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The role of PGR5 in the redox poising of photosynthetic electron transport ☆

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Abstract

The *pgr5* mutant of *Arabidopsis thaliana* has been described as being deficient in cyclic electron flow around photosystem I, however, the precise role of the PGR5 protein remains unknown. To address this issue, photosynthetic electron transport was examined in intact leaves of *pgr5* and wild type *A. thaliana.* Based on measurements of the kinetics of P700 oxidation in far red light and re-reduction following oxidation in the presence of DCMU, we conclude that this mutant is able to perform cyclic electron flow at a rate similar to the wild type. The PGR5 protein is therefore not essential for cyclic flow. However, cyclic flow is affected by the pgr5 mutation under conditions where this process is normally enhanced in wild type leaves, i.e. high light or low CO_2 concentrations resulted in enhancement of cyclic electron flow. This suggests a different capacity to regulate cyclic flow in response to environmental stimuli in the mutant. We also show that the *pgr5* mutant is affected in the redox poising of the chloroplast, with the electron transport chain being substantially reduced under most conditions. This may result in defective feedback regulation of photosynthetic electron transport under some conditions, thus providing a rationale for the reduced efficiency of cyclic electron flow.

Keywords: Cyclic electron transport; Non photochemical quenching; Photosynthesis

1. Introduction

Photosynthetic electron transport primarily occurs via a linear pathway in which photosystem I (PSI) receives electrons from photosystem II (PSII) to generate NADPH and a trans-thylakoid pH gradient (ΔpH) which is used to drive ATP synthesis [1]. In addition, cyclic electron flow (CEF) can occur, in which electrons from the acceptor side of PSI are re-injected into the photosynthetic electron transport chain (pETC), possibly via the cytochrome b_6f (cyt b_6f) complex. The occurrence and role of these pathways have been reviewed previously [2–6].

There is strong evidence for CEF in green alga (e.g. *Chlamydomonas* [7]) and cyanobacteria [8], as well as in bundle sheath chloroplasts of C_4 plants [9]. However, it is only recently that there has been an advance in understanding this process in C_3 plants, partly because of the difficulty in assaying CEF under conditions in which linear electron flow (LEF) predominates. Two roles for CEF have been suggested: (i) to supplement the ATP generated to maintain appropriate an ATP/NADPH ratio, and (ii) down-regulation of PSII via generation of ΔpH, resulting in non-photochemical quenching (NPQ). The occurrence of CEF under steady-state conditions has been under debate [5,10–12], although there is evidence that CEF does occur at significant rates during the induction period of photosynthesis [13].

We recently proposed a redox poise model to rationalise changes in the efficiency of CEF in C3 plants under different experimental conditions [14], which, builds on earlier work examining the effects of redox poising on CEF [15–18]. In this model, cyclic flow is assumed to be modulated by the competition between cyclic and linear flow pathways for reduced ferredoxin (Fd), the relative rates of the two processes being determined by the balance between PSI donor and acceptor redox states [14]. Thus, in the steady-state, when enzymes of the Benson–Calvin

Abbreviations: CEF, Cyclic electron flow; cyt b_6f, Cytochrome b_6f complex; ΔpH, pH gradient across the thylakoid membrane; LEF, Linear electron flow; pETC, Photosynthetic electron transport chain; PSI, Photosystem I; PSII, Photosystem II

☆ This work is dedicated to the memory of the late Mr. Narendra Dayalal Nandha.

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cycle are active, LEF will out-compete CEF, as carbon fixation forms an efficient sink for electrons from PSI. Under conditions when carbon fixation is down-regulated, such as under environmental stress or following a dark to light transition, CEF would have a competitive advantage.

One major difficulty in the study of cyclic electron flow has been the lack of mutants specifically affected in this process. A recent exception to this is proton gradient regulation-5 (*pgr5*), a mutant of *Arabidopsis thaliana* that has been reported to be impaired in CEF. This mutant was identified in a chlorophyll fluorescence screen as being deficient in non photochemical quenching and it has been proposed that this mutation specifically affects the Fd-mediated, antimycin-sensitive pathway of CEF, whilst being unaffected in the NDH-dependent one [19–21]. Furthermore, it is suggested that these two pathways are essential for normal growth but have a degree of redundancy, since a double mutant, impaired in both pathways, has greatly impaired growth and development [21]. It was suggested that the *pgr5* defect in PSI CEF leads to a reduction in the ATP/NADPH ratio during photosynthesis, causing over-reduction of the stroma. The resulting depletion of $NADP^+$ limits linear electron flow (LEF) specifically at high light. Nevertheless, the precise role of *pgr5* in CEF remains unclear. In particular, the question arises, does the PGR5 protein play a direct, essential role in CEF or does it only affect this process in a more indirect, regulatory manner (see [6] for a review).

To this end, , we have employed a range of *in vivo* assays to characterize CEF in intact leaves of the *pgr5* mutant [2,13,14,22]. We see that the *pgr5* mutant is capable of carrying out CEF *in vivo* — with approximately the same maximum efficiency as wt plantss. Thus PGR5 is not essential for the occurrence of Fd-mediated CEF. However, we observe that CEF is affected in the mutant under different experimental conditions. We attribute therefore the main pgr5 phenotype to either a disruption in the redox poising of the chloroplast or to a different sensing of redox poising in the mutant. The observed changes in CEF would stem for such impairment in redox regulation, consistent with the notion that CEF is modulated by the redox state of the pETC.

2. Materials and methods

2.1. Plant material

Experiments were carried out on 6 to 7 week old wild-type (wt; Columbia background strain *gl*1) and proton-gradient regulation-5 (*pgr5*) *Arabidopsis* plants [20] and *crr4-2 pgr5* double mutant [21] grown in Viking MM peat-based compost at a photon flux density (PFD) of 100 μmol m^{-2} s^{-1} with an 8-h photoperiod at 22–23 °C. Plant was dark-adapted for at least 16 h prior to taking measurements.

2.2. Reduction of oxidized P700

$P700^+$ reduction following a flash was assayed as detailed by Golding et al. [23]. Changes in absorbance at 830–870 nm were used as a measure of the P700 redox state and were performed using a Walz PAM 101 fluorometer in combination with an ED-P700DW-E emitter-detector unit (Walz, Effeltrich, Germany), and recorded using a National Instruments PCI-6220 data acquisition card, running software written using Labview 7 (National Instruments, Austin, USA). Leaves were vacuum infiltrated with 1 mM 3-(3, 4-dichlorophrenyl)-1, 1-dimethylurea (DCMU, Sigma, UK) in 300 mM sorbitol (Sigma, UK). A 200-ms saturating flash of red light (λ_{max}=627 nm; 8,000 μmol m^{-2} s^{-1}), supplied by a Luxeon LXHL-PD09 LED (Phillips-Lumiled, San Jose, USA) in a laboratory built lamp, was delivered to the leaf through a five-arm fibre optic (Walz). The accumulation of five flashes at 2 min intervals was recorded. Data were fitted with theoretical curves using GraFit (Version 5, Erithacus Software, Staines, UK).

2.3. P700 oxidation kinetics

Spectrophotometric measurements were made on a flash spectrophotometer as described previously [22].

P700 oxidation kinetics were determined using an 810-nm broadband LED source (30 nm). RG780 filters were used to protect the photodiodes from actinic light. Actinic FRL was provided by a Far red LED (λ_{max}=720 nm) filtered through three Wratten filters 55 that block wavelengths below 700 nm. Green light was provided by an inbuilt array of LEDs λ_{max}=530 nm). The atmosphere in the leaf chamber was controlled by pumping air through either water, or water and soda lime for 0 ppm CO_2.

2.4. Chlorophyll fluorescence parameters

Simultaneous chlorophyll fluorescence and $P700^+$ measurements were made using an experimental system described previously [24]. Attached leaves were enclosed in an environmentally controlled chamber with a gas stream containing CO_2 controlled by an ADC LC Pro+ infrared gas analyser (ADC Bioscientific, Hitchin, UK). The induction of NPQ was determined by delivering a saturating flash of white light (8000 μmol m^{-2} s^{-1}) to measure maximal fluorescence in the light (F_m') at 2 min intervals during 25 min of actinic illumination (800 μmol m^{-2} s^{-1}). This was followed by 5 min in darkness, after which, F_m' was determined at 2-min intervals for 10 min, in the absence of actinic illumination. Rapidly and slowly relaxing NPQ were then estimated as described previously [25]. Photochemical quenching, qP was used as an indicator of the redox state of the primary PSII electron acceptor and was estimated as defined in [25]. The relative concentration of $P700^+$ was measured as outlined in [24].

3. Results and discussion

3.1. Re-reduction of $P700^+$ following a flash in the presence of DCMU

The re-reduction of $P700^+$ following either FR illumination or illumination in the presence of DCMU has widely been used as an indicator of CEF (see e.g. [5,26,27] for discussion). Leaves of *pgr5* and wild-type were vacuum infiltrated with DCMU and were exposed to short (500 ms) saturating flashes of red light which were sufficient to largely oxidise P700. Re-reduction of $P700^+$ was then followed using absorbance changes in the near infrared (Fig. 1). The resulting decay followed a complex curve, as described previously [5]. The fast component in this decay has previously been attributed to CEF and the slower reduction phases are thought to indicate the slower processes of redox equilibration occurring in the chloroplast [5,24].

The overall decay of $P700^+$ was slower in *pgr5* than in the wt, however, the decay kinetics were very similar. Fitting with exponential curves gave variable results, with up to 4 components being required to obtain a satisfactory fit over a full 1 s decay curve (not shown). Nevertheless, no qualitative differences were seen — the same number of exponential components were needed for both wt and *pgr5*. Taking the initial slope of the $P700^+$ as an estimate of CEF, we determine a rate of CEF to be approx. 20–

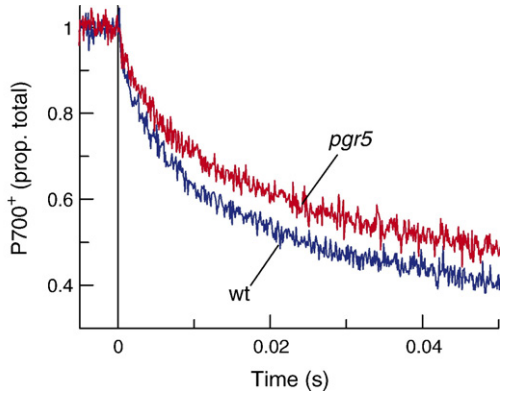

Fig. 1. The reduction of $P700^+$ following a 500-ms flash of saturating red light (~8,000 μmol m^{-2} s^{-1}) in leaves of wt and *pgr5* infiltrated with 1 mM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). Leaves were infiltrated in the presence of 300 mM sorbitol, to avoid osmotic effects.

30% slower in *pgr5* than wt (not shown). Thus, these data are consistent with the conclusion that *pgr5* and wt leaves are both capable of performing CEF but that the competitiveness of this is reduced in the mutant.

3.2. CEF capacity of pgr5 is sensitive to stromal redox poise

The above approach to quantifying CEF is open to criticism as it uses an invasive technique (DCMU infiltration) and does not correspond well to physiological conditions. An alternative approach to assaying CEF is to follow the kinetics with which P700 is oxidised by FRL, which preferentially excites PSI, [2,28]. Using this technique, sustained occurrence of CEF has been documented after a dark–light transition in dark-adapted leaves, where a slow net oxidation of P700 is accompanied by a continuing rate of electron flow, as indicated by the generation of electrical field across the thylakoid membrane. Joliot and Joliot [13] conducted experiments to quantify the extent of CEF in the induction phase of photosynthesis, and found evidence that CEF operates efficiently (~130 s^{-1}), during the induction of photosynthesis, while LEF functions at a much lower rate (~15 s^{-1}), due to the inactivation of the Benson–Calvin cycle in the dark-adapted state [22].

Fig. 2 illustrates the kinetics of P700 oxidation in dark-adapted leaves of wt, *pgr5* and the *crr4-2 pgr5* double mutant [21] in the presence of FRL only (open circles), after a short actinic pre-flash followed by FRL (closed circles), and in light-adapted conditions (squares).

In the absence of the flash, the wt displays a biphasic kinetic with a fast P700 oxidation phase followed by a lag and then a slower oxidation phase. The fast phase of these kinetics have previously been interpreted as representing the reduction of an efficient acceptor pool (probably $NADP^+$) with the slower component attributed to gradual leak of electrons from the cyclic pathway to stromal electron acceptors [2,14,29]. In *pgr5* (Fig. 2B) and *crr4-2 pgr5* (Fig. 2C), there is a less clear separation of two phases. A fast oxidation, similar to wt, is followed by a slower phase which is not clearly separated by a lag.

The kinetics of P700 oxidation are extremely variable among dark-adapted leaves illuminated with FRL, this variability probably reflecting variation in the dark redox poise of the chloroplast [28,29]. The application of a short (200 ms) pulse of saturating green light prior to measuring P700 oxidation was shown to remove this variability by pre-reducing the electron donor pool without activating the Benson–Calvin cycle [2,14]. The application of such a pulse induces a lag in P700 oxidation in all cases, this being followed by a slow monophasic slow oxidation of P700 (Fig. 2), as expected if all the PSI complexes were engaged in CEF. This supports the notion that *pgr5* has essentially the same ability to perform CEF as the wt following a dark to light transition.

The lag observed in P700 oxidation, which is of a longer duration in wt (~4 s) than *pgr5* or *crr4-2 pgr5* (~2 s) could be due to blockage of electron flow. Indeed, Siebke et al. [29] argued that initial reduction of the acceptor pool resulted in a complete blockage of electron transport. However, as seen previously [14], we observed that the generation of an electrical potential gradient across the membrane continues throughout the lag phase in P700 oxidation in both wt and mutant plants (data not shown). Hence it can be concluded that electron transport is

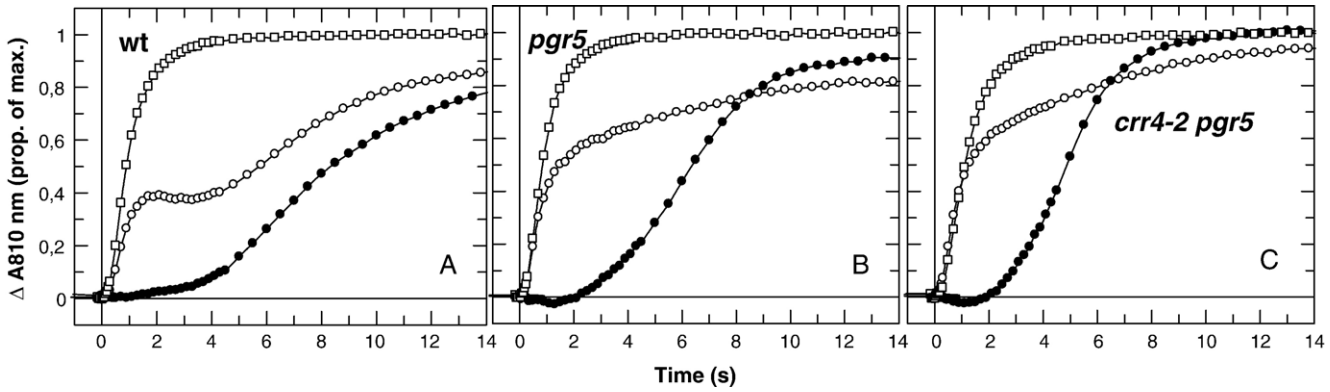

Fig. 2. Representative traces of (A) wt, (B) and *pgr5* and (C) *crr4-2 pgr5* double mutant P700 oxidation assayed in the presence of FRL with (closed circles) and without (open circles) 200 ms flash of green light (3000 μmol m^{-2} s^{-1}) given 5 s before the beginning of FRL. Signals were normalized to the maximum P700 oxidation recorded in leaves subjected to 20 min actinic illumination (330 μmol m^{-2} s^{-1}) followed by 4 min dark adaptation (open squares), a treatment fully activating the Benson–Calvin cycle. Leaves had been dark-adapted for > 16 h prior to experimentation.

maintained at a high rate throughout the initial lag and that this electron transport corresponds to the oxidation of reduced secondary PSI donors, plus CEF.

Finally, light adaptation of the leaves for several minutes greatly increases the rate of P700 oxidation, which becomes maximal and strictly monophasic. It has been shown that this rate corresponds to that of pure linear flow, as shown by the lack of further increase in P700 oxidation rate upon addition of the electron acceptor methyl viologen [2,28]. We also checked that addition of this compound enhanced the rate of P700 oxidation in dark adapted leaves (not shown, see however [28]), indicating that the slowing down of P700 turnover could be ascribed to sustained occurrence of CEF.

As with data in Fig. 1, these results are consistent with the conclusion that *pgr5* retains the ability to perform CEF. In principle, redundancy between PGR5 and NDH driven cyclic flow processes may explain lack of cyclic flow phenotype in *pgr5* (and in NDH lacking plants, not shown). However, we found that the same behaviour observed in *pgr5* could be reproduced in the double mutant *crr4-2 pgr5*, where both CEF pathways should be inhibited.

Taken together, these data suggest that (i) PGR5 in itself is not essential for cyclic flow, however its presence clearly enhances the efficiency of CEF under conditions where the pETC is largely oxidised (i.e. without the short saturating pulse) and (ii) as shown by the identical phenotype of the *pgr5* and *crr4-2 pgr5* mutants, the occurrence of CEF in the former cannot be explained in terms of a compensation between the putative PGR5 and the NDH pathways. A corollary of this finding is that the relative contribution of the NDH-pathway to CEF is probably not predominant in *Arabidopsis*, when compared to the Fd- driven one, in contrast to previous suggestions [30].

3.3. Activation of the Benson–Calvin cycle under ambient CO_2 progressively inhibits CEF in wt and pgr5

When leaves were exposed to actinic illumination, to activate enzymes of the Benson–Calvin cycle, CEF under FRL was progressively inhibited, as illustrated by the acceleration of P700 oxidation observed after varying periods of actinic illumination (Fig. 3A, B). P700 oxidation kinetics of wt and *pgr5* at various time points during continuous illumination were determined by interrupting actinic illumination and irradiating with weak FRL. Induction of photosynthesis causes acceleration of P700 oxidation by FRL, as the Benson–Calvin cycle becomes progressively more activated and the rate at which the electrons leak from CEF intermediates increases [2]. In wt, a short period of actinic illumination (30 s) still results in a slow oxidation of P700 leading to the conclusion that CEF is likely to be occurring. Longer periods of actinic illumination resulted in faster P700 oxidation, demonstrating that CEF becomes less competitive. The same effect was observed in *pgr5* (Fig. 3B), however, P700 oxidation was already faster after 30 s of illumination than was

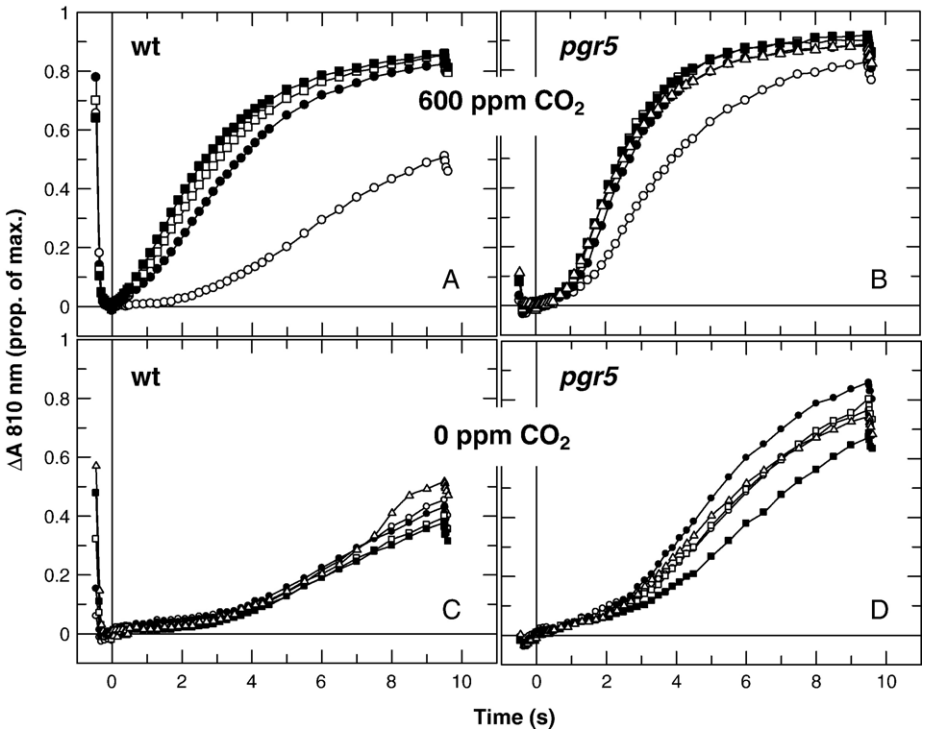

Fig. 3. Representative traces of P700 oxidation kinetics in single leaves of wt (A and C) and *pgr5* (B and D) at various time points during green actinic illumination (332 μmol m^{-2} s^{-1}) under conditions of 600 ppm (A and B) and 0 ppm CO_2 (C and D). Leaves were illuminated for periods of time varying from 30 s to 8 min Actinic illumination was interrupted at each time points and after 200 ms, FRL was applied for 10 s to oxidise P700. Immediately after each measurement, actinic illumination was resumed. Data have been normalised to the maximum P700 oxidation recorded after 4 min of dark following illumination of the leaf for 8 min. Time points: 30 s (open circles) and 2 (closed circles) 4 (open squares) 6 (closed squares) and 8 (open triangles) min of actinic illumination.

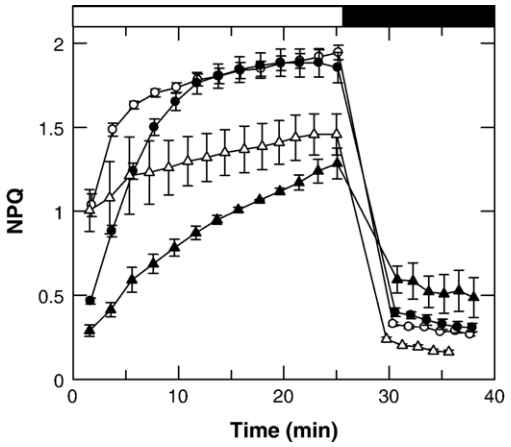

Fig. 4. NPQ induction and recovery in wt (open symbols) and *pgr5* mutant (closed symbols) under 2000 ppm (triangles) and 0 ppm CO_2 (circles). Error bars represent ± S.E. of the mean of 3 replicates. White bar indicates time under actinic illumination (800 μmol m^{-2} s^{-1}). Black bar represents time without actinic illumination.

the case in wt, implying that CEF was less able to compete with LEF in the mutant.

3.4. CEF is differently affected in wt and pgr5 plants under stress conditions

Previously we have observed an activation of CEF when plants are exposed to stress conditions, such as exposure to low CO_2 concentrations and drought [23,24]. Consistent with this, when P700 oxidation in wt and *pgr5* was recorded in the absence of CO_2 (Fig. 3C, D), a slowing of P700 oxidation was observed, relative to the situation with CO_2 present, in both wt and *pgr5*. This is consistent with the low CO_2 concentration causing a substantial increase in the proportion of CEF, in both wt and *pgr5*. This effect was, however, more marked in wt than in *pgr5,* as indicated by the more prominent lag and slower overall P700 oxidation. Nevertheless, in *pgr5* there is still a clear effect of removing CO_2, consistent with the idea that this promotes CEF.

A suggested role for CEF is the generation of a trans-thylakoid ÄpH required for NPQ. At low CO_2, for example, there is an elevation of CEF and an increase in NPQ is typically seen [23,31]. Munekage et al. [20] previously reported suppression of NPQ in the *pgr5* mutant. We observed the induction of NPQ during actinic illumination (800 μmol m^{-2} s^{-1} white light) at 2000 ppm and at 0 ppm CO_2 (Fig. 4). At 2000 ppm, NPQ was induced in both wt and *pgr5*, however, the rate of induction differed substantially between the two. After 20 min illumination, a similar extent of NPQ was reached in the wt and mutant, however, this was less readily reversed following a return to darkness in *pgr5*. This suggests a greater extent of photoinhibitory quenching in *pgr5*, compared to wt leaves, where rapidly relaxing, primarily pH dependent, quenching is predominant. At 0 ppm CO_2, a difference in the kinetics with which NPQ was induced was still seen between the wt and *pgr5*. However, not only was the final extent of quenching identical, but so was the reversibility of this following illumination, implying that high energy state quenching formed the same proportion of total NPQ in both cases. Thus we conclude that in both wt and *pgr5*, enhancement of CEF upon reducing the CO_2 concentration is sufficient to induce NPQ response, because of a large generation of ΔpH. see that it is possible to drive CEF to produce a large ΔpH in both wt and *pgr5* but that that ability is somewhat lowered in *pgr5*. The same intrinsic ability to generate a ΔpH does nevertheless exist in both plants.

Another condition where CEF becomes prominent is under high light [2,32]. With increasing irradiance during actinic illumination, oxidation of P700 by FRL became progressively slower in both wt and *pgr5* (Fig. 5). This suggests that there is an increase in the competitiveness of CEF relative to LEF with rising actinic irradiance in both wt and *pgr5* leaves. In Fig. 6A it is seen that reversible NPQ (an indicator of high energy state quenching) in *pgr5* is lower at any given irradiance and slowly relaxing NPQ (an indicator of photoinhibition) generally greater. In the irradiance range examined, rapidly relaxing NPQ did not attain the same level in *pgr5* as in wt, in contrast to observations at low CO_2, nevertheless a substantial degree of quenching is induced, implying that a certain amount of CEF is still occurring

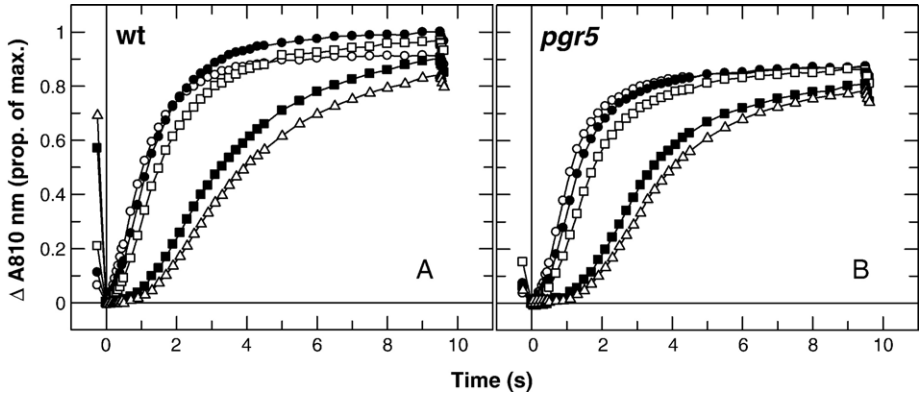

Fig. 5. Light response curves of a single leaf of (A) wt and (B) *pgr5* obtained after illumination at various intensities of green light. The light regime for a single leaf was as follows: 22 μmol m^{-2} s^{-1} for 10 min (open circles), 5 min at each of the following light intensities (μmol m^{-2} s^{-1}): 70 (closed circle), 140 (open square), 332 (closed square) and 2 min at 460 (open triangle). P700 oxidation levels were normalized to maximum P700 oxidation levels for each leaf. Assays were conducted in the presence of weak FRL, as in Fig. 3.

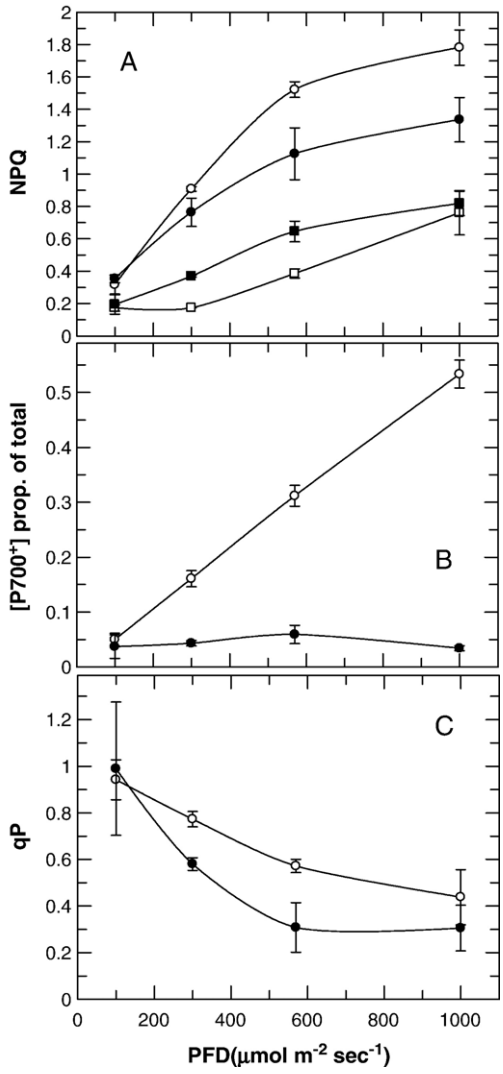

Fig. 6. Chlorophyll fluorescence and P700 parameters in wt (open symbols) and *pgr5* (closed symbols) at various white actinic light intensities: (A) reversible (circles) and slowly relaxing NPQ (squares); (B) proportion of oxidised P700 and (C) qP. Error bars indicate ± S.E. of the mean (n = 3 or 4).

and that this gives rise to a ΔpH that drives quenching. Importantly, the extent of inhibition of NPQ overall is small in *pgr5*, less marked than has been reported previously [20]. However in previous reports, plants were illuminated for short periods. This emphasises that the effect of *pgr5* deletion on NPQ is primarily kinetic and has a smaller effect on the final levels of NPQ attained.

3.5. Redox posing of the photosynthetic electron transport chain

The redox state of P700 in actinic light differed substantially between wt and *pgr5*, in line with previous observations [20]. In wt, P700 became more progressively oxidised in actinic light as the leaf approached steady-state, as indicated by $P700^+$ decay during the dark period immediately following actinic illumination (Fig. 6B). This can be explained as being due to the limiting step in electron transport being prior to PSI, specifically at the oxidation of PQH_2 by the cyt b_6f complex [33]. In *pgr5,* little or no net oxidation of P700 was observed (Figs. 3, 5, 6B).

Under the same conditions the acceptor pool of PSII is more reduced in *pgr5* than wt, as indicated by the lower levels of photochemical quenching (qP) across a range of irradiances (Fig. 6C). Cyt *f* was also found to be largely reduced (data not shown). The pETC is thus maintained in a more reduced state in the mutant compared to the wt. From this we can conclude that the reduction of cyt b_6f is not the rate-limiting step of photosynthetic electron transport in the mutant. Previously it has been suggested that the cyt b_6f complex is regulated in response to the stromal redox poise [34], and that this determines the redox state of P700 during illumination. Clearly an impairment in such regulation in *pgr5* would account for its reduced capacity to oxidise P700 when the light intensity is increased.

4. Conclusion

Results presented here provide evidence that *pgr5* is capable of performing CEF *in vivo*, allowing us to conclude that the PGR5 protein does not play a direct, essential role in that process. This is as expected based on sequence analyses—there is no indication that PGR5 itself binds any redox centres that might act in the CEF pathway [6].

The *pgr5* mutant has previously been suggested to be deficient in CEF. However, Munekage et al. [20] used an *in vitro* assay to test CEF in which reduction of PQ by NADPH, in the presence of Fd, was inhibited. This reaction would have been too slow to be compatible with rates of cyclic flow *in vivo*. Avenson et al. [35] attempted to quantify the relative rates of cyclic and linear electron flow *in vivo* by comparing the rate of LEF with the decay of the electrochemical gradient across the thylakoid membrane. They concluded that there was only a small difference (~ 13%) in the extent of cyclic flow between wt and *pgr5*. It seems therefore that our conclusion that the capacity to perform CEF *in vivo* is slightly affected in *pgr5* is not in contrast with previous literature.

Our results, however, do support the notion that *pgr5* is defective in the regulation of this process, in that CEF is less able to compete with LEF across a range of physiological conditions. What is the mechanism responsible for such down regulation of CEF? Previously, we presented evidence that the redox poise of the chloroplast is a major determinant of this competition [14], consistent with early work suggesting that redox poising of the chloroplast is crucial in determining the extent of CEF [15,17]. Our results suggest that that redox poise is altered in *pgr5*. This is indicated by data in Fig. 2, where traces obtained without any flash preillumination (FRL only), therefore without standardizing redox poise for wt and *pgr5* chloroplasts, show a systematic lack of biphasic kinetic in *pgr5*. On the other hand, these differences could be eliminated when the redox poising of the leaves was normalized with a flash. Siebke et al. [20] previously observed biphasic kinetics in initial rates of P700 oxidation under FRL in dark-adapted leaf. These they explained as being due to a limitation in the reduction of the phosphoglycerate (PGA) pool, this being due to the requirement for light-activation of glyceraldehyde-3-phosphate dehydrogenase. This

would imply that the NADP pool represents the main acceptor pool for electrons in dark-adapted leaves.

According to this hypothesis, a modified electron flow downstream of PSI may account for the *pgr5* phenotype. A striking feature of the *pgr5* mutant which has not been fully explained is that P700 is maintained in a largely reduced state even at high light. This suggests that, in contrast to wt plants, the effective slowest step in electron transport is after PSI. However, no deficiency in PSI to NADP electron flow has been found in thylakoids from *pgr5* plants [20] implying that the pETC is not modified *per se* in this mutant. Conversely, it is likely that the absence of PGR5 on the stromal side of the thylakoid membrane disrupts the balance between LEF and CEF, in a redox poise dependent manner, such that CEF is less competitive for electrons under steady-state conditions. The acceptor side of PSI and donor side of PSII were found to be in a more reduced state (Fig. 6) clearly indicating that the pETC is maintained in a reduced state in the mutant. It has been suggested that the cyt b_6f complex is regulated in response to stromal redox poise [34] and it therefore seems likely that this regulation is impaired in the *pgr5* mutant. The impaired feedback to the cyt b_6f complex would lead to a highly reduced redox state of the pETC in the steady-state This, we suggest, limits CEF competing with LEF and results in a lower ΔpH under any conditions.

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References

[1] J.F. Allen, Photosynthesis of ATP — electrons, proton pumps, rotors, and poise, Cell 110 (2002) 273–276.
[2] P. Joliot, A. Joliot, Cyclic electron flow in C3 plants, Biochim. Biophys. Acta. Bioenerg. 1757 (2006) 362–368.
[3] J.F. Allen, Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain, Trends Plant Sci. 8 (2003) 15–19.
[4] U. Heber, Irrungen, Wirrungen? The Mehler reaction in relation to cyclic electron transport in C3 plants, Photosynth. Res. 73 (2002) 223–231.
[5] G.N. Johnson, Cyclic electron transport in C-3 plants: fact or artefact? J. Exp. Bot. 56 (2005) 407–416.
[6] T. Shikanai, Cyclic electron transport around photosystem I: genetic approaches, Annu. Rev. Plant Biol. 58 (2007) 199–217.
[7] G. Finazzi, A. Furia, R.P. Barbagallo, G. Forti, State transitions, cyclic and linear electron transport and photophosphorylation in *Chlamydomonas reinhardtii*, Biochim. Biophys. Acta. Bioenerg. 1413 (1999) 117–129.
[8] J.J. van Thor, R. Jeanjean, M. Havaux, K.A. Sjollema, F. Joset, K.J. Hellingwerf, H.C.P. Matthijs, Salt shock-inducible Photosystem I cyclic electron transfer in Synechocystis PCC6803 relies on binding of ferredoxin:NADP+ reductase to the thylakoid membranes via its CpcD phycobilisome-linker homologous N-terminal domain, Biochim. Biophys. Acta (BBA) — Bioenerg. 1457 (2000) 129–144.
[9] A. Kubicki, E. Funk, P. Westhoff, K. Steinmuller, Differential expression of plastome-encoded ndh genes in mesophyll and bundle-sheath chloroplasts of the C-4 plant Sorghum bicolor indicates that the complex I-homologous NAD(P)H-plastoquinone oxidoreductase is involved in cyclic electron transport, Planta 199 (1996) 276–281.
[10] D.M. Kramer, T.J. Avenson, G. Edwards, Dynamic flexibility in the light reactions of photosynthesis governed by both electron and proton transfer reactions, Trends Plant Sci. 9 (2004) 339–348.
[11] C. Miyake, S. Horiguchi, A. Makino, Y. Shinzaki, H. Yamamoto, K. Tomizawa, Effects of light intensity on cyclic electron flow around PSI and its relationship to non-photochemical quenching of Chl fluorescence in tobacco leaves, Plant Cell Physiol. 46 (2005) 1819–1830.
[12] A. Laisk, H. Eichelmann, V. Oja, R.B. Peterson, Control of cytochrome b (6)f at low and high light intensity and cyclic electron transport in leaves, Biochim. Biophys. Acta 1708 (2005) 79–90.
[13] P. Joliot, A. Joliot, Cyclic electron transfer in plant leaf, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 10209–10214.
[14] C. Breyton, B. Nandha, G.N. Johnson, P. Joliot, G. Finazzi, Redox modulation of cyclic electron flow around photosystem I in C3 plants, Biochem. 45 (2006) 13465–13475.
[15] D.I. Arnon, R.K. Chain, Role of oxygen in ferredoxin-catalysed cyclic photophosphorylation, FEBS Lett. 82 (1977) 297–302.
[16] J.F. Allen, Protein-phosphorylation-carburetor of photosynthesis, Trends Biochem. Sci. 8 (1983) 369–373.
[17] U. Heber, H. Egneus, U. Hanck, M. Jensen, S. Koster, Regulation of photosynthetic electron transport and photophosphorylation in intact chloroplasts and leaves of Spinacia-Oleracea L, Planta 143 (1978) 41–49.
[18] J.E. Backhausen, C. Kitzmann, P. Horton, R. Scheibe, Electron acceptors in isolated intact spinach chloroplasts act hierarchically to prevent over-reduction and competition for electrons, Photosynth. Res. 64 (2000) 1–13.
[19] T. Shikanai, Y. Munekage, K. Shimizu, T. Endo, T. Hashimoto, Identification and characterization of Arabidopsis mutants with reduced quenching of chlorophyll fluorescence, Plant Cell Physiol. 40 (1999) 1134–1142.
[20] Y. Munekage, M. Hojo, J. Meurer, T. Endo, M. Tasaka, T. Shikanai, PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis, Cell 110 (2002) 361–371.
[21] Y. Munekage, M. Hashimoto, C. Miyake, K.-I. Tomizawa, T. Endo, M. Tasaka, T. Shikanai, Cyclic electron flow around photosystem I is essential for photosynthesis, Nature 429 (2004) 579–582.
[22] P. Joliot, D. Beal, A. Joliot, Cyclic electron flow under saturating excitation of dark-adapted Arabidopsis leaves, Biochim. Biophys. Acta 1656 (2004) 166–176.
[23] A. Golding, G. Finazzi, G.N. Johnson, Reduction of the thylakoid electron transport chain by stromal reductant-evidence for activation of cyclic electron transport upon dark-adaptation or under drought, Planta 220 (2004) 356–363.
[24] A.J. Golding, G.N. Johnson, Down-regulation of linear and activation of cyclic electron transport during drought, Planta 218 (2003) 107–114.
[25] K. Maxwell, G.N. Johnson, Chlorophyll fluorescence — a practical guide, J. Exp. Bot. 51 (2000) 659–668.
[26] T. Joët, L. Cournac, G. Peltier, M. Havaux, Cyclic electron flow around photosystem I in C-3 plants. In vivo control by the redox state of chloroplasts and involvement of the NADH-dehydrogenase complex, Plant Physiol. 128 (2002) 760–769.
[27] N. Bukhov, R. Carpentier, G. Samson, Heterogeneity of Photosystem I reaction centers in barley leaves as related to the donation from stromal reductants, Photosynth. Res. 70 (2001) 273–279.
[28] P. Joliot, A. Joliot, Quantification of cyclic and linear flows in plants, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 4913–4918.
[29] K. Siebke, A. Laisk, S. Neimanis, U. Heber, Evidence that NADP-

dependent glyceraldehydephosphate dehydrogenase, but not ferredoxin-NADP reductase, controls electron flow to phosphoglycerate in the dark–light transition. Planta 185 (1991) 337–343.

[30] M. Havaux, D. Rumeau, J.M. Ducruet, Probing the FQR and NDH activities involved in cyclic electron transport around Photosystem I by the 'afterglow' luminescence, Biochim. Biophys. Acta. Bioenerg. 1709 (2005) 203–213.

[31] G. Cornic, Drought stress and high light effects on leaf photosynthesis, in: N.R. Baker (Ed.), Photoinhibition of photosynthesis: from molecular mechanisms to the field, Bios, Oxford, 1994, pp. 279–313.

[32] J.E. Clarke, G.N. Johnson, In vivo temperature dependence of cyclic and pseudocyclic electron transport in barley, Planta 212 (2001) 808–816.

[33] W. Haehnel, Photosynthetic electron transport on higher plants, Annu. Rev. Plant Physiol. 35 (1984) 659–693.

[34] G.N. Johnson, Thiol regulation of the thylakoid electron transport chain — a missing link in the regulation of photosynthesis? Biochem. 42 (2003) 3040–3044.

[35] T.J. Avenson, J.A. Cruz, A. Kanazawa, D.M. Kramer, Regulating the proton budget of higher plant photosynthesis, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 9709–9713.