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Cyclic electron transport in C_3 plants: fact or artefact?

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Abstract

The phenomenon of cyclic electron transport was first characterized in higher plant chloroplasts 50 years ago, yet there is still a debate about whether or not this is a physiological process. The recent isolation of mutants that appear to lack cyclic electron transport, as well as new data providing functional evidence for its occurrence, support the notion that this pathway plays an important role in plant responses to stress, providing a pH gradient across the thylakoid membrane to trigger non-photochemical quenching of chlorophyll fluorescence. At present, little is known about the regulation of cyclic electron transport, but it is possible that this is activated in response to a low redox potential in the chloroplast stroma.

Key words: Cyclic electron transport, cytochrome b_6f, NPQ pgr5, photosystem I, photosystem II, ΔpH.

Introduction

A pathway of cyclic electron transport (CET) around photosystem I (PSI) in the thylakoid membranes of plants was first described 50 years ago (Arnon *et al.*, 1955). Since then, in spite of a large body of literature relating to the process either *in vitro* or *in vivo*, little progress has been made in the understanding of this pathway. Indeed, whilst the pathway has been described in C_4 plants, green algae and cyanobacteria, until recently a consensus seemed to be developing that CET did not occur in C_3 plant leaves under 'normal' physiological conditions (Herbert *et al.*, 1990). However, a number of papers have been published presenting indirect and direct evidence that CET not only occurs in C_3 plants but is essential to growth. Notably, Munekage *et al.* (2004) have presented a description of a mutant that is thought to lack both the principal pathways of CET and which is grossly impaired in growth and development. If CET is really so important, why was it missed for so long in C_3 leaves? In this paper, the evidence for and against CET as a significant process in C_3 plants is briefly reviewed. Accepting that it does really occur, then its function and what is known about its regulation will be considered. For a more complete view of CET, including CET in cyanobacteria and algae, the reader is referred to other recent reviews (Fork and Herbert, 1993; Bendall and Manasse, 1995; Heber, 2002; Allen, 2003; Joliot *et al.*, 2005).

The pathway of CET

Although the *in vivo* occurrence of CET has been a subject of some controversy, especially in C_3 plants, the possibility of such a pathway existing is well established in isolated systems. In considering whether this process is simply an artefact of the conditions used in *in vitro* experiments, it is necessary to understand the route taken by electrons in such experiments (Fig. 1).

The earliest measurements that gave an indication that a cyclic flow of electrons could occur around the PSI reaction centre involved the addition of artificial electron mediators. However, it was the observation that ferredoxin could also act as an electron mediator that made it seem plausible that CET could occur in intact plants (Arnon, 1965). Given that CET generates a ΔpH, it was postulated that an enzyme must exist that could use ferredoxin to reduce plastoquinone, which would then feed electrons into the cytochrome b_6f complex, i.e. ferredoxin quinone oxidoreductase (FQR). Early observations indicated that this pathway was sensitive to the electron transport inhibitor antimycin (Tagawa *et al.*, 1963), although more recent data in intact leaves have questioned this (Joet *et al.*, 2002; Joliot *et al.*, 2004). Antimycin is known to bind to the Q_i (stromal)

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Abbreviations: CET, cyclic electron transport; FNR, ferredoxin NADP oxidoreductase; FQR, ferredoxin quinone oxidoreductase; ndh, NAD(P) dehydrogenase; PSI, photosystem I; PSII, photosystem II; ΔpH, pH gradient across the thylakoid membrane.

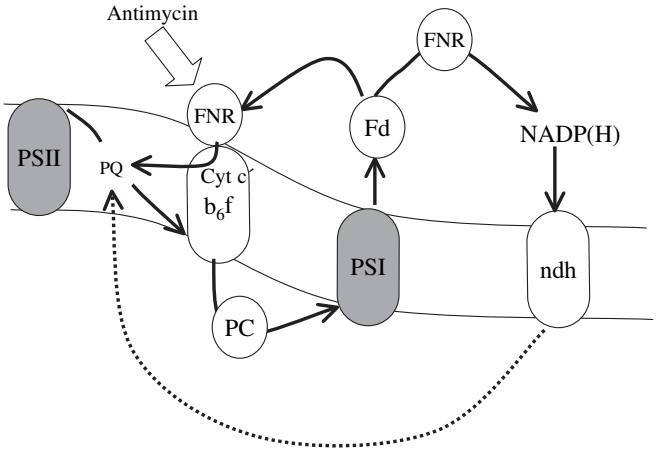

Fig. 1. Possible pathways for cyclic electron transport around photosystem I. Electrons pass from the acceptor side of PSI to ferredoxin. From there they may be transferred to a ferredoxin NADP oxidoreductase (FNR) molecule on the stromal side of the cytochrome b_6f complex and, via cytochrome c' to plastoquinone, from where they pass through the normal electron transport chain to PSI. Alternatively, electrons from ferredoxin might be passed, via FNR, to NADPH, which can then reduce plastoquinone in a reaction mediated by NADP dehydrogenase (ndh). Arrow labelled 'antimycin' indicates probable site of action of antimycin.

quinone binding pocket of the cyt b_6f complex, leading to the suggestion that this site was involved in electron flow. However, Moss and Bendall (1984) noted that 2-heptyl-4-hydroxy-quinoline N-oxide (HQNO), an inhibitor of cytochrome *b*, did not affect CET, leading to the suggestion that FQR is a distinct enzyme. No biochemical evidence exists for such a distinct protein and the recent observation of a novel *c*-type cytochrome in the cyt b_6f complex throws into question the evidence against this complex having a role in CET (Kurisu *et al.*, 2003; Stroebel *et al.*, 2003). Furthermore, the observation that ferredoxin NADPH oxidoreductase (FNR) binds tightly to the cyt b_6f complex provides a possible ferredoxin binding site on this complex (Zhang *et al.*, 2001). Thus, the most probable route for electron flow is from the acceptor side of PSI, via ferredoxin, FNR and cyt c' in the cyt b_6f complex to plastoquinone. From there, electrons would follow the normal route through cyt *f* and plastocyanin to P700.

In addition to the above ferredoxin dependent–antimycin sensitive pathway, evidence also exists for a pathway that does not directly involve ferredoxin and which is insensitive to antimycin. This would involve the transfer of electrons from NADPH to plastoquinone. The observation that a complex homologous to the mitochondrial NADH dehydrogenase (ndh; complex I) exists in the chloroplast provides a possible mediator for this pathway (Shinokazi *et al.*, 1986; Sazanov *et al.*, 1995). There is some evidence that the ndh complex can mediate a cyclic electron flow *in vivo*, but concentrations of this enzyme are extremely low so it remains unclear if this flux would ever be significant under physiological conditions (Sazanov *et al.*, 1998; Joet *et al.*, 2002).

Does cyclic electron transport occur in C_3 plants?

One of the principal problems in studying CET has been the difficulty in assaying it. This is a problem that is common to all cyclic processes which, by their nature, do not involve a net flux. The flux through individual components believed to take part in the pathway, most readily in PSI and cyt b_6f, can, however, be measured. The problem is that these electron carriers are also involved in linear electron transport from water, via photosystem II (PSII) to NADP. Hence, it is necessary to be able to determine the relative contribution of linear flow to the overall flux through the shared components. This has been achieved in two ways; either the flux through PSII can be measured at the same time, mostly using chlorophyll fluorescence, or it can be suppressed, using an inhibitor such as DCMU or by illuminating the leaf only with far-red light (λ >700 nm, which excites only PSI and not PSII). These two approaches will be classified here as 'steady-state' and 'non-steady-state', respectively. Both approaches have given rise to contradictory results which will be discussed.

Non-steady-state measurements of CET

Perhaps the simplest assay that can be performed to measure CET is to illuminate a leaf with far-red light or in the presence of DCMU and measure the electron flux through PSI. The most common assay of this is to stop the illumination abruptly and to measure the rate at which oxidized P700, the primary electron donor of PSI is re-reduced (Maxwell and Biggins, 1976). The redox state of P700 can be measured as an absorbance change at 700 nm or, more commonly, in the near infrared (800–900 nm) (Harbinson and Woodward, 1987). The rate of reduction of P700 immediately following the cessation of the light is taken as a measure of the rate of electron flow that was occurring in the light. Measurements of this kind have been reported in a variety of species, including both C_3 and C_4 species, as well as in algae and cyanobacteria.

An additional approach, often used in combination with measurements of P700 kinetics, is to assay the product of CET. Although there is no net flux to measure, CET does nevertheless result in the generation of a product; a proton gradient across the thylakoid membrane (ΔpH). This cannot be directly measured, however, using the technique of photoacoustics it is possible to assay the 'energy storage' associated with electron transport (Herbert *et al.*, 1990). In addition, the formation of a ΔpH is associated with the generation of a potential gradient across the membrane and this can be assayed using a shift in the spectrum of a carotenoid, that gives rise to an apparent absorbance change around 515 nm (Joliot and Joliot, 2002). Furthermore, the swelling of the thylakoid membrane induced upon generation of the ΔpH produces a change in the light-scattering properties of the chloroplast, which can be seen as an apparent absorbance change around

535 nm (Cornic *et al.*, 2000). All of these signals have been measured in whole leaf studies of CET.

Many measurements using the above approaches have led to the conclusion that, whilst cyanobacteria, green algae, and C_4 plants are capable of CET, C_3 plants are not (Herbert *et al.*, 1990). In the first three groups, the decay of P700 following a far-red light–dark transition is seen to follow a complex kinetic with at least two exponential components. The 'fast' component, which has a half-time of the order of 200 ms is taken to be indicative of CET and is much slower ($t_{½}$ >1 s) or absent in C_3 plants. At the same time, photoacoustic measurements of energy storage in far-red light support the notion that CET does not occur under such conditions in C_3 plants; whilst cyanobacteria, algae, and C_4 plants all show significant energy storage under far-red light, C_3 plants generally do not (Herbert *et al.*, 1990).

There are, however, problems with the above approach. Firstly, much of the early work on CET has emphasized the importance of redox poising of the cyclic chain. For CET to persist, there must be a mix of both oxidized and reduced electron transporters available. If all the acceptors from PSI are reduced or all the donors oxidized, then electron flow will stop. Under 'normal' conditions (i.e. with PSII active) electron flow into the electron transport chain is ensured. There will always be a pool of reduced plastoquinone providing reducing equivalents that can be used in CET. The danger under such conditions is that too many electrons will come from PSII and that the cyclic pathway will become overreduced (Heber *et al.*, 1992). Under far-red light, however, this source of electrons is absent. CET generates reduced iron–sulphur centres on the acceptor side of PSI. These are liable to be oxidized by molecular oxygen (the Mehler reaction). Thus, there will always tend to be a leak of electrons away from the cyclic pathway, which, during prolonged far-red illumination, will soon result in the cessation of CET.

In C_4 plants, CET is presumed to be a phenomenon associated with bundle sheath cells, where PSII is largely absent and so CET can be the only significant form of photosynthetic electron transport (Bassi *et al.*, 1985). In these cells, malate exported from the mesophyll is broken down generating CO_2 and NADPH. This results in there being a rather negative redox potential in the chloroplast, even in the absence of PSII activity. There is evidence that algae also maintain a low redox potential in their chloroplasts and, in cyanobacteria, the co-existence of photosynthesis and respiration in the same membrane means that a supply of electrons to maintain the redox poise of the cyclic pathway is always available. Thus, the redox poise of the cyclic pathway is maintained even under far-red illumination.

That leaching of electrons from the cyclic pathway is responsible for the apparent absence of CET under far-red light in C_3 plants has been confirmed by recent measurements by Joet *et al.* (2002), as well as by earlier work (Heber *et al.*, 1978; Katona *et al.*, 1992). If far-red illumination is provided in the absence of oxygen, thus ensuring the maintenance of reducing conditions, the 'fast' component in P700 relaxation can be seen and a significant ΔpH across the thylakoid membrane is generated.

From the above discussion, as well as from the large body of *in vitro* literature, it can be deduced that C_3 plants are quite capable of performing CET, i.e. they have all the necessary 'equipment' to support this pathway. This does not, however, determine whether they actually do perform CET at a significant rate under conditions of normal photosynthesis, i.e. in the presence of linear ET. Under the best conditions, the 'fast' component of P700 decay is rather slow, compared with the rate at which PSII is capable of delivering electrons to PSI (half-time for P700 reduction of a few ms). Even if this rate of electron flow is maintained in the presence of linear flow, its contribution to overall electron flux would be minimal, begging the question, does it have any real physiological role or is it simply a curious artefact? Some recent data suggest, however, that such measurements significantly underestimate the capacity of electron flow.

Joliot and co-workers have recently examined the effects of short periods of saturating illumination on electron transport in dark-adapted spinach (Joliot and Joliot, 2002) and *Arabidopsis* (Joliot *et al.*, 2004) leaves infiltrated with DCMU to suppress PSII. They measured absorbance changes at 515 nm as an indicator of the *trans*-thylakoid potential gradient and used the decay of this field during short interruptions in illumination to assay the rate of electron transport. They concluded that in *dark-adapted* leaves very substantial rates of CET can be sustained, although this is only a transient phenomenon, presumably as electrons are leached to oxygen. In plants that have previously experienced high light, however, this transient state is absent.

Support for the above observations comes from measurements that have been performed on barley (Golding *et al.*, 2004). Measurements of the decay of oxidized P700 following short light flashes (100–200 ms) to dark-adapted leaves infiltrated with DCMU resulted in a decay with two exponential components. The *slow* phase measured in this decay was of the same order of magnitude as the *fast* phase described by Joet *et al.* (2002) and others. Golding *et al.*'s measurements (Golding *et al.*, 2004) were made over short time periods and did not resolve the slow component well. Careful measurements of these longer phases reveal the presence of a third, even slower decay component (Fig. 2). Thus, these results suggest that there are three populations in the P700 decay, the two slower components previously described and a third, much faster component that is absent in previous measurements (probably due to electrons leaching from the cyclic pathway). The fast phase observed in these measurements has a rate constant close to that seen for P700 reduction in the presence of PSII activity and so could be competitive with linear electron transport.

A key feature of the above measurements was the length of the flash that was given. With increasing flash length, the amplitude of the fast phase decreased. Thus long flashes of

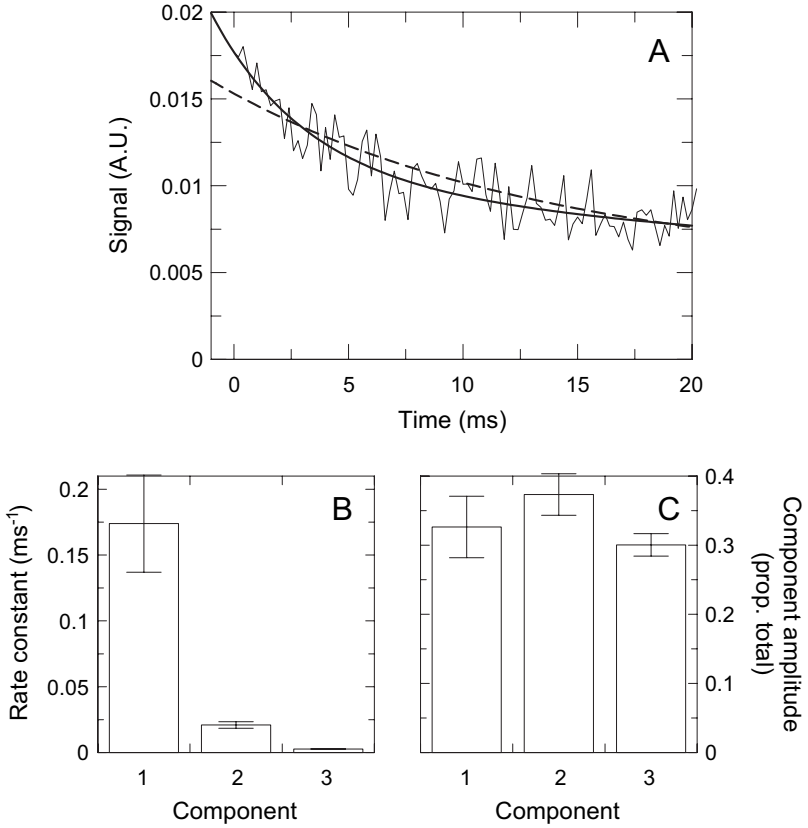

Fig. 2. Reduction of P700 following a 100 ms saturating light flash given to a leaf previously infiltrated with the herbicide DCMU (diuron) to block turnover of photosystem II. (A) Example data set. Data were recorded using a Walz dual wavelength detector linked to a laboratory-built data acquisition system. Data were fitted with a three-component exponential curve (solid line) or a two-component exponential curve (dashed line). The latter can be seen to fit poorly to the data. (B) Rate constants for the three components. (C) Relative amplitudes of the three components. (B, C) Data are the mean of three measurements with standard error bars shown.

light gave results that were indistinguishable from earlier measurements on C_3 plants, consistent with the idea that electrons are being leached from a cyclic pathway. A further characteristic, also seen by Joliot and co-workers (Joliot and Joliot, 2002; Joliot *et al.*, 2004), was that, to observe the fast phase relaxation of P700, leaves had to be dark-adapted for several hours. Once actinic light was given, this state rapidly disappeared (within a few minutes) and took hours to reappear. However, if leaves were subjected to drought stress, this fast phase could be seen even in the absence of dark-adaptation.

In conclusion, it appears that the chloroplasts of C_3 plants are probably capable of performing cyclic electron transport, at least under carefully defined conditions. What is not certain from such measurements is whether this CET is occurring under conditions that are physiologically relevant, i.e. under conditions of steady-state photosynthesis, when PSII is active.

Steady-state measurements of photosynthesis

The challenge, in measuring CET under conditions where linear flow is present, is to distinguish the contribution of the two flows to flux through PSI. To do so, it is common to gain a measure of PSII electron transport using chlorophyll fluorescence. The parameter ΦPSII (Genty *et al.*, 1989) is widely used and, provided necessary precautions are taken, gives a robust measure of PSII electron transport. Typically, electron transport through PSI and PSII are measured over a range of conditions and the ratio of one to the other compared. If this varies, it can be concluded that CET could be occurring. However, if CET remains constant as a proportion of total electron transport, this could not be detected in this way, since measurements using fluorescence are uncalibrated, i.e. the *absolute* rates of electron flow through the two reaction centres are not known, only the *relative* rates.

A number of studies have been published that take this approach to measurements of CET, providing contradictory results. Some, but not all, of these contradictions can be explained by differences in experimental approach, with the technique adopted to measure PSI electron transport being crucial. A widespread approach to this has been to assume that the proportion of P700 in the reduced state provides a measurement of the quantum efficiency of PSI (Harbinson and Woodward, 1987). The basis of this assumption is that

the limitation to PSI electron transport lies at the level of electron donation to P700. Thus, if P700 is reduced, it is capable of undergoing charge separation, if it is oxidized, it is not. No state analogous to the 'closed' state of PSII, with the electron donor and acceptor both reduced, exists. There are, however, conditions (e.g. low CO_2 and O_2) where this is not the case (Harbinson, 1994). To overcome, this problem, Klughammer and Schreiber (1994) proposed that by providing a short saturating flash of light to the sample and measuring the signal induced it is possible to determine the proportion of PSI centres that are 'open', i.e. oxidized on the acceptor side and reduced on the donor side. A further potential problem with this approach is that the size of the PSI antenna may vary. This is clearly the case if state transitions occur, however, these are generally confined to very low light conditions in higher plants. There is evidence, however, that PSI located in the stromal lamellae have significantly smaller antennae than those in the granal margins (Albertsson, 2001). Thus the contribution of these centres is going to tend to be overestimated under non-saturating illumination, distorting the relationship between irradiance and PSI electron transport. Recent measurements modelling flux through multiple components have suggested that the redox poise of P700 is a poor indicator of electron flux through PSII (Oja *et al.*, 2003).

An alternative approach to estimating PSI electron transport has been to measure the rate at which P700 is reduced following a light-to-dark transition, in the same way has been done following far-red illumination (see above). This is an intuitively more obvious approach, however, it is also not without complications. The principal complication that has been noted is related to the portioning of electrons within the electron transport chain (Sacksteder and Kramer, 2000). The limiting step in this chain is the oxidation of plastoquinol by the cyt b_6f complex. Thus, under high-light conditions, cytochrome *f*, plastocyanin, and P700 will all tend to be oxidized. The equilibration between these is very rapid and, given the difference in redox potentials, electrons entering this portion of the chain will tend to go straight to P700. Under conditions of low light, however, this is not necessarily the case and net reduction of other components may occur which will need to be taken into account. In practice, there is only a narrow range of conditions in which this presents a significant problem, when P700 is less than 20% oxidized, and this can be taken into account by making measurements of cytochrome *f* reduction (Sacksteder and Kramer, 2000; Golding *et al.*, 2005).

One of the earliest reports, comparing PSI and PSII under conditions of varying irradiance, used P700 redox state as a measure of PSI electron transport and found no evidence for CET (Genty *et al.*, 1990). Other reports using the same approach but varying CO_2 reached the same conclusion (Harbinson, 1994; Genty and Harbinson, 1996). Curiously, evidence was found for CET in the absence of CO_2, and varying the irradiance (Harbinson and Foyer, 1991).

More recently, the light and CO_2 dependence of CET have been re-examined in barley leaves and it was concluded that both high light (Clarke and Johnson, 2001) and low CO_2 (Golding and Johnson, 2003) induce this process. This conclusion is valid using both the decay of P700 and its steady-state redox state (Klughammer and Schreiber, 1994) as assays of PSI turnover. So, how can the discrepancy with earlier studies be explained? An important distinction between this work and the earlier studies relates to the methodology. Taking the case of low CO_2, it was observed that the proportion of PSI in the oxidized state falls with decreasing CO_2, consistent with earlier data. However, measurements of the pool of *active* P700, i.e. which can be oxidized by a flash of saturating light and then be reduced rapidly in the dark, reveals that this increases at low CO_2. Hence, whilst the pool of oxidized P700 rises, the total 'reduced-active' pool (i.e. centres in which P700 is in the reduced state and which are capable of undergoing stable charge separation) does not fall correspondingly. Consistent with this, the rate of PSI turnover is maintained at a higher rate than expected from the earlier measurements. This is a surprising result, as it implies that there is a population of PSI centres that are inactive under optimal conditions and which become active when plants are exposed to stress. These centres would not have been observed in those earlier studies which did not use saturating flashes to measure PSI activity, leading to an underestimate of PSI flux.

In contrast to the above results, there remain some recent studies that have failed to observe any evidence for CET in C_3 plants. A study from Sacksteder and co-workers, in greenhouse-grown tobacco, compared the decay of the absorbance change at 515 nm (taken by Joliot and Joliot as a measure of PSI+PSII flux) with the flux through the high potential portion of the electron transport chain (cyt *f* to P700) and with PSII flux measured using fluorescence (Sacksteder *et al.*, 2000). They observed a strict correlation between these three parameters over a range of different irradiances from limiting to nearly saturating. These measurements provide a robust indication that either no CET was occurring under these conditions or, if it was, it was at a rate that was locked to the linear flow. However, these authors did not examine situations under which PSII electron transport was saturated. The studies also differed in the light sources used to illuminate leaves. While the studies recording CET at high light have used white light sources, rich in far-red light, the Sacksteder and Kramer (2000) study used narrow band red light which preferentially excites PSII. Given the differences in the excitation spectra of the two photosystems, such differences might be crucial.

In summary, whilst there is clear evidence that CET occurs in C_3 plants, it seems to be a phenomenon of stress conditions, appearing when linear electron flow is saturated either at high light or under conditions when carbon fixation is limited. This does not exclude the possibility of it

occurring under light-limited conditions, however, if this were the case, either the rate would need to be small (within the measuring error of the methods available) or strictly locked to the rate of linear electron flow.

The role of CET in C_3 plants

Cyclic electron transport around the PSI reaction centre is generally accepted to generate a pH gradient across the thylakoid membrane. This ΔpH might serve a number of functions; however, the most obvious to consider in the context of CET are the generation of ATP and the regulation of light harvesting, via the process of non-photochemical quenching.

The idea that CET might be required to generate the ATP required for carbon fixation has a long history, much of which has revolved around the question of what is the stoichiometry of protons pumped per electron transferred through the linear pathway and how many protons have to pass through the ATPase to generate a molecule of ATP. The current consensus on these questions is that there is an H^+/e^- of 3 and an H^+/ATP of 4.7 (Allen, 2003). If these ratios are correct, this only corresponds to 2.55 ATP molecules being synthesized per CO_2 fixed, a shortfall of about half an ATP molecule. Thus, there might be a requirement for additional ATP generation via CET. Under light-limiting conditions there is little evidence for CET, however, and, given that the shortfall in ATP synthesis is small, other electron transport pathways may be sufficient to cover this. For example, electron transfer to nitrogen assimilation or to oxygen in the Mehler reaction both generate ΔpH without being coupled to ATP consumption. A recent paper by Munekage and colleagues has suggested that CET is essential for photosynthesis to occur (Munekage *et al.*, 2004). This is on the basis that plants lacking both the ndh complex and a protein termed pgr5, which has been implicated in ferredoxin-dependent CET, have severely inhibited growth. However, whether the inhibition of growth in these plants is related to a lack of ATP for carbon fixation has not been established.

The idea that CET might function to generate a ΔpH to induce non-photochemical quenching of chlorophyll fluorescence (NPQ) was first proposed by Heber and Walker (1992) and has recently gained experimental support from different directions. The isolation of the pgr5 mutant was achieved by screening for plants which lack the ability to perform NPQ (Munekage *et al.*, 2002). This mutant is clearly inhibited in its ability to generate a ΔpH and is thought to be impaired in ferredoxin-mediated (antimycin-sensitive) CET. At present, the definition of this phenotype has only been indicated on isolated thylakoid membranes, examining the reduction of plastoquinone in the presence of NADPH and ferredoxin (Munekage *et al.*, 2002, 2004). This, however, needs confirming using *in vivo* assays for CET. A curious feature of the pgr5 mutant, which has yet to be fully explained, is that P700 remains in a largely reduced state at high light, in contrast to the wild type where P700 becomes progressively more oxidized with increasing irradiance. This might be explained in two ways: down-regulation of the linear electron transport chain, which is required for oxidation of P700 to occur, might require a large ΔpH, which is not generated in pgr5; or, alternatively, it might be the feedback regulation of electron transport which is itself the primary lesion in the mutant. In the latter case, CET might still be inhibited, as such a phenomenon would interfere with the redox poising of the cyclic pathway. Clearly, a better understanding of the phenotype of this mutant will provide a clearer picture of how electron transport is regulated.

In addition to the genetic support for Heber and Walker's model (Heber and Walker, 1992) provided by the pgr5 mutant, functional measurements of CET also provide evidence in favour of a protective role for this pathway. Measurements of NPQ show it to primarily be a phenomenon of stress conditions; high light, low temperature, drought etc. Under such conditions, it is commonly observed that NPQ will vary substantially under conditions where linear electron transport is saturated and, indeed, NPQ will even rise under conditions where linear flow is inhibited, for example, at low CO_2.

Consider the case of drought stress. It is well established that either drought or conditions simulating drought (low CO_2) can lead to an increase in NPQ. A number of different studies have indicated that CET increases under such conditions, using both steady-state and non-steady-state approaches. For example, data from Gerst *et al.* (1995) indicated that PSII electron transport was more sensitive to the progressive development of drought, induced by cutting the petiole of an illuminated leaf, than was PSI. In this study, PSI electron transport was assayed using the approach of Klughammer and Schrieber (1994). Harbinson and Foyer noted evidence of CET at low CO_2 when varying light, using the PSI redox state as a simple measure of PSI quantum efficiency (Harbinson and Foyer, 1991), however, Harbinson did not find such evidence when varying CO_2 at a fixed irradiance using the same approach (Harbinson, 1994). This latter discrepancy can be explained by the recent data using either the Klughammer and Schreiber (1994) approach or the kinetics of P700 relaxation as indicators of PSI turnover, both of which indicate that CET increases with decreasing CO_2 (Golding and Johnson, 2003).

Similar conclusions can be drawn when examining the case of high light. NPQ saturates at a significantly higher irradiance than linear electron transport or oxygen evolution. Cornic and co-workers noted that the sensitivity of PSI to far-red illumination decreased following periods of illumination with high irradiance, i.e. the intensity of FR light needed to oxidize P700 fully increased (Cornic *et al.*, 2000). This was suggested to result from an activation of the cyclic pathway under such conditions, making it harder to oxidize PSI. It was observed that saturation of PSI

electron transport under steady-state conditions occurred at substantially higher irradiances than PSII, again consistent with CET being activated at high light (Clarke and Johnson, 2001). In the same study, it was noted that PSI electron transport is less sensitive to low temperature than is PSII. Under the same conditions, NPQ was seen to rise.

A further incidence where high levels of NPQ are induced is following a transition from darkness into light, where high levels of ΔpH are transiently generated prior to the activation of the Benson–Calvin cycle (see Finazzi *et al.*, 2004, for a discussion). During the initial illumination, it has been established that CET proceeds at a high rate. This might explain the transient occurrence of high NPQ under such conditions.

Taking all the above observations, it seems clear that there is a coincidence in the occurrence of CET and of high levels of NPQ. It has, however, recently been questioned whether the levels of CET observed would be sufficient to support the ΔpH required (Kramer *et al.*, 2004). Kramer and colleagues have suggested that to attain the necessary ΔpH levels for NPQ, CET would have to occur at up to five times the linear rate. In these measurements, the maximum CET was around twice the linear rate at low CO_2, or two-thirds that at high CO_2 (Golding and Johnson, 2003). Kramer and colleagues argument depends on the interpretation of the relationship between NPQ and linear electron flow measured at a range of different CO_2 concentrations (Kanazawa and Kramer, 2002). At low CO_2, the rate of linear flow at a given level of NPQ was around 5–6 times smaller. So, they argue, to compensate for this shortfall, a similar increase in CET is needed. This argument fails to take into account, however, that, whilst linear electron flow to CO_2 fixation is coupled to ATP consumption, CET is not. Thus, linear flow to the Benson–Calvin cycle will not be a net producer of ΔpH, but, as discussed above, will actually consume more ATP than it can produce. Changes in linear flow will hence give no information about the cyclic rate needed to sustain a given ΔpH.

Regulation of cyclic electron transport

Given that much of the discussion concerning CET in C_3 plants has revolved around the question of whether it is occurring at all, consideration of the regulation of this pathway remains at a very basic level. As discussed above, the bulk of the evidence suggests that the major role for CET in C_3 plants is to regulate the ΔpH, so controlling light harvesting via an NPQ mechanism. As such, most CET will be occurring under conditions where light absorption and linear electron transport tend to be in excess of the capacity for carbon fixation by the Benson–Calvin cycle. Under such conditions, the flow of electrons through the linear chain is down-regulated, via regulation of the cytochrome b_6f complex (Laisk and Oja, 1994; Genty and Harbinson, 1996; Ott *et al.*, 1999). This leads to a situation where the plastoquinone pool is largely reduced and the components of the electron transfer chain after plastoquinol oxidation (P700, cyt *f*, plastocyanin) oxidized. If CET is to occur under such conditions, it must be able to inject electrons into the plastoquinone pool, in competition with PSII. Given that PSII will be turning over at high rates with Q_A^- being largely reduced at high light or low CO_2, it is open to question whether the activity of any ferredoxin quinone oxidoreductase could be sufficiently competitive. If it is feeding electrons into the same PQ pool as PSII then it would probably need to be highly efficient at doing this. An alternative, however, which excludes this dilemma, is to consider that there might be some degree of isolation between the two pathways.

Two models for the separation of cyclic and linear flows have been proposed. Joliot and co-workers (Joliot and Joliot, 2002; Joliot *et al.*, 2004, 2005) have considered the possibility that CET might involve the existence of supercomplexes, containing cytochrome b_6f, plastocyanin, PSI, ferredoxin, and FNR in a more or less tightly bound complex. To date, however, no biochemical evidence has been found for such a complex under a variety of different conditions (C Breyton and G Finazzi, personal communication). Alternatively, segregation might occur at a higher organizational level. Unlike in other photosynthetic organisms, higher plant thylakoid membranes show a high degree of organization, with clear separation of stacked granal regions and unstacked stromal lamellae. The distribution of proteins within these membranes is also heterogeneous, with PSII being concentrated in the stacked regions and PSI in the unstacked regions and on the granal margins (Albertsson, 2001). Cytochrome b_6f is uniformly found in the membrane. Optimization of linear electron transport requires a minimization of the distances between the components involved in electron transfer, i.e. a random distribution of all components. This is not what is seen in higher plants. Furthermore, given the very high concentration of protein in the granal regions, diffusion of plastoquinol in the membrane is expected to be highly restricted (Joliot *et al.*, 1992; Kirchhoff *et al.*, 2000; Lavergne *et al.*, 1992). Thus, it is difficult to envisage that a significant rate of electron transfer could occur from PSII to cytochrome b_6f complexes in the stromal lamellae. Such considerations have led a number of authors to suggest that PSI and cytochrome b_6f in the stromal lamellae might be primarily involved in CET, with PSI at the margins and cytochrome b_6f in the granal stacks being involved in linear electron transport (Albertsson, 2001; Joliot *et al.*, 2004).

Measurements of the distribution of protein complexes in different regions of the thylakoid membrane reveal that approximately 30% of PSII complexes are found in the stromal lamellae, with the remainder being located in the granal margins. In a recent analysis of CET under drought conditions (Golding and Johnson, 2003), it was noted that under optimal conditions (high CO_2, moderate light)

approximately 30% of PSI (measured relative to the total pool of PSI that could be oxidized in a dark-adapted leaf illuminated with far-red light) did not turn over in the light. This effect was measured by determining the signal change in 830 nm absorbance when a flash of saturating light was given to the leaf, followed by immediate transfer to total darkness. This allowed the three types of reaction centre to be defined. Those that were reduced but could be oxidized by a flash; those that were oxidized and were reduced rapidly following a transition to darkness; and those whose redox state did not change during the measurement. The extent of the latter varied from 30% of the total under optimal conditions to 0% at low CO_2. This phenomenon could be explained either as a change in the net balance of electron flow through PSI centres, such that PSI centres were switching continuously through all three states or that the 30% of centres that could not be assayed under optimal conditions represented a distinct pool. Thus the change in signal between optimal and low CO_2 conditions would either represent a switch in the probability of centres being at any moment in time in the 'inactive' state or a sustained switching on and off of centres. Given the coincidence of the proportion of centres in the stromal lamellae and those that were inactive under optimal conditions, it is suggested that the latter was the case and that this difference in behaviour reflected their location in the membrane (Golding and Johnson, 2003).

If the above model is correct, it is necessary to consider what might be the activation step involved, what is the nature of the CET switch. A recent paper by Rajagopal *et al.* (2003) allows one possible mechanism to be suggested. These authors noted that in thylakoid membranes or isolated PSI preparations a proportion of PSI (about 25%) is sensitive to the NADP/NADPH ratio, being activated under conditions where this ratio is low. The fact that the size of this population is similar to the pool of stromal lamellae PSI and of stress-activated PSI leads to the hypothesis that these three pools are identical and that CET is activated in response to an overreduction of the chloroplast stroma under stress conditions (Fig. 3). The NADP(H) sensitivity of a pool of PSI might be explained by their different environment (possibly involving interaction with lipids in the membrane) or may indicate a difference in peptide composition. Many of the minor polypeptides associated with PSI have poorly defined function and might not be uniformly distributed (Jensen *et al.*, 2003). Furthermore, some of these exists in different isoforms, including PsaE which has been implicated in CET in cyanobacteria (Thomas *et al.*, 2001).

Coincident with the activation of CET is an inhibition of linear electron flow (Golding and Johnson, 2003). This can be explained according to two alternative models. Early studies of the effects of pH on electron transport showed that electron flow through the cytochrome b_6f complex is sensitive to the presence of a low pH in the thylakoid lumen (Haehnel, 1984). Since CET generates ΔpH which is not

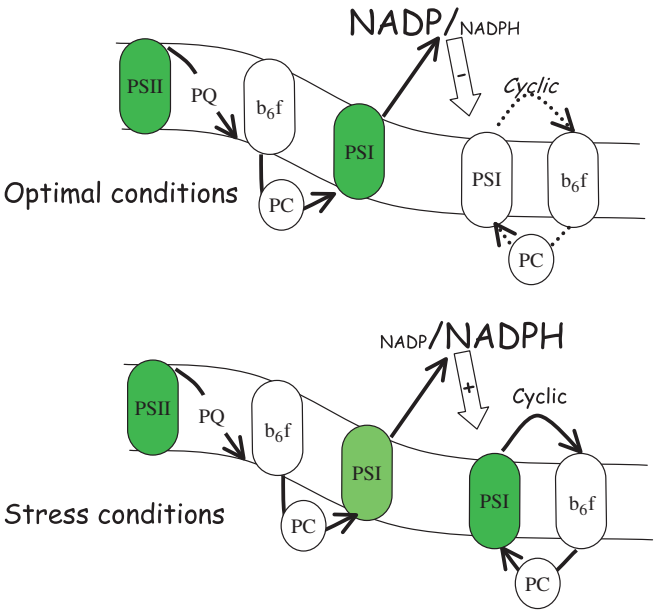

Fig. 3. Model for the organization and regulation of cyclic electron transport. Under optimal conditions (moderate light, high CO_2) linear electron transport proceeds using PSI centres at the margins of the granal stacks. PSI centres in the stromal lamellae are inactive. Under conditions of stress, the NADP/NADPH ratio falls and PSI in the stromal lamellae are activated. These are involved in cyclic electron transport.

dissipated through ATP consumption, free-running CET would cause the ΔpH to rise, until its rate became limited by low lumenal pH. However, if the pH did fall so low, it would also be expected to inhibit PSII, causing the release of Ca^{2+} from the oxygen-evolving complex. There is little evidence for this occurring under steady-state conditions in plants (Krieger *et al.*, 1993; Johnson and Krieger, 1994). In an alternative model, evidence has recently been published that the cytochrome b_6f complex might be regulated by the redox potential of the chloroplast stroma, via a thioredoxin-linked mechanism (Johnson, 2003). This requires confirmation as a physiological process, however, if this were the case, the coincidence of down-regulation of linear flow and activation of CET would be explained by their both responding to a common signal: stromal redox poise.

Given the observations that suggest a substantial rate of CET persists under steady-state conditions, the question must be posed, what prevents the ΔpH from rising to the point where it becomes damaging to the photosynthetic apparatus? Measurements of the decay of the electrical field across the thylakoid membrane following a light–dark transition might give some clues to this.

The rate with which the potential gradient decays following a sudden transition to darkness provides a measure of the rate of PSI+PSII electron transport (Joliot and Joliot, 2002), but has also been argued to be indicative of the flux through the ATPase, coupled to ATP synthesis (Sacksteder *et al.*, 2000). Comparing a leaf at high or low CO_2, it could be expected that there would be a substantially different flux

through the ATPase, in line with the different demands for ATP in the chloroplast. However, measurements of the decay of the potential gradient reveal that the rate of this varies only slightly between these conditions (Sacksteder *et al.*, 2000). This might suggest that the rate of ATP consumption is also only weakly dependent on the activity of the Benson–Calvin cycle, however, this seems unlikely. Alternatively, it is necessary to suggest that the decay of the field is only poorly coupled to ATP consumption under low CO_2 conditions, i.e. that the membrane becomes leaky to protons. If this were the case, the ΔpH produced under any set of conditions would be a function of the rate of proton pumping and the overall resistance of the membrane (including ATP synthesis and any passive leaks). Evidence from isolated thylakoids indicates that the ATPase can become uncoupled in the absence of adenosine nucleotides, but it has been argued that *in vivo* concentrations of these will be sufficient to prevent such a slip (Groth and Junge, 1993). However, it has also been discussed that high light conditions or a strong potential gradient might also induce such a slip (Berry and Rumberg, 1999).

Conclusion

After a history of 50 years, it is only recently that research into cyclic electron transport is starting to make progress. Evidence from *Arabidopsis* mutants (Munekage *et al.*, 2002, 2004) suggests for the first time that this process plays an essential role in higher plants, however, much remains to be determined concerning its mechanism and regulation. However, significant advances in the understanding of this enigmatic process can be expected in the next few years.

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