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Review

Physiology of PSI cyclic electron transport in higher plants ☆

Giles N. Johnson *

Faculty of Life Science, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

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ABSTRACT

Having long been debated, it is only in the last few years that a concensus has emerged that the cyclic flow of electrons around Photosystem I plays an important and general role in the photosynthesis of higher plants. Two major pathways of cyclic flow have been identified, involving either a complex termed NDH or mediated via a pathway involving a protein PGR5 and two functions have been described—to generate ATP and to provide a pH gradient inducing non-photochemical quenching. The best evidence for the occurrence of the two pathways comes from measurements under stress conditions—high light, drought and extreme temperatures. In this review, the possible relative functions and importance of the two pathways is discussed as well as evidence as to how the flow through these pathways is regulated. Our growing knowledge of the proteins involved in cyclic electron flow will, in the future, enable us to understand better the occurrence and diversity of cyclic electron transport pathways. This article is part of a Special Issue entitled: Regulation of Electron Transport in Chloroplasts.

1. Introduction

The concept of there being a cyclic flow of electrons involving only a single photosystem can be dated back to some of the earliest work on photosynthetic electron transport [1,2]. It is only in the last 10 years, however, that a consensus has grown up that this is actually a physiological process (see [3] for a discussion). Even now, details of the pathway involved in this electron flow remain obscure. Nevertheless, we are beginning to develop a view of how cyclic transport works and more importantly of the conditions under which it occurs and the role it plays in the physiology of the plant. It is not the intention of this review to give a comprehensive account of the history of research into cyclic flow—the reader is referred to other reviews for that [3–5]. Rather it is my intention in this short review to examine recent work examining how cyclic flow may function *in vivo* and in particular what roles it might fulfil under different conditions.

At least two distinct pathways and two major functions of cyclic flow have been suggested for higher plants. The recognised pathways have often been referred to as the ferredoxin-dependent (or sometimes FQR) and NADPH-dependent pathways, to reflect the proposed reducing agent re-injecting electrons into the electron transport chain. Recent data have, however, thrown doubt on this distinction, with both pathways appearing to direct involve ferredoxin (T. Shikanai, personal communication), and so these will be referred to as the PGR5 and the NDH pathways respectively. Functioning of either of these pathways is suggested to result in the generation of a pH gradient across the thylakoid membrane (ΔpH) which in turn drives the synthesis of ATP and which also may function to regulate light harvesting, by inducing high energy state quenching (qE) [6]. Recently, however, it has been proposed that a high rate of cyclic flow might occur in an uncoupled manner [7]. If this is the case, a third function (possibly using a third pathway) maybe directly to dissipate energy absorbed by Photosystem (PS) I.

A major hurdle limiting our understanding of cyclic electron transport has been the difficulty of measuring a pathway that does not result in a new flux. Various approaches have been used, in particular techniques examining electron flow in the absence of PSII turnover (either due to the use of light in the far red, which is only weakly absorbed by PSII or by addition of PSII inhibitors), measuring the oxidation (or lack of oxidation) and re-reduction of the PSI primary donor, P700. The generation of an electrochemical gradient under such conditions is an indicator of cyclic flow generating a proton motive force. Measurements of a transient rise in chlorophyll fluorescence following a short period of illumination have been used with great effect in identifying mutants lacking components required for types of cyclic flow [5]. Such approaches are however only of limited use in addressing the *physiology* of cyclic flow, as the flow that they measure is only being seen under non-physiological conditions. Measurements of steady state fluxes through each of the two reaction centres are more physiologically relevant but also have limitations, especially as neither photosystem can be accurately measured in a totally quantitative way. In this review I will try to

Abbreviations: FNR, ferredoxin NADP oxidoereductase; NDH, NADH oxidase like complex; NPQ, non-photochemical quenching; PC, plastocyanin; PSI, photosystem I; PSII, photosystem II; PQ, plastoquinone; qE, high energy state quenching

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* Tel.: +44 161 275 5750; fax: +44 161 275 5082.
E-mail address: giles.johnson@manchester.ac.uk.

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consider to what extent we can understand the physiology of cyclic flow, based on the limitations of the methodology currently available to us.

2. The pathways of cyclic electron flow in higher plants

There is now clear biochemical and spectroscopic evidence for the occurrence of at least two distinct pathways for cyclic electron flow being present, to a greater or lesser extent, in higher plants—the NDH and the PGR5 pathways. It is likely, though not certain, that these perform distinct roles or are subject to distinct regulation. At the same time, there is, to some degree at least, redundancy between them.

The NDH pathway requires the presence of a large, multisubunit complex which has been known to exist for some years but the full nature of this is only now becoming clear (see [5] for a review). The isolation of many different mutants deficient in various subunits has allowed it to be concluded that this complex is not, in fact, an NADPH-dependent plastoquinone reductase but rather takes electrons directly from ferredoxin. This is both exciting, it resolves the long term problem of the missing or unidentified NADPH binding domain, but is also perplexing as it eliminates what was thought to be a distinction from the PGR5 pathway.

The PGR5 dependent pathway of cyclic electron transport remains much more poorly defined. The isolation of the mutant of Arabidopsis *pgr5* [8] and the subsequent isolation of a second mutant lacking a different protein, PGRL1 (for PGR5-like 1) [9] indicate clearly that a previously unknown protein complex exists and that in the absence of that complex redox poising of electron transport is perturbed and cyclic electron transport suppressed. Data from Nandha and co-workers [10] have indicated that plants lacking PGR5 are still capable of cyclic electron flow, indicating that PGR5 is not an essential co-factor for cyclic flow. Similar conclusions were reached for PGRL1 [9]. In both cases, however, there is a clear suppression of cyclic flow. In the mutants, electron carriers between photosystems I and II are found to be reduced under high light conditions [9,10]. This contrasts with wild type plants, where increasing light results in an increasing degree of oxidation of electron carriers after the plastoquinone pool, with plastoquinol oxidation by the cytochrome b_6f complex being the effective slowest step in electron transport [11,12]. Early work indicated that redox poising of electron transport is essential to efficient cyclic flow with over reduction or oxidation of the electron transport chain being inhibitory [13–15]. Thus, it seems likely that it is the role of the PGR5/PGRL1 complex in regulating redox poise that gives rise to its effect on cyclic flow. Work from Hald et al. [16] has indicted that the cytochrome b_6f complex is regulated in response to NADP/H redox state and it maybe this regulation that is failing in *pgr5* and *pgrl1* mutants.

Based on *in vitro* work, it seems probable that the PGR5 pathway of cyclic flow involves electron transfer from ferredoxin to plastoquinone. In higher plants, this process is characterised as being sensitive to the inhibitor antimycin A [17]. In mitochondria, antimycin A is known to bind to and block the quinone binding site Q_N on the cytochrome bc complex and this was originally supposed to explain its action in chloroplasts. However the subsequent observation that the equivalent site in the cytochrome b_6f complex is insensitive to antimycin A lead to proposal that there must be a distinct, antimycin A sensitive, ferredoxin quinone oxidoreductase (FQR) in the chloroplast [18]. No biochemical evidence for such a complex exists. It is also the case that the effect of antimycin A on cyclic flow may not result from its binding to a quinone site. Antimycin A has multiple effects on components of the photosynthetic apparatus, including inhibiting non-photochemical quenching by interfering with the aggregation of LHCII [19]. The effect of antimycin A on cyclic flow may therefore be a result of a similar effect, e.g. interfering with the formation of complexes involving cytochrome b_6f. Most current research suggests that cyclic flow is mediated via the cytochrome b_6f complex.

The observation that the cytochrome b_6f complex contains an additional c-type haem compared to the cytochrome bc complex [20] has lead to the idea that this cytochrome c' maybe required to mediate cyclic flow [5]. This is an attractive idea, that explains the presence of this additional haem, however direct experimental evidence for this is still required.

The enzyme ferredoxin NADP reductase (FNR) has been suggested to be bound to the stromal side of the cytochrome b_6f complex in a stoichiometric manner [21]. This is suggested to provide a possible docking site for ferredoxin and possibly to mediate electron flow to plastoquinone, via the cytochrome c' and/or b. Nevertheless, direct evidence for this role is still lacking. Breyton et al. [22] examined the distribution of FNR and binding to the thylakoid membrane, under conditions favouring or disfavouring cyclic flow, and found no evidence for changes in the distribution of FNR. Indeed FNR was seen to bind to many different membrane complexes, opening the question of how specific FNR binding to membrane complexes is. A rhodonase like protein, TROL has been described as specifically binding FNR to the membrane and plants lacking TROL are impaired in linear electron transport at high light [23]. Measurements of cyclic electron flow were reported for this mutant, suggesting no change and the observation that NPQ is enhanced would tend against a role in cyclic electron flow. It has also been suggested that FNR binds directly to the lipid phase of the membrane [24] and even that this is able to reduce quinones [25], offering the possibility that FNR alone could act as an FQR. The physiological relevance of these observations remains to be confirmed. Regardless of whether FNR has a direct role in cyclic flow, alterations in the distribution of the protein in the cell or in its activity may play a role in regulating the partitioning of electrons between different electron transport pathways.

In addition to the above, widely recognised cyclic pathways, it has been proposed by Laisk and co-workers that a high rate of cyclic flow may occur under some conditions that is not coupled to the generation of a ΔpH [7]. This pathway is tentatively suggested to occur via a short circuiting of normal electron flow, e.g. electron transfer from cytochrome c' to cytochrome f, perhaps via the two b haems and the rieske FeS complex. Such a pathway would not involve PQH2 generation and so would not generate a pH gradient.

3. Cyclic electron transport as a source of ATP

Early work in the field of photosynthesis research referred to cyclic and linear photophosphorylation [1,26,27], and these two distinct sources of ATP are often still discussed in undergraduate textbooks. The evidence supporting a role for cyclic transport in being required to generate ATP in higher plants remains mixed. This may in part be because of the continuing difficulty in actually directly measuring cyclic fluxes. Many methods for the occurrence of cyclic flow are indirect and do not measure under conditions that are relevant to physiology. For example, measurements using far red light or in leaves infiltrated with PSII inhibitors provide valuable information about the *possibility* and the *mechanisms* of cyclic flow, but do not actually show that it is occurring in competition with linear flow. For this, evidence is required under steady state conditions using light sources that are relevant to (if not identical to) those found in nature. Under such light conditions, evidence for cyclic flow can come from comparing rates of PSII and PSI turnover. If there is a variation in the relative rates of cyclic and linear flow then the ratio of PSI:PSII turnover should vary. Generally, the data available indicate that under low light conditions there is a linear relationship between PSI and PSII turnover, implying either that cyclic flow does not occur at low light, or that it forms a constant proportion of linear electron transport e.g. Ref. [28].

Much of the argument for a role of cyclic electron transport in generating ATP has focused on the stoichiometry of NADPH to ATP synthesis during linear flow and whether these match the requirements of CO_2 fixation. Firstly the proton pumping efficiency of the cytochrome b_6f complex (i.e. the Q-cycle) and more recently the H^+/ATP required by the thylakoid ATPase have been subject to scrutiny and the reader is referred to Ref. [29] for further discussion of this point. To some extent, however, these arguments are futile. Photosynthetic electron transport is not simply and tightly coupled to the Benson–Calvin cyclic. Rather, many different metabolic pathways, with different requirements for ATP and reducing equivalents, compete with each other. At the same time, there is a growing recognition that the chloroplast is not energetically isolated within the cell. Both reducing equivalents and ATP maybe directly or indirectly transferred across the chloroplast envelope, in either direction, as determined by the requirements of cellular metabolism. If an obligate role for ATP generation during photosynthesis exists, then it would be predicted that the relative rates of cyclic and linear flow should be constant and so the rates of PSI and PSII turnover linearly related. Under low light conditions, this is generally seen, however there is no way to distinguish no cyclic flow from an obligate proportion of cyclic flow [30–32].

The most widely cited example where cyclic flow is probably important in generating ATP is in the bundle sheath of C4 plant. In C4 plants, CO_2 is fixed in the mesophyll to form a C4 sugar, malate, which is then transferred to the bundle sheath where it is decarboxylated to release CO_2 for fixation by Rubisco. Mesophyll chloroplasts contain similar amounts of PSI and PSII implying that linear electron flow predominates to produce NADPH and ATP to drive C4 CO_2 fixation. Decarboxylation in the bundle sheath produces NADPH at the ratio of one molecule per CO_2. This is thought to lead to an imbalance in the production of NADPH:ATP, which is rectified through the generation of ATP through cyclic flow. The evidence that this is the case relates to observation that bundle sheath chloroplasts generally contain only low concentrations of PSII (see [33] for a review). At the same time, they have been shown to contain high concentrations of both PGR5 [34] and subunits of the NDH complex [34,35] depending on species. Interestingly, NDH appears to be present in much higher concentrations, relative to other electron transport components than is the case in C3 leaves or mesophyll cells of C4 [36]. The NDH complex is often dismissed as playing a substantial role in cyclic flow in C3 plants, due to its low concentration (although there can be conditions where this is important e.g. [37,38]). The elevated concentrations in C4 bundle sheath strongly suggest that this is important in such cells.

Another case where cyclic flow has been postulated to generate ATP is in plants growing at very low light. In Arabidopsis, growth at low light results in leaves having a high PSI:PSII ratio [39]. Natural low light, caused by shading from other plants, is enriched in far red light. Far red light is more effective at driving PSI than PSII turnover. Generating tissues that are preferentially involved in cyclic flow to generate ATP maybe a way in which cells make the best use of the light available under such conditions. This has not however been demonstrated experimentally.

In conclusion, the process of cyclic electron flow certainly can and probably does result in the generation of ATP. The extent to which this is a requirement for photosynthesis, in particular for CO_2 fixation, remains open to debate. There is little evidence to support a substantial cyclic flow at limiting light, unless this is strictly coupled to linear flow, and so undistinguishable by the methods used. It is probably not the case that there is a strict coupling of cyclic and linear flows, certainly no mechanism by which such a coupling might be achieved is obvious. Nevertheless it is clear that both the synthesis of ATP and the generation and use of reducing equivalents in the chloroplast are highly regulated processes and that cyclic electron flow contributes to that regulation.

4. Cyclic flow to generate ΔpH for high energy state quenching

The concept that cyclic electron flow generates a high ΔpH required to drive high energy state quenching was first proposed by Heber and Walker in 1992 [6]. Direct experimental evidence to support this model did not arrive until some years later. Harbinson compared the rates of PSI and PSII electron transport under various light conditions in pea and concluded that they were generally linearly related [28], however at low CO_2 there was some indication of "excess" PSI turnover at low CO_2 [40]. In contrast, Clarke and Johnson performed similar measurements under various temperature and light conditions in barley and concluded that cyclic flow increased at high light and at low temperature [41]. The difference between these conclusions might be related to the way in which PSI flux was estimated, a topic that has been subject to some debate (see [3] for further discussion) however Golding and Johnson [42] used the approaches of both previous studies and confirmed the occurrence of cyclic flow at high light, under low CO_2 and upon exposure to drought. This was also confirmed by Miyake in tobacco [43]. Also in tobacco, Sacksteder and Kramer [32] saw little if any evidence for cyclic flow across a range of irradiances. The measuring approach they were using, taking measurements of the decay of the trans thylakoid potential gradient to indicate fluxes though the ATPase, were different and this may explain the discrepancy, however this difference may not reflect technical but, rather, physiological differences—i.e. the conditions to induce cyclic flow were not achieved. ΔpH dependent NPQ is induced at irradiances where linear electron flow starts to become saturated and continues to rise with increasing light, after PSII turnover is clearly saturated. PSI turnover saturates at a higher irradiance than PSII, implying that cyclic electron transport occurs.

The *pgr5* mutant of Arabidopsis was isolated initially as lacking in the ability to induce non-photochemical quenching [44]. It was later shown to lack NPQ due to a lack of ΔpH, and to be inhibited in cyclic flow [8]. This provides strong evidence that the PGR5 dependent cyclic pathway plays a substantial role in generating ΔpH for NPQ. Mutants lacking the NDH complex have not been shown to lack NPQ.

5. Regulation of cyclic electron flow—competition with linear flow and the need for segregation

Both PSII and cyclic electron flow involve the reduction of plastoquinone in the thylakoid membrane. Under conditions where electron flow is limiting (e.g. high light, low CO_2) this pool will be largely reduced, meaning that the relative ability of the two electron transport pathways to reduce PQ may determine their relative efficiencies. This poses a problem in that, if cyclic flow is required to generate additional ΔpH for qE, it will function poorly in competition with linear flow under conditions where it is most required but where the PQ pool will be largely reduced. For this reason alone, it is widely believed that there needs to be segregation of the two pathways, maintained at some structural level.

In algae, recent exciting work points to the formation of supercomplexes involving PSI, the cytochrome b_6f and FNR [45]. At present, there is no direct experimental evidence for the formation of such supercomplexes in higher plants [22] although there is some evidence for an association between the NDH complex and components of PSI [46]. It may simply be that the evidence has not yet been found, however there are good reasons for thinking that such complexes may not be required. A characteristic of higher plants, which is more or less absent in algae and totally absent in cyanobacteria is the extensive stacking and tight segregation of functional PSII in the appressed regions of the membrane. This means that there is always a pool of both PSI and of cytochrome b_6f complex that is physically distant from and therefore probably functionally isolated from the bulk of PSII [47]. Given that diffusion of plastoquinone in the thylakoid membrane is highly restricted, due to the presence of very high protein concentrations, it

is likely that the PQ pool reduced by PSII is functionally isolated from cytochrome b_6f complexes located in the non-appressed regions of the membrane [48,49].

Cross talk between the two pathways may also occur at the level of plastocyanin. There is some evidence that under some conditions there maybe distinct pools of plastocyanin, based on observations of the apparent non-equilibrium of cytochrome f, PC and P700, possibly due to constrictions in the thylakoid lumen preventing free PC diffusion [50]. Such observations are however confined to measurements at low light or during induction. Measurements at high light and in the absence CO_2, where the electron transport chain is largely oxidised, showed a consistent equilibration of the high potential chain, implying free PC diffusion [51]. This is, however, less problematic. Once a pair of electrons originating from either PSII or ferredoxin has successfully been injected into the electron transport chain it can be regarded as committed to one or other pathway. Competition is at the point of injection and all electrons arriving at PC are essentially equivalent, probably also regardless of the PSI unit from which they exit. There is no need to maintain separate PC pools.

The final, possibly most important, point where cyclic-linear competition must be considered is for the oxidation of ferredoxin. Ferredoxin may be oxidised by the enzyme FNR to produce NADPH or may supply electrons directly to other metabolic pathways. These will all compete with the reduction of PQ in the cyclic pathway. Is there therefore a need for a strict segregation of cyclic electron transport components from stromal reactions? I would argue that there is not and that, indeed, experimental evidence suggests that free competition for ferredoxin oxidation maybe a major site for the regulation of cyclic flow.

Experiments measuring the rate of oxidation of P700 by far red light, or the reduction of P700 following far red light have been widely used as indicators of cyclic electron flow. Such measurements do not give information about how cyclic flow operates under steady state conditions as they eliminate the competition from PSII and linear flow. They do however give information on the fate of electrons that reach the PSI acceptor pool under different conditions. For example, P700 oxidation by far red light is observed to be slow in leaves that have been dark adapted, increase under conditions favouring CO_2 fixation (high light and CO_2) and to decrease under conditions where CO_2 fixation is limited (low CO_2) [22,52,53]. Such observations lead to the conclusion that competition for the oxidation of ferredoxin is the major control point for cyclic vs. linear electron flow. Plants in which electron flow is limited by lowering the activity of PSI are still able to maintain a significant rate of cyclic electron flow, suggesting that electrons are partitioned between linear and cyclic pathways after PSI [54]. In contrast, plants overexpressing ferredoxin have been shown to have increased capacity for cyclic flow, leading to the suggestion that it is the concentration of ferredoxin that limits cyclic flow [55]. Given that ferredoxin is the last common intermediate in all pathways of chloroplast electron transport, it is certain that its fate will play a major role in determining the extent of cyclic flow.

Regardless of whether it is directly involved in cyclic electron transport, FNR features as a possible regulatory point in the partitioning of electrons to different pathways [22]. Electrons are injected into the cyclic pathway directly from ferredoxin and the reactions involved occur in competition with reduction of NADP by FNR. The activity of the Benson Calvin cycle is highly regulated and there is some evidence that FNR is the first regulated step [56]. Thus, FNR activity could affect distribution of electrons between linear and cyclic flow. If FNR is directly involved in cyclic flow, either bound to PSI, cytochrome b_6f or elsewhere, then the extent of that binding may act as a regulatory step, although there is no experimental evidence of this being the case in plants [22]. Even if direct binding to PSI or cytochrome b_6f is not required for electron transport, binding to membranes may play a role in regulating the activity of FNR. This binding may be modulated depending on physiological conditions. Moolna and Bowsher [57] examined the properties of different isoforms of FNR in wheat. Most species contain at least two genes for photosynthetic FNR, with different pK's. The relative binding of each isoform to the membrane may vary depending on the pH of the stroma, which will in turn vary in a light and stress dependent manner. Relative concentrations of the two isoforms varied with growth conditions and developmental stage. FNR isoforms are also subject to post-translational modification, in particular cleavage of N-terminal sequences, which alter membrane binding properties [57,58]. Such mechanisms open the possibility of both long term (acclimative) and short term (regulatory) changes in cyclic electron flow. Such regulation remains however highly speculative.

Evidence for a role of stromal redox poising in controlling the relative flows through cyclic and linear pathways comes from examination of different mutants with impairments in Calvin cycle enzymes. Plants lacking glyceraldehyde-3-phosphate dehydrogenase [16,59], fructose-1,6-bisphosphatase [38] or Rubisco [59] show elevated non-photochemical quenching at low (i.e. sub saturating) light, suggesting that cyclic electron transport has increased. Livingston et al. [59] reported however that plants lacking Rubisco did not show elevated cyclic flow. In contrast, plants with reduced levels of FNR do not show an elevation of NPQ at low light, although they do at saturating irradiances [16]. The significance of these different responses remains to be elucidated.

What is clear is that cyclic electron flow *tends* to increase under conditions where CO_2 fixation is limited, either due to low CO_2 concentrations in the leaf or suppression of enzyme activity. Under such conditions, the concentrations of NADPH and ATP will *tend* to rise. This is accompanied by a feedback inhibition of the cytochrome b_6f complex which results in oxidation of the high potential portion of the electron transport chain (Cyt f, PC and P700) and reduction of the plastoquinone pool [16]. Reduced PSI acceptors (ferredoxin or NADPH) are then able to reduce the high potential chain, via whatever pathway. Since the pool of PQ associated with PSII will be largely reduced under such conditions, it is plausible to suppose that a distinct PQ pool, isolated by diffusion limitation [48,49] and associated with cytochrome b_6f complexes in non-appressed regions of the membrane takes electrons into a (more or less) common pool of plastocyanin and P700 (Fig. 1).

6. Adaptation and acclimation of cyclic electron transport

Adaptation refers to genetically determined differences seen between plants from different environments. The term acclimation relates to changes in the composition of tissues in response to changes in conditions whether this be during development or dynamically in response to changes in the environment. Importantly, in talking about acclimation we need to be clear that we are discussing changes in phenotype resulting ultimately from changes in the protein content of the leaf. This is different to rapid regulatory responses, e.g. the induction of cyclic flow or of NPQ, which do not require changes in gene expression. By examining how the capacity for cyclic flow varies in different plant material depending on provenance or growth conditions, occurrence of mutations or exposure to stress we might learn something about the physiological function of the processes involved. Given the difficulty of accurately quantifying cyclic flow, it is difficult to establish for certain whether a particular phenotype is associated with a change in the capacity for cyclic flow or simply an increase in the degree to which it is active. However, where proteins (especially PGR5 and subunits of the NDH complex) have been identified as involved then we can ask the question of whether these vary between plant materials.

There is evidence that proteins involved in cyclic flow are present from early on in development. Plants lacking either PGR5 or the NDH complex develop normally and are largely indistinguishable from wild type plants [8,60]. Double mutants, lacking both complexes, do not however grow normally, perhaps suggesting a role at an early stage in leaf development [61]. Recently Okegawa and colleagues [62] examined

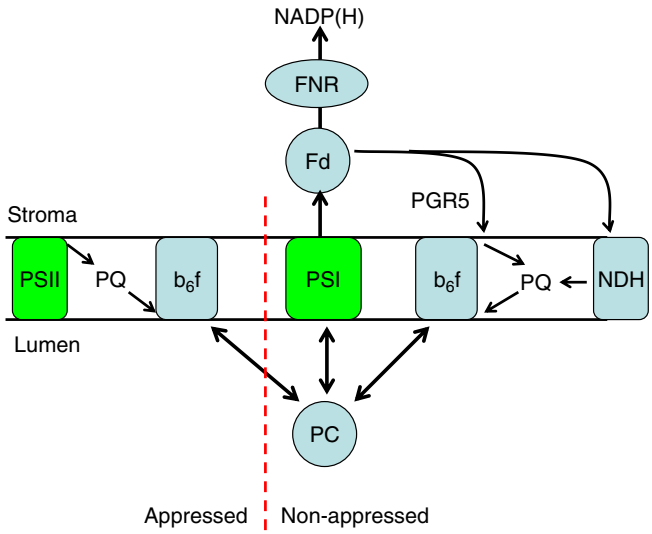

Fig. 1. Model of possible interactions between linear and cyclic electron transport pathways. Electrons flowing into a plastoquinone pool from Photosystem II reduce cytochrome b_6f complexes in the appressed regions of the thylakoid membrane. These flow via plastocyanin to PSI and from there to ferredoxin. Reduced ferredoxin can reduce NADP, via FNR or can feed electrons to a plastoquinone pool in the stromal lamellae via either a PGR5 dependent pathway or an NDH dependent pathway. Reduced PQ then reduces cytochrome b_6f complexes in non-appressed membrane regions. This in turn reduces plastocyanin and PSI. Plastoquinone in the stacked and unstacked regions represent isolated pools due to restricted diffusion in the membrane. Plastocyanin is able to move more or less freely in the thylakoid lumen and exists in equilibrium with P700 and cytochrome f. Partitioning of electrons between linear and cyclic pathways occurs at the step of ferredoxin oxidation only.

the development of plants lacking either or both pathways in combination with a lack of the plastid terminal oxidase (immutans), a plastoquinone oxygen oxidoreductase and showed that the phenotype of plants lacking immutans was suppressed in plants lacking PGR5 and to a lesser extent NDH. The growth phenotype in plants lacking both cyclic pathways was however suppressed if immutans was also missing. These results suggest that the redox poising of the PQ pool plays an essential role in chloroplast development.

There have been a few reports of environmental conditions that cause an induction of components associated with cyclic flow in Arabidopsis. For example, exposure to drought and heat has been observed to induce expression or activity of both PGR5 and NDH subunits. Given that such stresses have been reported to increase cyclic electron flow, up regulation of these proteins may make sense. There is no clear evidence, however, that increases in *PGR5* message levels or PGR5 protein will actually give rise to increased cyclic flow. Indeed, overexpression would suggest otherwise [63], although this study was conducted before it became apparent that PGR5 does not act alone but in a complex along with PRGL1.

Perhaps the most informative comparisons may come from identifying situations where one complex or pathway or the other is up-regulated alone. We still have very little idea as to why two pathways exist and indeed it is generally argued that the NDH pathway has only a very small capacity relative to linear flow. Nevertheless a number of observations have emerged to indicate that this complex can be significant under some conditions. For example, plants lacking fructose-1,6-bisphosphotase show increased expression of NDH [38], suggesting that the conditions favouring this complex occur in such plants. Ibanez et al. [64] reported an increase in NDH induced by stress in both a sun tolerant species *Chrysanthemum morifolium* and a shade plant *Spathiphyllum wallisii*. Only the former increased PGR5 in response to stress and also contained more PGR5 under non-stress conditions. Such comparative approaches, combined with functional measurements, have significant potential to inform us as to the relative roles and importance of the two cyclic pathways (and indeed of chlororespiratory process that may be occurring).

7. Conclusion

It is now clear that cyclic electron flow, long debated and often doubted is real and important in higher plants. The coming years will no doubt throw up new and much more detailed evidence as to how this pathway functions and which polypeptides and co-factors are required. It will also no doubt lead to extensive continued debate as to the relative importance of different proteins and probably even as to whether certain pathways exist at all. It should be remembered, when taking part in such debates, that nature is not simple and clean and does not always come up with the same answer. Just because tobacco contains only trace amounts of the NDH complex, for example, this can not be extrapolated to different species of the same genus, let alone to different genera or families. Plant physiologists, especially ecophysiologists, have long recognised that the differences between species are far more important than looking for a single answer to a simple question. A particular pathway may be dominant in one species but largely or totally absent in another and understanding this in the context of the ecology of the plants concerned will give us powerful insights into the roles of limitations of different pathways. Recent advances in high throughput sequencing and quantitative proteomics will mean that we will be able to gain detailed molecular insights into a much broader range of species in the future, however a true understanding of cyclic electron flow, or of any other physiological process, will only be possible if research is lead by an appreciation of the physiology and ecology of the plants concerned.

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