II and light-induced regulatory changes in energy transfer efficiency, *Biophys. J.*, 12, 858, 1972.
103. **Ried, A. and Reinhardt, B.,** Distribution of excitation energy between photosystem I and photosystem II in red algae. II. Kinetics of the transition between state I and state II, *Biochim. Biophys. Acta,* 460, 25, 1977.
104. **Ried, A. and Reinhardt, B.,** Distribution of excitation energy between photosystem I and photosystem II in red algae. III. Quantum requirements of the induction of a state 2-state 1 transition, *Biochim. Biophys. Acta,* 592, 76, 1980.
105. **Barber, J.,** Ionic regulation in intact chloroplasts and its effect on primary photosynthetic processes, in *Topics in Photosynthesis,* Vol. 1, Barber, J., Ed., Elsevier, Amsterdam, 1976, 89.
106. **Barber, J.,** Influence of surface charge on thylakoid structure and function, *Ann. Rev. Plant Physiol.*, in press.
106a. **Biggins, J.,** Thylakoid conformational changes accompanying membrane protein phosphorylation, *Biochim. Biophys. Acta,* 679, 479, 1982.
107. **Millner, P. A., Widger, W. R., Abbott, M. S., Cramer, W. A., and Dilley, R. A.,** The effect of adenine nucleotides on inhibition of the thylakoid protein kinase by sulphydryl-directed reagents, *J. Biol. Chem.*, in press.

108. **Telfer, A. and Barber, J.,** ATP-dependent state 1-state 2 changes in isolated pea thylakoids, *FEBS Lett.,* 129, 161, 1981.
109. **Chow, W. S., Telfer, A., Chapman, D. J., and Barber, J.,** State 1-state 2 transition in leaves and its association with ATP induced chlorophyll fluorescence quenching, *Biochim. Biophys. Acta,* in press.
110. **Sinclair, J. and Cousineau, C.,** The effect of adenosine-5'-triphosphate on oxygen-evolution, fluorescence emission and the Emerson enhancement effect, *FEBS Lett.,* 136, 213, 1981.
111. **Horton, P. and Black, M.,** On the nature of the fluorescence decrease due to phosphorylation of chloroplast membrane proteins, *Biochim. Biophys. Acta,* in press.
112. **Horton, P. and Black, M.,** Light-induced redox changes in chloroplast cytochrome f after phosphorylation of membrane proteins, *FEBS Lett.,* 132, 75, 1981.
113. **Melis, A. and Harvey, G. W.,** Regulation of photosystem stoichiometry, chlorophyll *a* and chlorophyll *b* content and relation to chloroplast ultrastructure, *Biochim. Biophys. Acta,* 637, 138, 1981.
114. **Allen, J. F. and Bennett, J.,** Photosynthetic protein phosphorylation in intact chloroplasts. Inhibition by DCMU and by the onset of CO_2 fixation, *FEBS Lett.,* 123, 67, 1981.