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Review

Cyclic photophosphorylation and electron transport

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Contents

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′,4,4′-trinitrodiphenyl ether; DSPD, disulfosalicylidenepropane-1,2-diamine; FQR, ferredoxin-plastoquinone reductase; FNR, ferredoxin-$NADP^+$ reductase; HQNO, 2-heptyl-4-hydroxyquinoline *N*-oxide; HQNOBr, 2-heptyl-3-bromo-4-hydroxyquinoline *N*-oxide; MOA-stilbene, (*E*,*E*)-methyl-3-methoxy-2-(styrylphenyl)propenoate; NQNO, 2-nonyl-4-hydroxyquinoline *N*-oxide; PMS, *N*-methylphenazinium methyl sulfate; UHDBT, 5-n-undecyl-6-hydroxy-4,7-dioxobenzothiazole.

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1. Introduction

Cyclic photophosphorylation has long been the Cinderella of chloroplast energetics, but has been receiving increased attention of late. The neglect resulted partly from a lack of definitive methods for studying it and partly, perhaps, from its uncertain significance. But a lack of information about cyclic phosphorylation and its regulation has become a major impediment to the full understanding of the energetics of photosynthesis. Fortunately, new techniques for observing cyclic electron flow have become available and make it possible to investigate the role of cyclic phosphorylation in intact cells and tissues. The limiting factor in studying its regulation has become the lack of knowledge concerning the molecular details of the key reactions concerned with closing the cycle. This is where the main emphasis of the present review will lie, although an overview of the subject as a whole is attempted.

Two recent reviews have been dedicated to cyclic electron transport and phosphorylation. Heber and Walker [1] have introduced the idea that cyclic electron transport can function as a means of protection against photoinhibition. Fork and Herbert [2] have reviewed the subject in more general terms, but with particular emphasis on physiological aspects.

2. Characteristics of cyclic electron transport and phosphorylation

By the early 1960s, four basic characteristics of the electron transport system assumed to underly PS-I-dependent cyclic phosphorylation had been established by Arnon and co-workers.

(i) With thylakoid preparations from higher plants it is always necessary to add a soluble redox cofactor. Membrane preparations from cyanobacteria appear to behave similarly, although they have been less thoroughly studied. The question of whether it might be possible, under appropriate conditions, to obtain cyclic photophosphorylation without a cofactor is discussed below in the section on pathways.

(ii) Although many naturally occurring low molecular weight redox cofactors, and also many artificial dyes, have been shown to catalyse cyclic phosphorylation, the discovery that ferredoxin was a good cofactor immediately seemed to identify the physiological cofactor [3].

(iii) A key point, first discussed by Grant and Whatley [4], and confirmed by others, is that appropriate redox poising of the system is essential. In a cyclic electron transport system the rate of electron flow must fall to zero at the two extremes, when the components of the cycle are either fully oxidised or fully reduced. Redox poising is the basis for any physiological system of regulation of cyclic electron flow.

(iv) Finally, the conclusion that antimycin is a specific inhibitor of cyclic electron flow at low concentration (about 1 μM) [3] provided an important experimental tool and indicator of the occurrence of a cyclic process. Only much later did it become apparent that antimycin provided an ambiguous label. On one side it reads cyclic, and on the other cytochrome *b*, but the latter has proved misleading and should be replaced by "quinone-binding site". The site of action of antimycin is discussed further below.

3. Chloroplast energetics

The biochemistry of chloroplast energetics has been understood in outline for about 30 years, following the discovery of photophosphorylation and the formulation of the Z scheme and the chemiosmotic hypothesis. Surprisingly, however, a fundamental problem remains unresolved, that is how the right balance between the rates of production of ATP and NADPH is maintained. The central question concerns the protonmotive Q cycle. Is there slip, or is there not, in the proton pump of the cytochrome *bf* complex? Chemiosmotic stoichiometries are notoriously difficult to establish. In a very careful study of photophosphorylation by washed spinach thylakoids under oxidising conditions, however, Davenport and McCarty [5] obtained a maximum $P/2e^-$ ratio for non-cyclic phosphorylation of 1.33. This was an extrapolated value which took into account any loss by a passive proton leak. It was consistent with an H^+/e^- ratio of 2 if the widely accepted value of 3 was assumed for the H^+/ATP ratio of the ATP synthase. This result implied that the Q cycle was not operating under the range of light intensities used. On the other hand, Rich reached a conflicting conclusion when he was unable to find conditions, including a wide range of light intensities, under which the Q cycle clearly did not operate [6]. A key point in the discrepancy is that when ferricyanide is used as an acceptor a significant proportion of the electron flux may pass directly from the acceptor side of PS II to ferricyanide, so bypassing the cytochrome *bf* complex entirely.

The above discussion leads directly to the question of whether cyclic phosphorylation is required in steady-state photosynthesis in order to provide the additional ATP to drive the Calvin cycle and other metabolic processes.

4. Cyclic photophosphorylation in vivo

That cyclic photophosphorylation and electron transport can occur in vivo is well established. The normal technique in earlier work was to establish experimental conditions (e.g., anaerobiosis with addition of DCMU) under which cyclic was the only possible source of the ATP required to drive some physiological process. The ability of cyclic electron transport to drive processes such as ion

uptake, assimilation of organic substances, nitrogen fixation and protein synthesis was established [7,8]. Under optimum conditions rates of phosphorylation in excess of 100 μmol ATP/mg Chl per h can be observed [9–11], which would satisfy all estimates of the maximum physiological rates of cyclic phosphorylation.

4.1. Measurement of cyclic electron transport in vivo

Recently, new measuring techniques have been used to provide evidence for the occurrence of a cyclic process in vivo, especially photoacoustic spectroscopy [12–20] and measurements of the redox state of P700 [21–25] or of its rate of rereduction following strong illumination [26–30]. An early example of the latter was the work of Maxwell and Biggins [30] with various species of algae and a cyanobacterium. These methods still do not provide a specific measure of cyclic turnover, however, and results need to be interpreted with caution. P700 measurements can provide direct information only on the total electron flux through PS I and experimental conditions must be carefully designed to provide information on cyclic electron flow. Photoacoustic spectroscopy in far-red light can detect storage of light energy by PS I. Cyclic phosphorylation is the most likely mechanism for this, but in algae and cyanobacteria there is the possibility of a linear electron flow dependent on the donation of electrons into the plastoquinone pool by respiratory substrates (in higher plants the activity of the chloroplast NADH dehydrogenase is thought to be very low). Both processes would give rise to energy storage in the form of ATP. In general, the electron acceptor for a linear process could be either oxygen or $NADP^+$, but the behaviour in photoacoustic measurements would be markedly different. Oxygen reduction should be detectable in either by the direct "photobaric" effect (a modulated pressure change in response to the modulated exciting light), or by the large heat release associated with electron transfer from, say, NADH to oxygen. Both responses should depend on the frequency of modulation of the measuring light. Although oxygen reduction has been detected in heat shocked tobacco leaves by its photobaric effect [31], in general there is no evidence for the unusual photothermal effect associated with oxygen reduction under conditions in which a PS-I-dependent energy storage has been reported. On the other hand, photoreduction of $NADP^+$ with NADH as the electron donor would give rise to no large heat release, only an energy storage due to ATP reduction that would be indistinguishable from that associated with cyclic electron flow. In fact, some such linear flow seems inevitable when PS II is inactive in order to provide the correct redox poising for cyclic electron flow. Thus, a PS-I-dependent energy storage is likely to be due to a mixture of cyclic and non-cyclic electron transport, with the proportions dependent on the physiological conditions. A further complication is that a cycle might be formed by a transhydrogenase reaction between NADPH and NAD^+, or by direct electron donation from NADPH to the plastoquinone reductase.

4.2. Roles for cyclic phosphorylation in cell metabolism

Recent work has strengthened the view that cyclic phosphorylation can provide ATP for a variety of cellular processes. Studies of the postillumination CO_2 burst by suspensions of *Anacystis nidulans* grown in air support the conclusion that the accumulation of CO_2 during illumination is driven by cyclic phosphorylation [32]. The effect of DCMU, and the action spectrum, show that a PS I reaction is involved, and inhibition by O_2 and nitrite, which tend to drain the electrons out of the cycle, are consistent with an involvement of cyclic electron transport. Experiments with fluorescent indicators applied to *Fuchsia* leaves show that a PS-I-dependent reaction, probably cyclic, is responsible for energising vacuolar acidification [33]. Fluorescence measurements on nitrogen limited cells of the green alga *Selenastrum minutum* have shown that assimilation of NH_4^+ induces a state transition and an increased activity of PS I [34]. NH_4^+ assimilation requires a relatively high ATP/NADPH ratio and the extra ATP is probably provided by cyclic phosphorylation. At the same time there is a stimulation of dark respiration and a decreased rate of CO_2 assimilation, which can be explained by a transfer of intermediates from the Calvin cycle into the citric acid cycle for amino acid synthesis [35]. These are conditions that would lead to an accumulation of reducing equivalents on the acceptor side of PS I which would, in turn, favour cyclic electron transport.

An important extension of the role of cyclic phosphorylation is the idea that it is often involved in the response to stress. For example, photoacoustic measurements on pea leaves suggest increased cyclic activity under heat stress [19]. Photoacoustic measurements have also demonstrated increased energy storage by PS I in photoinhibited *Chlamydomonas* [18] and salt-stressed *Dunaliella salina* [17]. Measurements of photophosphorylation by incorporation of $[^{32}P]P_i$ into cell suspensions of *Scenedesmus* and *Synechococcus* have shown that cells acclimated to growth at high light intensities have a greater potential for cyclic phosphorylation relative to non-cyclic phosphorylation [36].

4.3. Role of cyclic phosphorylation in steady-state photosynthesis

The above considerations leave open the question of whether or not cyclic phosphorylation makes an essential contribution to steady-state photosynthesis under stress-free conditions. It is not easy to see how the appropriate redox poise alone can activate cyclic in both the presence and absence of stress, but more elaborate regulatory processes, as yet unknown, may make this possible. In reviewing the experimental evidence, a distinction must be made between C_3 and C_4 plants.

4.4. C_4 plants

C_4 photosynthesis is less efficient and requires a total of 5 or 6 ATP molecules per CO_2 fixed. The additional ATP (over the 3 molecules required to drive the Calvin cycle) is usually assumed to come mainly from cyclic phosphorylation but there have been few experimental studies of cyclic processes in these plants. Evidence for cyclic activity in isolated chloroplasts from mesophyll cells of *Digitaria sanguinalis* was obtained by Huber and Edwards [37,38]. Pyruvate-dependent CO_2 fixation (which only requires ATP), under an atmosphere containing 2% O_2 to inhibit the Mehler reaction, was taken as evidence of cyclic phosphorylation. The rate was stimulated by 0.8 μM DCMU, as expected for a cyclic process, and inhibited by antimycin, although the sensitivity was lower than would be expected (5 μM required for good inhibition). From experiments with isolated bundle sheaths from maize it was calculated that most of the additional ATP probably came from cyclic phosphorylation because measurements of $^{18}O_2$ uptake during CO_2 fixation showed that little could come from pseudocyclic phosphorylation [39]. Recent photoacoustic measurements [13,16] and observations on the redox behaviour of P700 [28] with leaves of C_4 plants have supported the general conclusion that cyclic phosphorylation plays an important role in C_4 photosynthesis.

4.5. C_3 plants

With C_3 plants, on the other hand, the evidence is conflicting. Evidence in favour of a contribution from cyclic rests mainly on the effects of inhibitors, especially antimycin. The most compelling evidence comes from a study by Furbank and Horton of CO_2 – dependent O_2 evolution by isolated barley protoplasts, which is a system in which photosynthesis is occurring under conditions close to those of the intact leaf [40]. Antimycin at a concentration of 1 μM inhibited the rate at the ceiling of the light response curve by 75%, but had no effect on the initial slope. This result is in agreement, qualitatively at least, with an earlier study with spinach chloroplasts by Heber et al. [41], from which they concluded that the extra ATP for photosynthesis was provided by pseudocyclic phosphorylation at low light intensities, but that additional cyclic phosphorylation was necessary at high intensities. Woo [42] also obtained antimycin inhibition of spinach chloroplasts, although in his case the light response was different. Low concentrations of antimycin are known to have two different effects on chloroplast processes. One is the classical effect on cyclic electron transport, and the other a more recently discovered effect on energy quenching in PS II [43,44]. The latter could not provide an explanation for inhibition at saturating light intensities. Mitochondrial oxidative phosphorylation would have been powerfully inhibited by 1 μM antimycin, but is unlikely to have been providing phosphorylation equivalents for steady-state photosynthesis and cannot explain effects on isolated, intact chloroplasts. This conclusion has been confirmed by recent work showing that oligomycin (an inhibitor of mitochondrial ATP synthesis) has only small effects on the rate of photosynthesis in barley protoplasts [45].

The recent evidence against a necessary cyclic contribution rests mainly on measurements of the redox state of P700, which can be made with intact leaves by making use of the 830 nm absorbance band of $P700^+$. Harbinson and colleagues have made extensive determinations of the quantum efficiencies of the two photosystems (PS I from P700 and PS II from fluorescence) under a variety of conditions [21–23,46] and have shown that under normal conditions the two efficiencies (and activities) remain linearly related to each other as the light intensity is varied [46]. In CO_2-free air, however, F_{II} declines more rapidly with increasing light intensity than F_I, suggesting a significant contribution from cyclic electron flow at high intensities. This interpretation is consistent with the role of cyclic electron transport in photodissipation proposed by Heber and Walker [1]. Harbinson et al. [22] have concluded "It appears that for leaves photosynthesising in air (i.e., photorespiratory conditions), or actively photosynthesising in non-photorespiratory conditions, a balanced flux through both photosystems can be maintained, and electron flow is predominantly linear. In such a situation the total demand for ATP and reductants can be balanced against the combined pmf and reduced ferredoxin generating capacity of linear electron flow, and the pmf generating capacity of pseudocyclic electron flow to O_2. Cyclic electron flow may occur in this situation; however, it must represent only a small proportion *or a fixed proportion* of the total electron flux from plastoquinol to PS I...Under more extreme physiological conditions (CO_2 compensation point, or low CO_2) the relationship between F_I and F_{II} is predominatly non-linear which is inconsistent with a predominant role for linear electron flow and may indicate a substantial cyclic electron flux about PS I" (our italics). A fixed proportion of the total electron flux from cyclic is what would be predicted if it makes a necessary contribution to the ATP turnover in steady-state photosynthesis. Thus, the conclusion that it is not involved under normal conditions from this and other work [47] is plausible but is in need of confirmation. Observations on the behaviour of P700 had earlier been made with cyanobacteria by Myers [24,25], who reached the conclusion that cyclic electron flow could not be more than 3 times the respiratory flow, which itself was only a few per cent of the photosynthetic rate; let us say that cyclic flow must be less than 10% of the non-cyclic flow in photosynthesis. Yu et al. [48] have recently estimated the cyclic flow rate to be about 3% of the non-cyclic rate in the cyanobacterium *Synechococcus* sp. PCC 7002. From a study of a *psaE*$^-$ mutant they concluded that this low rate does have a role in autotrophic

growth at low light intensity, although not at high intensity. The mutant strain could grow autotrophically, although at a reduced rate under a low light intensity, and was unable to grow photoheterotrophically. A role for the PsaE protein in cyclic electron flow was consequently suggested.

There seems to be a possible compromise which would allow a solution to this conundrum. We have pointed out above that the central question of chloroplast energetics is whether the Q cycle is constitutive or facultative. If we can accept that in fact it is constitutive (as the most recent evidence strongly suggests), then much depends on the value taken for the protonic stoichiometry of the ATP synthase. The commonly accepted value of 3 gives $P/2e^-$ = 2.0 or 1.33, depending on whether the Q cycle operates or not. The first figure is larger than required to drive the Calvin cycle and the second smaller. However, values of H^+/ATP of 4 have been reported for both spinach chloroplasts [49,50] and cyanobacterial membranes [51]. With preparations from cyanobacteria variable ratios above 3 have been found to depend on membrane composition [52] and pH [53]. A value of 4 for H^+/ATP would give $P/2e^- = 1.5$, which is exactly what is needed to drive the Calvin cycle. It may be objected that 1.5 is an ideal value which would not be obtained in practice, owing to leaks, and furthermore a small amount of extra ATP is required for starch or sucrose synthesis, quite apart from that required for protein sythesis and so on. Nevertheless, the need for "extra ATP" would have been reduced to a small value. This small amount might be large enough to explain a role for pseudocyclic or cyclic phosphorylation, as shown by mass spectrograph data and antimycin inhibition, and yet small enough not to have been ruled out by the in vivo measurements on P700.

5. Energy dissipation in Photosystem II

The most significant new concept arising out of recent work is the paradoxical one that coupled cyclic electron transport is an important factor in the promotion of energy dissipation by PS II and protection against photoinhibition. This idea was foreshadowed by Ridley and Horton's observation [54] that photodestruction of pigments of intact chloroplasts in the presence of DCMU only occurs at the high concentrations of DCMU (10 μM) that are necessary for inhibition of cyclic electron flow (presumably by over-oxidation) but which are higher than those necessary for inhibition of most of the PS II activity. Heber et al. have developed the concept of a physiological photoprotective function of cyclic electron flow, which has recently been reviewed [1]. Light scattering and fluorescence measurements on chloroplasts and leaves in the absence of CO_2 (such as could occur naturally on closure of the stomata to avoid excess loss of water) have demonstrated a role for cyclic electron transport in generating a low enough pH of the thylakoid lumen to initiate photodissipation in PS II [55,56].

6. Pathways of cyclic electron flow

6.1. Physiological cofactors

The distinction between physiological cycles, which include the plastoquinone pool, the cytochrome *bf* complex and plastocyanin, and cycles with artificial cofactors such as PMS and pyocyanine, was made clear by Trebst [57]. The former are inhibited by inhibitors of the cytochrome *bf* complex, such as DBMIB, whereas the latter are not. In addition, however, there is a class of artificial cofactors catalysing photophosphorylation that is sensitive to DBMIB but insensitive to antimycin. These compounds, including anthraquinone-2-sulfonate and methyl viologen [9,10], may be able to interact directly with the plastoquinone pool or possibly with the quinone reduction site of the cytochrome *bf* complex.

Possible physiological pathways of cyclic electron flow are illustrated in Fig. 1. Most of the proposed pathways involve ferredoxin as the cofactor. The need to add a soluble cofactor, whether it be ferredoxin or an artificial redox agent, distinguishes the plant and cyanobacterial type of cyclic from that of purple bacteria. Nevertheless, the bacterial type might possibly have been overlooked with thylakoid preparations because the appropriate redox poising had not been established. The presence of a quinone acceptor system in PS I (the naphthoquinone A1, and a second naphthoquinone of unknown function) emphasises this possibility. Cyclic phosphorylation has not been studied in green sulfur bacteria or heliobacteria, which have

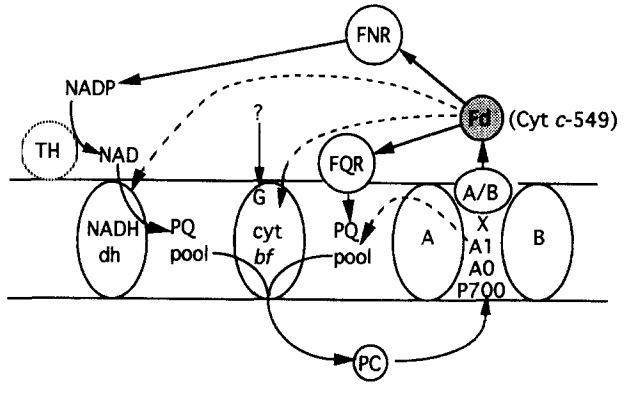

Fig. 1. Possible pathways of cyclic electron transport. NADH dh, NADH-plastoquinone reductase; cyt *bf*, cytochrome *bf* complex; A, B, reaction centre subunits (PsaA, PsaB) of PS I; P700, A0, A1, redox centres associated with the reaction centre; A/B, iron-sulfur centres F_A and F_B associated with subunit PsaC of PS I; Fd, ferredoxin; Cyt *c*-549, cytochrome *c*-549; FQR, ferredoxin-plastoquinone reductase; FNR, ferredoxin-$NADP^+$ reductase; TH, transhydrogenase; PC, plastocyanin. Dashed arrows indicate reactions that have been proposed but for which evidence is lacking.

photosystems related to PS I [58,59], although there is evidence for its occurrence in the former [60].

In many cyanobacteria and algae growing under iron-deficient conditions the synthesis of ferredoxin is suppressed and a flavoprotein called flavodoxin is induced. Like ferredoxin, this is a highly acidic protein, but it has about twice the molecular mass, a different secondary and tertiary structure, and flavin adenine dinucleotide as a cofactor rather than an iron-sulfur centre. Flavodoxin can substitute for ferredoxin in the photochemical reduction of $NADP^+$ by PS I and is known to interact with FNR, but its ability to substitute in cyclic electron flow does not seem to have been tested. It may be significant that *Nostoc* was found to produce appreciable amounts of ferredoxin I at iron concentrations low enough to cause maximum induction of flavodoxin, although ferredoxin II production was almost completely suppressed [61]. Some organisms contain a constitutive flavodoxin, and in the case of *Chondrus crispus* a low level of ferredoxin was detectable. These observations suggest that there may be a role for ferredoxin that is not satisfied by flavodoxin; nevertheless, loss of cyclic phosphorylation is probably not lethal.

Many cyanobacteria and some eukaryotic algae contain a low-potential cytochrome *c*-549 or *c*-550 which occurs either as a soluble protein or as an extrinsic membrane-bound protein. The two forms have not been reported to occur in the same organism and the relation between them is uncertain, although substantial N-terminal sequence similarity has been demonstrated [62]. In the cyanobacteria *Phormidium laminosum* [63] and *Synechococcus vulcanus* [64] it is associated with purified, oxygen evolving PS II particles, and in the latter case there is evidence for its occurrence as a stoichiometric component of PS II and for a function in the S-state transitions [65]. The soluble form has been shown to catalyse cyclic phosphorylation when added to isolated thylakoid membranes of *Anacystis nidulans* under anaerobic conditions [66]. Whether the electron transfer pathway in this system is the same as that used when ferredoxin is the cofactor is uncertain. The concentration of the soluble cytochrome *c*-549 in cells of *Anacystis nidulans* is variable, being almost undetectable in early logarithmic phase but approaching that of ferredoxin in late logarithmic and stationary phase.

6.2. The role of the plastoquinone pool and the cytochrome *bf* complex

Evidence for the involvement of the cytochrome *bf* complex in cyclic electron transport comes principally from studies of the effects of specific inhibitors. The inhibitors DBMIB [67–70], DNP-INT [69], UHDBT [68], HQNO [69], stigmatellin [67] and a variety of substituted benzoquinone analogues of DBMIB [68] have all been demonstrated to inhibit ferredoxin-catalysed cyclic electron transport in higher-plant thylakoids. DBMIB is thought to inhibit plastoquinol oxidation at the Q_o site of the *bf* complex [71,72] as are stigmatellin [73,74], DNP-INT [74] and UHDBT [68]. HQNO is thought to inhibit electron transfer at the quinone reduction (Q_i) site of the complex [75,76]. These effects are therefore consistent with the involvement of the *bf* complex in the cyclic pathway of higher plants.

Although the cyanobacterial system has been less well studied, there is evidence to support the involvement of the *bf* complex in ferredoxin-catalysed cyclic electron transfer in these organisms. DSPD has been reported to inhibit cyclic photophosphorylation in *Anacystis nidulans* thylakoids [77], and MOA-stilbene [78], DBMIB and stigmatellin (Manasse and Bendall, unpublished data) have all been shown to inhibit cyclic electron transport in *Phormidium laminosum* thylakoids.

Although all the inhibitors described above have been shown to affect the function of the higher-plant cytochrome *bf* complex, the degree of specificity of a number of these compounds (e.g. DBMIB) is known to be low. In addition, there are no known reports of studies of the possible effects of any of these compounds on PS I activity and possible effects at other sites in the electron transfer cycle have generally not been investigated either (but see [67]). Moreover, it should not be assumed that they necessarily have the same site of action in cyanobacteria as in higher plants, although DBMIB has been shown to inhibit the cytochrome *bf* complex in *Synechococcus* sp. [79,80], and effects of NQNO, UHDBT, stigmatellin, DNP-INT and DBMIB consistent with *bf* complex inhibition have been observed in *Oscillatoria limnetica* [81]. The effects of HQNOBr on the *bf* complex and ferredoxin-dependent plastoquinone reduction, for example, have been shown to differ between pea and *Phormidium laminosum* thylakoids [78]. Assumptions about the effects of the inhibitors on the role of the *bf* complex in cyclic electron transfer must therefore be made with these considerations in mind.

In addition to the evidence from inhibitor studies, there is direct evidence for the involvement of the complex from spectral measurements of turnover of cytochromes *b* and *f* during cyclic electron transport around PS I in PS-I-enriched subchloroplast vesicles [70,82], intact chloroplasts from spinach [83] and *P. laminosum* thylakoids [78].

Thus there is convincing evidence for the operation of the *bf* complex in cyclic electron transport in both cyanobacteria and higher plants.

6.3. H^+ and e^- movements within the cytochrome *bf* complex

Most of the relevant data indicate that the functioning of the *bf* complex is the same in cyclic as it is in linear electron transport [84]. De Wolf et al [70] report deconvolution of optical spectral signals during single-turnover cyclic electron transfer in PS-I-enriched subchloroplast vesicles from spinach. The reported data are consistent with an equivalent function of the *bf* complex in cyclic

and linear electron transport. The fact that the Q_i site inhibitor HQNO inhibits cyclic photophosphorylation in spinach chloroplasts [69] possibly indicates that the Q cycle is involved, although HQNO may also have other sites of action (see above).

The detailed pathways of electron transfer in the cytochrome *bf* complex remain controversial. Some form of Q cycle is widely accepted as providing an explanation for electrogenic proton pumping, although difficulties remain in matching theory to experiment [84]. Cramer and colleagues have proposed that there are different modes of operation during linear and cyclic electron transport [85,86]. Their proposal was based on three main observations. First, that the cytochrome *bf* complex can be isolated as an active dimer, and may well occur as such in vivo [87]. Secondly, that sequence comparisons of the cytochrome *b* components of cytochrome *bc* complexes suggested that in the case of the *bf* complex there may be no direct electron transfer between the b_H and b_L haems, a concept supported by some experimental observations [88]. Thirdly, that the extent of dark reoxidation of the cytochrome *b* following a flash is markedly increased when the b_H haem is initally reduced by exposure to NADPH and ferredoxin [88]. These observations led to the suggestion that the complex may function as a dimer under non-cyclic conditions and a monomer when cyclic electron flow is activated. According to this model the key step in the creation of a cycle was the reduction of haem b_H at the stromal surface by ferredoxin reacting at a site analagous to the Q_i site of the Q cycle model. There would be a concomitant oxidant-induced reduction of haem b_L at the Q_o site at the lumenal surface. The two reduced *b* haems would then cooperate in the reduction of a plastoquinone molecule held in a "quinone pocket" in the centre of the complex. In the dimeric form of the complex, on the other hand, there would be a cooperative two electron oxidation of a single plastoquinol molecule at the combined Q_o sites of the dimer, so that no plastosemiquinone was available for reduction of b_L (and no electrogenic process would occur). Control over the dimerisation of the complex would thus provide the possibility for a system of regulation of cyclic electron transport and phosphorylation.

The above model loses some of its force when one tries to reconcile it with the need for the complex to be able to function with an enhanced H^+ stoichiometry under non-cyclic conditions. Some of the reasons for its original proposal have been weakened by recent experimental evidence which strongly suggests that direct electron transfer between the two *b* haems does, in fact, occur [89], but that there is no quinol oxidation at the Q_o site without reduction of cytochrome *b* [90]. Furthermore, Rich *et al* [91] have explained some of the kinetic behaviour which is anomolous in terms of a conventional Q cycle (turnover in the presence of Q_i site inhibitors and the kinetics of reoxidation of cytochrome *b*) by a modification in which the fully oxidised complex needs priming with one electron to convert it into a form which can show rapid turnover. A key feature is the concerted two electron reduction of a plastoquinone molecule at the Q_i site. Although the model was proposed with a monomeric complex in mind, dimerisation might facilitate this two equivalent reduction. We discuss below evidence that may identify the site at which ferredoxin donates electrons back into a component of the non-cyclic chain, and mention here only that there is no compelling evidence for direct donation into the cytochrome *bf* complex as proposed in the Cramer model.

The above evidence suggests that during cyclic electron transport the cytochrome *bf* complex functions in much the same way as it does during non-cyclic electron transport, but the possible involvement of a Q-cycle needs further investigation. The remaining question is whether there is any direct electron transfer from a carrier in the stroma, in particular ferredoxin, into the cytochrome *bf* complex, and this is discussed in the next section.

6.4. Electron donation from the stroma into the cytochrome bf complex

A common textbook description of photosynthetic electron transport includes a direct reaction between ferredoxin and cytochrome *b*-563. This idea is based partly on the inhibition of cyclic phosphorylation by antimycin, mistakenly assumed to inhibit the cytochrome *bf* complex, and partly on the observation of a PS-I-dependent photoreduction of cytochrome *b*-563 [92] before the phenomenon of oxidant-induced reduction was understood. It can no longer be considered to be supported by experimental evidence, however, and is inconsistent with the observation by Cox [93] that ferredoxin does not accelerate the slow reduction of cytochrome *b*-563 by dithionite. The slowness of dithionite reduction implies that the haem is not readily accessible from the aqueous phase, which would be consistent with structural models based on sequence comparisons [86] and is required for proper operation of a Q cycle. The inhibitor evidence quoted above favours the view that electrons from ferredoxin reach the cytochrome *bf* complex via the plastoquinone pool. Chain interpreted his observation of reductant-induced oxidation of cytochrome *b*-563 in terms of mediation by the plastoquinone pool between ferredoxin and the cytochrome [94]. The term "ferredoxin-plastoquinone reductase" has been coined for the enzyme catalysing the reduction of the pool by ferredoxin [11], and the nature of this enzyme is discussed in a later section.

Following the work of Lavergne [95], Joliot and Joliot [96] identified a redox-active component "G" in *Chlorella sorokiniana*. Its oxidised-minus-reduced spectrum resembled that of a high-spin cytochrome *c'*. G was shown to undergo redox equilibration with the haem b_H of cytochrome *b*-563 as if it had a midpoint redox potential 10–30 mV more positive, and on this basis it was sug-

gested to be loosely associated with the stromal surface of the cytochrome *bf* complex; it has not been identified as a component of thylakoid membranes or of the complex isolated from another green alga, *Chlamydomonas reinhardtii* [97]. The suggestion was also made that G might be part of the cyclic pathway in green algae, but there is no experimental support for this.

7. Cycles involving NAD(P)H-plastoquinone reductase

If thylakoid membranes contain NADH- or NADPH-plastoquinone reductase activity there is a possibility of cyclic electron flow around PS I through the pyridine nucleotide pool. The surprising discovery that the plastid genome contains genes homologous to subunits of the mitochondrial complex I (NADH-ubiquinone reductase) [98,99] has caused a serious consideration of this possibility. The first seven genes described were homologous to the membrane embedded (mitochondrial encoded) subunits of the mammalian enzyme, which carry no cofactors. More recently, chloroplast genes have been identified that are homologous to some of the nuclear encoded mammalian subunits, but genes for the crucial flavoprotein subunits have not yet been identified.

The function of these complex I proteins in plastids has still to be elucidated, but the possibility is worth bearing in mind that an incomplete enzyme lacking the flavoprotein subunits might have a function. For example, a cyclic pathway can be imagined in which ferredoxin donates electrons directly to iron-sulfur proteins of the complex. A subcomplex of the enzyme, lacking the flavoprotein subunits, has been isolated from *Synechocystis* membranes [100].

7.1. Higher plants and algae

The protein products of the *ndhH* and *ndhK* genes have been found in stroma lamellae of higher-plant chloroplasts [101–104], suggesting that an active NADH dehydrogenase might be assembled. Nevertheless, NADH dehydrogenase activity has not been found in thylakoid membranes from higher plants, so that the presence of a complete enzyme similar to mitochondrial complex I is doubtful. On the other hand, NADPH is capable of reducing the plastoquinone pool in the dark by an antimycin-sensitive pathway [105,106]. A dark reduction of the plastoquinone pool has been demonstrated in leaves from a number of species [107], for which NADPH or ascorbate might be donors. The existence of an NADPH-plastoquinone reductase activity is adequately explained by an electron transfer sequence involving FNR and FQR. Presently available evidence, therefore, does not favour a role for a complex I-type enzyme in cyclic electron transport of higher plants.

In green algae, however, the situation is different. Godde and Trebst [108] showed that NADH is an effective donor into the photosynthetic electron transport system in *Chlamydomonas* thylakoid membranes, and Godde [109] partially purified an NADH-plastoquinone reductase from this source. Significant features of the enzyme were that it was sensitive to inhibition by rotenone (but only at about 100 μM), preferred plastoquinone to ubiquinone as the acceptor, and oxidised NADPH almost as well as NADH. Whether or not this preparation represented an expression of the chloroplast *ndh* genes is unclear. The existence of an NAD(P)H-plastoquinone reductase activity opens up the possibility of redox poising of a cyclic pathway in the absence of PS II activity, as well as chlororespiration. Maione and Gibbs [110] have shown that the photoreduction of CO_2 in hydrogen-adapted *Chlamydomonas* is sensitive to rotenone (also 100 μM), and suggest that an NAD(P)H-plastoquinone reductase is involved, but a role of FQR is not clearly ruled out. Antimycin was also an effective inhibitor, but only at a concentration significantly higher than that required in vitro. Interestingly, the dark oxyhydrogen reaction, which is also coupled to CO_2 fixation, was inhibited at much lower concentrations of antimycin. This implies an involvement either of FQR or of the mitochondrial electron transport chain.

Ravenel et al [111] have used the photoacoustic technique to study cyclic energy storage in *Chlamydomonas* cells. They argued from the synergistic effects of pairs of inhibitors for the presence of two distinct pathways. The inhibitors used were antimycin and *N*-ethylmaleimide, or antimycin and 2′-monophosphoadenosine-5′-diphosphoribose, which was introduced by electroporation. The antimycin-sensitive pathway was interpreted as a normal ferredoxin-dependent pathway. Although ferredoxin was assumed to donate directly to the cytochrome *bf* complex it seems more likely that FQR was involved. However, FQR has not been studied explicitly in *Chlamydomonas* and its inhibitor sensitivity is not known, but it could reasonably be assumed to be inhibited by antimycin. *N*-ethylmaleimide and 2′-monophosphoadenosine-5′-diphosphoribose are thought to inhibit $NADP^+$ reduction by FNR. Thus, NADPH might complete this cycle by reacting with the plastoquinone pool via an NAD(P)H-plastoquinone reductase. The inhibitor effects on which this interpretation rests need to be confirmed by studies with systems in vitro, and it would be especially pertinent to reconstitute an $NADP^+$ dependent cycle in vitro. The interpretation of these experiments is complicated by the knowledge that *N,N-p*-phenylenedimaleimide (which presumably acts in similar fashion to *N*-ethylmaleimide) inhibits FQR activity in pea chloroplasts [67].

The presence of two different cyclic pathways in spinach chloroplasts was suggested by Hosler and Yocum [112,113] as a result of their studies of the P/O ratios obtained under various conditions. Methyl viologen gave a Mehler reaction with a P/O ratio of about 1.25, which they took to be

the value expected for a purely non-cyclic process. When methyl viologen was replaced by ferredoxin a slower O_2 uptake was observed which gave a P/O ratio of 1.6 in the absence of antimycin and 1.25 in its presence. This was consistent with the additional ATP arising from an antimycin sensitive pathway of ferredoxin dependent cyclic. When methyl viologen was replaced by ferredoxin plus $NADP^+$ a high rate of electron transfer was observed which was also associated with a P/O ratio of about 1.6, but antimycin had no effect. The higher ratio in this case was suggested to be the result of a second type of cycle which involved FNR. It is unlikely that $NADP^+$/NADPH was involved, partly because of the lack of an NADPH dehydrogenase activity in spinach, but also because the apparent activity of the cycle was independent of the concentration of NADPH in the reaction mixture. In fact, Hosler and Yocum suggested that a reduced form of FNR (probably the semiquinone form) could donate directly to the plastoquinone pool. There is no evidence, however, that FNR possesses, or participates in, a quinone binding site. An alternative explanation is that in the presence of $NADP^+$ a Q-cycle was operating to enhance the H^+ stoichiometry of the cytochrome *bf* complex. The difficulty with this is that there is no simple kinetic explanation of why the Q-cycle did not operate with methyl viologen as acceptor. Possibly methyl viologen shortcircuits the Q-cycle by reacting directly with haem b_L or cytochrome *b*-563.

Asada et al. have developed a technique for estimating the size of the pool of electrons in the stroma that are available for reduction of $P700^+$ immediately following illumination and found numbers in the range 12–25/P700 in different species of plant [28,114]. It was thought that these figures were too large to be accounted for solely by ferredoxin, and must include the NADPH pool of the stroma and probably triosephosphate to some extent. The tentative suggestion was made that electron donation into the plastoquinone pool occurred through an NAD(P)H dehydrogenase. It seems more probable, however, that the pathway involved a reversal of the FNR reaction followed by reduction of the pool through FQR.

7.2. Cyanobacteria

NAD(P)H-plastoquinone reductases occur in cyanobacteria where they can be expected to have a respiratory function, because respiratory and photosynthetic electron transport are known to occur together in thylakoid membranes and to share some components [115]. Such enzymes can also occur in the plasma membrane, which is not photosynthetically competent. NADH and NADPH have been shown to donate electrons to PS I in cell-free preparations from *Aphanocapsa* 6714 [116] and there is evidence for the reduction of $P700^+$ by respiratory donors in whole cells of *Synechococcus* PCC 7002 [26,48] and *Synechocystis* PCC 6803 [27]. There are several reports that they may be involved in cyclic electron flow [27,101,117]. This possibility is more complicated than in eukaryotic algae because the thylakoid membranes are directly exposed to the total metabolic system of the cell. In addition more than one kind of enzyme has been described and the occurrence is species dependent.

As in bacteria [118], two types of cyanobacterial NADH-quinone reductase have been found. The bacterial NDH-1 has many distinct subunits, FMN as a prosthetic group and several iron-sulfur centres. It is also sensitive to rotenone inhibition and acts as a H^+ pump. These characteristics are similar to those of mitochondrial complex I. NDH-2, on the other hand, usually has a single polypeptide, FAD as prosthetic group and is inhibited only by high concentrations of rotenone ($\sim 100\ \mu M$). Enzyme activities and some polypeptides of the NDH-1 type have been reported in *Synechocystis* PCC 6803 [100,119] and *Anabaena* PCC 7120 [120]. Several *ndh* genes coding for subunits of the NDH-1 type enzyme have been identified in *Synechocystis* [117,119,121–123] and *Plectonema boryanum* [124]. There is some confusion regarding the localisation of the enzyme. In *Synechocystis* PCC 6803 the enzyme has been reported to occur in both thylakoid and plasma membranes [119] or to be restricted to the thylakoid membranes [117]. In the case of *Anabaena* PCC 7120 the NDH-1 enzyme seems to be confined to the plasma membrane and the thylakoid membranes contain an NDH-2 enzyme [120,125]. *Microcystis aeruginosa* has also been shown to have an NDH-2 enzyme, for which NADPH is the better substrate [126,127], although the *Anabaena* enzyme oxidises only NADH [120,125]. If the pyridine nucleotide pool is to participate in a cycle it is important to establish that the thylakoid dehydrogenase oxidises NADPH, or that there is an active transhydrogenase, for which evidence is lacking so far. The plasma membrane NDH-1 of *Anabaena* appears to oxidise NADPH, but the substrate specificity of the *Synechocystis* enzyme has not been established.

Mi et al have carried out an interesting series of studies on the pathways of dark rereduction of $P700^+$ in *Synechococcus* PCC 7002 [26] and *Synechocystis* PCC 6803 [27]. Kinetic arguments were used to identify a cyclic return of electrons from the reducing side of P700 to the oxidising side. Inhibitor effects, which are not in themselves conclusive, suggested that an NAD(P)H-plastoquinone reductase played a crucial role in the cycle, as well as in reduction by respiratory donors. Compelling evidence for this conclusion (assuming that the kinetic evidence for the cyclic flow is correct) came from the behaviour of mutant cells in which either the *ndhB* or the *ndhL* gene had been inactivated. It should be noted that these experiments were not performed under steady-state conditions, so their physiological significance needs to be established. A further point is that the conclusion that a cycle involving ferredoxin and FQR was not involved may not be justified, because it was based on a lack of inhibi-

tion by antimycin, which has not been established as an FQR inhibitor in cyanobacteria [78].

Further evidence for the role of NADH dehydrogenase in a cyclic pathway comes from the study of the inorganic carbon concentrating mechanism of cyanobacteria. Uptake of CO_2 and bicarbonate is driven by a PS-I-dependent process and requires ATP, so cyclic phosphorylation is likely to be involved [32]. The concentrating mechanism no longer functions in specific *ndh* mutants of *Synechocystis* PCC 6803 [117,128] or *Synechococcus* PCC 7942 [129]. The simplest explanation is that NADH dehydrogenase is part of the cyclic electron transport pathway, but there are some difficulties about this. The mechanism of energisation is not well understood, and it has been suggested that, in addition to ATP, electron transport *per se* is required [32,130]. NAD(P)H would probably be the electron donor and the dehydrogenase would have to be present in the plasma membrane. In this case the involvement of the dehydrogenase would be in a non-cyclic process, and the electron acceptor needs to be identified.

8. Ferredoxin-plastoquinone reductase (FQR)

8.1. Evidence for the existence of FQR

As mentioned above, in much of the earlier work on cyclic electron transport it was assumed that reduced ferredoxin donated directly to the cytochrome *bf* complex via an antimycin-sensitive site. However, attempts to demonstrate the direct effect of antimycin on the electrogenic turnover of the cytochrome *bf* complex were unsuccessful [11]. In these experiments care was taken to use a concentration of antimycin no higher than that required to inhibit cyclic phosphorylation specifically. The antimycin binding site [131] of thylakoid membranes was found to be localised in the PS I fraction (approximately 1 binding site per reaction centre) after treatment of thylakoid membranes with digitonin and not to occur in the cytochrome *bf* complex [132]. Antimycin binding could not be detected in purer samples of PS I prepared by Triton fractionation. The association may therefore be loose, but some structural modification by Triton cannot be excluded. So far, no method has been devised for testing PS I preparations for FQR activity. It is now generally accepted that antimycin is not a specific inhibitor of the cytochrome *bf* complex [91]. This conclusion is consistent with the failure of antimycin to inhibit linear electron flow, and the linkage of a Q-cycle to turnover of the cytochrome *bf* complex [84]. In order to explain the specific inhibition of cyclic phosphorylation by antimycin it was necessary to postulate that antimycin inhibited the electron transfer reaction closing the cycle, that is, ferredoxin-plastoquinone reductase (FQR).

FQR activity can be detected directly by monitoring the absorbance changes associated with the reoxidation of ferredoxin following a brief period of illumination of thylakoid membranes under anaerobic conditions in the presence of DCMU [67]. Ferredoxin-catalysed cyclic phosphorylation can be measured unambiguously under the same conditions and antimycin inhibits the two reactions in the same concentration range. The electron transfer assay allows a distinction to be made between the effects of inhibiting FQR and of inhibiting the cytochrome *bf* complex. With a preparation of cyanobacterial thylakoid membranes from *Phormidium laminosum* antimycin is not an effective inhibitor of either FQR or cyclic phosphorylation, but both can be inhibited by HQNOBr [78]. With pea thylakoids, on the other hand, HQNOBr is an inhibitor of the cytochrome *bf* complex. The failure of antimycin to inhibit cyclic phosphorylation is consistent with a similar observation made with preparations from *Anacystis nidulans* [77,133]. Although it is dangerous to generalise from results with only two species, they are taxonomically sufficiently distinct to make it likely that the cyanobacterial system in general is not sensitive to antimycin.

There has been no direct demonstration that plastoquinone is the immediate acceptor of FQR. That this is the case is concluded from the kinetic evidence for the involvement of a pool of the right size, the effects of inhibitors such as DBMIB which inhibit plastoquinol oxidation by the cytochrome *bf* complex [67], and the fact that antimycin and HQNOBr behave as quinone/quinol analogues in their inhibitory effects. Attempts to demonstrate an assay for the enzyme with artificial quinone acceptors have failed because in aqueous solution there is a rapid uncatalysed oxidation of reduced ferredoxin by quinones. The native plastoquinone, however, is firmly anchored in the lipid phase of the membrane and the effects of antimycin or HQNOBr show that a specific quinone binding site is involved. The problem of identifying the polypeptides concerned with FQR activity can thus be described as identification of a quinone (or inhibitor) binding site on the one hand and, on the other, of a ferredoxin binding site. In addition, one may ask whether any additional redox active group is involved. There is some evidence that FNR may be involved, and if so it could provide both a flavin cofactor and a ferredoxin binding site. This question is considered in the next section.

Thus, FQR provides a pathway of cyclic electron flow in both plants and cyanobacteria that is independent of the presence of $NADP^+$/NADPH.

8.2. Is FNR involved in FQR activity?

The idea that FNR might be involved in cyclic electron transfer [106,134] and thus represent an integral part of FQR is an attractive one. Part of the total FNR activity is firmly bound to the membrane and provides a possible ferredoxin binding site for FQR. Although there is no evidence for a quinone binding site (for example, FNR in

solution does not bind antimycin), one might be provided by an unidentified membrane protein which acted as the binding site for FNR. In this case FNR would play a crucial role in the regulation of cyclic phosphorylation, because it would include a switching mechanism by which electrons could either reduce $NADP^+$ or be diverted to an alternative bound quinone acceptor. The evidence in favour of a role for FNR is unfortunately rather flimsy, but it has proved difficult to eliminate the possibility entirely, in the absence of a deletion mutant for the enzyme.

Evidence in favour of an involvement of FNR is twofold. First, cyclic electron flow is inhibited by disulfodisalicylidenepropane-1,2-diamine, which is considered to inhibit FNR specifically [134]. Although the original demonstration of cyclic inhibition depended on an effect on the slow phase of the electrochromic effect at 518 nm, which is no longer considered a specific assay for cyclic flow, FQR activity is inhibited (Cleland and Bendall, unpublished observations). Similarly, maleimides inhibit binding of ferredoxin to FNR [134] and also inhibit FQR activity [67]. Secondly, a rapid absorbance transient attributed to the half-reduced form of FNR has been described [135,136], but the significance of this is unclear because it was found to be independent of the presence of ferredoxin.

Against the involvement of FNR one may state first that antibodies to FNR tend not to inhibit cyclic electron flow [67,134], but this may be because they bind to the $NADP^+$ domain. Secondly, some inhibitor experiments may be interpreted as indicating that FNR is not involved in the antiymcin-sensitive pathway. Hosler and Yocum [112] reached this conclusion from some discrimination between the effects of heparin and maleimides on cyclic phosphorylation and FNR. They suggested that the antimycin-sensitive reaction was more sensitive to low concentrations than is FNR. This distinction needs a more detailed study, however. Another possible approach is to remove FNR from thylakoid membranes by washing procedures and to test simultaneously the effect on FQR activity and, if necessary, to attempt reconstitution with added FNR. Attempts in the authors' laboratory with both pea and *Phormidium* thylakoids favour the conclusion that FNR is not necessary for FQR activity (unpublished) but a decisive result has not yet been possible because of the difficulty of completely removing FNR without damaging the membrane sufficiently to inactivate FQR.

8.3. Photosystem I subunits and FQR activity

So far FQR activity has only been detected in preparations containing intact thylakoid membranes. The localisation of the antimycin binding site [132] indicates association with PS I, but this may be loose (see section 8.1). Nevertheless, for want of more positive identification it seems worthwhile to consider whether the ferredoxin binding site associated with FQR activity is amongst the extrinsic stroma exposed polypeptides of PS I, which are PsaC, PsaD and PsaE. A concise review of the functions of these polypeptides in the context of the structure of PS I has recently been published [137]. Electron microscopy of two-dimensional crystals of PS I has demonstrated that these proteins form a ridge on the stroma exposed surface of the complex [138]. PsaC is an iron-sulfur protein which acts as the immediate electron donor to ferredoxin [139]. What of PsaD and PsaE?

PsaD (PsI-D, subunit II) is a basic protein of about 16 kDa in cyanobacteria and about 17.5 kDa in higher plants. Its structure is unknown, but it contains no redox cofactors. As a result of cross-linking studies with the water-soluble zero-length cross-linker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, PsaD has been dubbed the ferredoxin docking protein. FNR is the only other protein associated with PS I which has been shown to be cross-linked to ferredoxin by this reagent [140,141], although a minor cross-linked product has been observed which indicates that an unidentified polypeptide with a molecular mass slightly lower than that of PsaD may also bind to ferredoxin [140]. Recent work has identified a cross-link between Glu-93 of ferredoxin and Lys-106 of PsaD in the case of the *Synechocystis* proteins [142]. PsaD is also known to be required for the correct functional reconstitution of PsaC into the reaction centre; PsaC contains the iron-sulfur centres F_A and F_B, so it is reasonable to postulate that PsaD also orientates ferredoxin correctly for photoreduction by PS I. Support for this model comes from the properties of a PsaD deletion mutant of *Synechocystis* [143].

PsaE is a basic polypeptide of approximately 8 kDa in cyanobacteria and 10 kDa in higher plants, and contains no redox cofactors. It has been implicated in stabilising the interaction of PsaD and PsaC with the PsaA/PsaB core complex of PS I [144] and cross-linking experiments suggest that it is involved in binding FNR in higher-plant thylakoids [145]. In cyanobacterial preparations it has been shown to be required for rapid photoreduction of ferredoxin [146,147]. Nevertheless, PsaE deletion mutants of *Synechocystis* PCC 6803 [148] and *Synechococcus* PCC 7002 [149] show a normal rate of photoautotrophic growth under optimum conditions (high light, high CO_2), so that rapid photoreduction of ferredoxin does not seem to be necessary for this purpose. The *Synechococcus* mutant, however, grew more slowly at a low partial pressure of CO_2 or under a low light intensity, and would not grow at all under photoheterotrophic conditions (DCMU and glycerol present). By contrast, a PsaD deletion mutant of *Synechocystis* shows the converse behaviour, growing well under photoheterotrophic conditions but very slowly photoautotrophically [143].

The above results have led to the conclusion that PsaE has a role in cyclic electron transfer [149] and further support for this idea has come from measurements of rates of $P700^+$ reduction in cell suspensions [48]; in the double mutant *psaE⁻ndhF⁻* the rate of reduction of $P700^+$ was

very low, as if there were no cyclic return of electrons. The simplest explanation is that PsaE is the crucial component of FQR, providing both ferredoxin and plastoquinone binding sites. An additional redox cofactor would not be required if these binding sites were close enough to bring the FeS centre of ferredoxin and the headgroup of plastoquinone within 15–20 Å. The three-dimensional solution structure of PsaE of *Synechococcus* sp. PCC 7002 has recently been reported [150,151]. It shows that the conserved residues Lys-7, Lys-9 and Arg-12 form a basic patch on the surface. These are close to the hydrophobic core of the molecule and there are several aromatic residues which might be involved in a quinone binding site. A plausible model is one in which the quinone binding site is formed by a pocket between PsaE and either the PsaA/PsaB core of the reaction centre or one of the smaller hydrophobic subunits. This is speculative, but a direct test would be to measure the FQR activity of thylakoid membranes from the PsaE deletion mutant. It is worth noting that a cyclic pathway involving NADPH could not explain the behaviour of the PsaE deletion mutants.

An obvious objection to the model described above is that cross-linking between ferredoxin and PsaE has not been reported. It seems possible, however, that in normal thylakoid membranes ferredoxin would bind preferentially to PsaD, the implication being that PsaD may exert some regulatory function over cyclic electron flow. An alternative way of overcoming this objection is to propose that the ferredoxin binding site is provided by the form of FNR which is bound to PsaE, so that the role of PsaE would then be to provide or contribute to the quinone binding site. The crucial distance would then be that between the flavin group and the bound quinone. Convincing evidence for a role of FNR in cyclic activity is lacking, however, as discussed above. Another possibility for the role of PsaE in cyclic electron transfer, as suggested by Falzone et al. [151], is that it is indirect, by regulating the binding of a minor redox protein of the PS I complex, for example.

9. Regulation of cyclic electron transport

Cyclic electron transport differs from non-cyclic in that an appropriate redox poise must be maintained if optimum rates are to occur. Over-oxidation and over-reduction (too few or too many electrons in the cycle) are equally detrimental. Appropriate conditions are relatively easy to establish artifically in vitro, but under aerobic conditions, which normally prevail physiologically, there is always the danger that electrons will drain away to $NADP^+$ and CO_2 or to O_2. A further point, relevant to algae and cyanobacteria, is that if there are NADP-dependent and NADP-independent pathways these should have different physiological functions and different systems of regulation. A distinction may also need to be made between cyclic electron flow occurring in response to a need for extra ATP, and that occurring as a response to forms of stress which lead to down regulation of PS II via a low intrathylakoid pH.

At first sight the plastoquinone pool is an attractive candidate for redox regulation. A high degree of reduction of the pool, as might occur in steady-state photosynthesis at high light intensity, would leave relatively little opportunity for cyclic return of electrons. When conditions favoured activity of PS I over PS II the pool would become more oxidised and could act as an effective acceptor for cycling electrons. This simple picture is difficult to reconcile with the fact that high rates of cyclic phosphorylation can be observed in vitro under strongly reducing conditions (about 50% reduction of the added ferredoxin). Hence FQR activity is more likely to be a limiting factor.

An alternative source of redox regulation would be the NADP(H) pool. A relatively oxidised state would favour linear electron transport and a more reduced state the cyclic return of electrons to the plastoquinone pool. Such an effect would be enhanced by an allosteric inhibition of FNR activity by NADPH [152]. A regulatory mechanism of this kind has been proposed by Tagawa et al [3], Arnon and Chain [153], and Hosler and Yocum [112,113]. Laisk et al. [154] have made use of it in an interesting discussion of the origins of oscillations in the rate of photosynthesis induced by sharp changes in light intensity or CO_2 concentration. This type of mechanism assumes a kinetic control over the availability of reduced ferredoxin to FQR, which is known to have a sufficiently low affinity for reduced ferredoxin [67]. Attempts to show an allosteric effect of NADPH on FQR activity only revealed a weak inhibition (the opposite of what is required) at high (probably unphysiological) concentrations of NADPH [67]. Only low rates of cyclic phosphorylation would be possible under steady-state conditions at high light intensity, when the acceptor side of PS I is largely oxidised [47], and the redox state of the NADP pool appears to change little with light intensity [155]. Redox control by NADPH may be able to explain regulation of cyclic electron flow under transient conditions [154] and light stress induced by the absence of CO_2 [1], but is not adequate for the regulation of cyclic photophosphorylation by processes which require solely ATP (e.g., supplementary ion transport under conditions of salt stress [17,156]).

The mechanism by which changes in ATP demand are translated into redox regulation of cyclic electron flow is unknown and attempts to demonstrate a direct effect of ATP or ADP on FQR activity have been unsuccessful [67]. However, an interesting inhibition of FNR activity by ATP has been demonstrated by Hodges and Miginiac-Maslow [157]. Non-competitive inhibition occurs with K_i for ATP of 2.5 mM, which may be physiologically significant. Presumably this is an allosteric effect which does not involve the NADP binding site. Phosphorylation of the N-terminal region of FNR was also shown to occur. In the phosphorylated enzyme the K_m for ferredoxin is increased

by a factor of 4, but there is also a small increase in V_{max}. These effects might have a role in diverting electron flow away from $NADP^+$ reduction into other pathways, including cyclic.

There is also evidence that state transitions, which lead to changes in the relative rates of delivery of excitation energy to PS I and PS II, play a role in regulation by ATP demand [34,158,159]. A change in intracellular demand for ATP has been shown to induce a state transition in *Chlamydomonas* [160]. A study of the kinetics of photooxidation of cytochrome *f* in cell suspensions of green algae [161] suggested that close association of PS I and the *bf* complex is correlated with ATP depletion, and would be consistent with a stimulation of cyclic phosphorylation under these conditions. An alternative explanation is that the effect is controlled by the intrathylakoid pH. Nevertheless, direct evidence for a lateral redistribution of cytochrome *bf* complexes during state transitions was obtained by Vallon et al. [159] with both maize thylakoid membranes and *Chlamydomonas* cells. The role of state transitions has been discussed in more detail by Fork and Herbert [2] and Anderson [158]. Anderson has developed the idea further by proposing that different thylakoid membrane domains are responsible for linear electron transport (granal compartments) and cyclic electron transport (stroma lamellae) [158,162]. This concept is based on the presence of two populations of PS I, one confined to stroma lamellae and the other to the grana margins and end membranes [163]. The outstanding question here is whether the increase in PS I activity causes an increase in the state of reduction of ferredoxin or NADP which could cause a significant increase in cyclic activity, or whether some other redox switch needs to be invoked. The idea of a separate cyclic domain raises the possibility of a localised reduction of ferredoxin.

Many of the features of regulation discussed above apply to both NADPH dependent and NADPH independent cyclic pathways. The crucial question, however, is the nature of the switch from linear to cyclic electron flow, which is likely to be different in the two cases. The molecular mechanisms of cyclic electron transfer need to be more completely understood before this question can be answered.

10. Conclusions

(1) Knowledge of the pathways and regulation of cyclic electron transport and phosphorylation is necessary for understanding the overall energetics of photosynthesis.

(2) New methods have recently become available, particularly techniques involving photoacoustics and measurement of the redox state and kinetics of P700, which give new insight into the role of cyclic phosphorylation in whole cells and tissues.

(3) There is evidence that cyclic electron transport may serve three distinct functions.

(i) Supplementation of ATP coupled to linear electron transport in order to keep the ATP/NADPH ratio high enough to drive the Calvin cycle in steady-state photosynthesis.

(ii) Provision of ATP for a variety of cellular processes, under both normal and stress conditions.

(iii) Protection against photoinhibition by down-regulation of PS II through the generation of a low intrathylakoid pH.

The maximum rate of cyclic processes is probably not more than about 3% of non-cyclic. Under some circumstances the rate of cyclic phosphorylation may be significant, but too low for ready detection in intact cells.

(4) There is good evidence that physiological pathways of cyclic electron transport involve the plastoquinone pool and the cytochrome *bf* complex. The key step is the return of electrons from the reducing side of PS I to the plastoquinone pool. Studies in vitro strongly suggest that in plants and cyanobacteria a soluble cofactor is always required at this point in the cycle. The physiological cofactor is usually ferredoxin, but the role of flavodoxin has not been investigated. In some cyanobacteria a soluble, low-potential cytochrome *c*-549 can catalyse cyclic phosphorylation, but its physiological significance in this role is uncertain.

(5) Plants and cyanobacteria possess a cyclic pathway in which electrons are returned directly to the plastoquinone pool from reduced ferredoxin via an unidentified enzyme known as ferredoxin-plastoquinone reductase (FQR), which is probably associated with PS I although a firm link has not been established. This pathway is likely to occur in all oxygenic organisms, although its distribution has not been thoroughly studied. The characteristic inhibitor of FQR in higher plants is antimycin. In those cyanobacterial systems which have been tested antimycin is not effective, but HQNOBr does inhibit.

(6) In some algae and cyanobacteria there is evidence for a second cyclic pathway which involves NADP and an NAD(P)H-plastoquinone reductase occurring in the thylakoid membranes. Although the chloroplast genome of higher plants is known to include genes homologous with those of mitochondrial complex I, evidence for a significant level of expression is lacking.

(7) Direct electron donation from reduced ferredoxin to the cytochrome *bf* complex has frequently been postulated, but there is no convincing evidence that it occurs at a significant rate.

(8) The protein components of FQR have not been identified, but genetic and kinetic evidence for a role of PsaE in cyclic electron transport in a cyanobacterium suggests that this subunit may be involved.

(9) The key question for understanding the regulation of cyclic electron transport is how the distribution of electrons from reduced ferredoxin is controlled. The main distinction is between linear electron flow to CO_2 or other acceptors on the one hand, and back into the plastoquinone

pool on the other. Kinetic control via the redox state of ferredoxin or NADP may be adequate under some circumstances, but an unidentified allosteric mechanism is likely to be required to explain the variety of physiological demands placed upon the cyclic pathway. In particular, the mechanism by which a deficiency of ATP is translated into redox control of the cycle is unknown. There is no evidence for an allosteric effect of either NADPH or ATP on the activity of FQR.

(10) Different regulatory systems should be sought for the NADPH-dependent and NADPH-independent pathways.

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