Plant Biotechnology 22, 361–369 (2005)

Review

Cyclic electron transport through photosystem I

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Received August 26, 2005; accepted September 14, 2005 (Edited by A. Yokota)

Abstract Ferredoxin (Fd)-dependent cyclic electron transport through photosystem I (PSI) was first discovered to be cyclic photophosphorylation coupled with ATP synthesis in the chloroplast. Although pioneer studies provided important information, the physiological significance of this electron transport has been underestimated in C_3 plants. The discovery that the *Arabidopsis pgr5* (*proton gradient regulation*) mutant shows impaired Fd-dependent cyclic electron transport was the first piece of evidence of molecular-scale information on this electron transport pathway and its physiological function. The recent advance of techniques for measuring the activity of cyclic electron transport *in vivo* has allowed a re-evaluation of its activity using wild type and *pgr5*. In this review, we discuss the major role played by Fd-dependent cyclic electron transport, especially in 1) induction of thermal dissipation, 2) contribution to ATP synthesis and 3) photoprotection of PSI.

Key words: *Arabidopsis*, chloroplast, cyclic electron transport, photoinhibition, photosynthesis.

Sunlight is the essential energy source of photosynthesis, which supports all life on Earth. The primary process in photosynthesis is the conversion of light energy into chemical energy as a consequence of photosynthetic electron transport. The chemical energy produced is used to sustain CO_2 assimilation in the chloroplast. In nature, light intensity fluctuates, and specific environmental conditions such as drought, nutrient deprivation and changes in temperature often decrease CO_2 assimilation and consequently cause excess absorption of light energy or over-reduction of photosynthetic electron transport carriers, ultimately leading to the production of active oxygen species that damage the photosynthetic apparatus (reviewed in Demmig-Adams and Adams 1992; Asada 1999). Plants have therefore developed adaptive mechanisms to control the efficiency of light energy utilization and photosynthetic electron transport.

Linear electron transfer from water to $NADP^+$ is driven by photosystem II (PSII) and PSI and results in O_2 evolution at PSII and generation of NADPH (Figure 1). Protons are released from water and translocated across the thylakoid membrane, coupled with electron transport through the cytochrome (cyt) b_6f complex; the resulting ΔpH is utilized in ATP synthesis. On the other hand, cyclic electron transport is driven solely by PSI. In this type of electron transport, electrons are recycled from reduced Fd or NADPH to plastoquinone, which increases electron flux through the cyt b_6f complex involved in ΔpH generation. The activity of PSI cyclic electron transport was discovered 50 years ago (Arnon et al. 1954). Since cyclic electron transport can generate ΔpH without accumulation of NADPH, it can also modify the ratio between proton translocation and electron transport and in turn the ATP/NADPH production ratio. It has been thought for some time that cyclic electron transport confers flexibility on all light reactions; however, there is a conflicting hypothesis that PSI cyclic electron transport operates at very low levels, especially in C_3 plants. This discrepancy results from the lack of a definitive method for evaluating its activity *in vivo* and from the limited information available on PSI cyclic electron transport at the molecular level.

To prevent photoinhibition caused by the excessive absorption of light energy, plants have developed multiple strategies to regulate the efficiency of light energy utilization (Niyogi 2000). One of the most effective mechanisms is thermal dissipation, in which excessive light energy is dissipated safely from PSII as heat (Müller et al. 2001). Since the induction of thermal dissipation may reduce the efficiency of photosynthesis under low-light conditions, heat dissipation is regulated by monitoring the pH of the thylakoid lumen, which becomes acidified under illumination. Alternative electron transport pathways, including PSI cyclic

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Abbreviations: AG, afterglow; cyt, cytochrome; DCMU, (3-(3,4-dichlorophenyl)-1,1-dimethylurea); Fd, ferredoxin; FNR, ferredoxin-$NADP^+$ reductase; FQR, ferredoxin-quinone reductase; NDH, NAD(P)H dehydrogenase; NPQ, nonphotochemical quenching; PSI, photosystem I; PSII, photosystem II; qE, ΔpH-dependent NPQ.

This article can be found at http://www.jspcmb.jp/

electron transport and the water-water cycle, may regulate the induction of thermal dissipation by modifying the rate of generation of ΔpH (Heber and Walker 1992; Asada 1999).

We have carried out a genetic approach by mutant screening of *Arabidopsis* to clarify the regulatory mechanisms of photosynthesis by alternative electron transport pathways (Shikanai et al. 1999). If cyclic electron transport really is involved in the induction of thermal dissipation, it should be possible to isolate specific mutants based on high chlorophyll fluorescence emissions caused by lack of thermal dissipation (Niyogi et al. 1998). This assumption is based on the fact that the yield of room temperature chlorophyll *a* fluorescence emitted by PSII decreases when excitation energy is used for photochemistry (photochemical quenching) or is dissipated as heat (nonphotochemical quenching, NPQ) (Krause and Weis 1991). State transition regulated by phosphorylation of light-harvesting complex II (qT) and photoinhibitory quenching (qI) also contribute to NPQ. In higher plants, the major component of NPQ is thermal dissipation, identified as ΔpH-dependent quenching, qE (Horton et al. 1996). *Arabidopsis npq* (*nonphotochemical quenching*) mutants (*npq1*, deficient in violaxanthin deepoxidase; *npq4*, deficient in PsbS), which are affected in the crucial dissipation processes were also isolated using chlorophyll fluorescence imaging (Niyogi et al. 1998; Li et al. 2000). One mutant, *pgr5*, which has impaired Fd-dependent PSI cyclic electron transport, was isolated based on its high level of chlorophyll fluorescence at high light intensities (Shikanai et al. 1999). The characterization of this mutant provided new molecular information and clarified the physiological importance of this Fd-dependent PSI cyclic pathway (Munekage et al. 2002). In this review, we revisit the model of cyclic electron transport studied since the 1960s and discuss its physiological function based on the recent genetic and physiological characterization of *pgr5*.

Discovery of cyclic phosphorylation

PSI cyclic electron transport was first discovered as cyclic phosphorylation by Arnon and co-workers (1954) before the proposal of the Z-scheme, a working model of photosynthetic electron transport driven by two photosystems, which is now accepted (Hill and Bendall 1960). Arnon and co-workers tested whether ATP was formed in isolated chloroplasts under illumination. Their idea was challenging because the ATP used for carbon fixation was at the time believed to derive from mitochondrial respiration. ATP formation was observed under illumination in isolated chloroplasts where CO_2 uptake was detected, indicating that the chloroplasts are the unique site of photosynthesis in plants and do not require the participation of any other organelles (Arnon et al. 1954; Arnon 1955). In the original experiments, Arnon showed that ATP was synthesized in a closed system without electron donor or acceptor, implying that it was driven by activity of cyclic electron transport, also terms as cyclic photophosphorylation (Arnon 1959). After the discovery of artificial cofactors, photophosphorylation was shown to consist of two pathways: cyclic electron transport and noncyclic (linear) electron transport coupled with oxygen evolution and reduction of a pyridine nucleotide analogue of $NADP^+$ (Tagawa et al. 1963a; Arnon et al. 1967). Although the rate of cyclic electron transport was lower than linear electron transport, it was believed in early studies that both electron transport pathways were essential for carbon fixation.

In vitro assay using cofactors and inhibitors

Understanding of the model for the pathway of the cyclic electron pathway was much improved by the discovery of a catalytic cofactor, Fd, a soluble protein with redox potential of −350 to −450 mV. The rate of cyclic photophosphorylation is dramatically increased by adding Fd (Tagawa et al. 1963a). Current basic knowledge of PSI cyclic electron transport was for the most part established by these early studies on cyclic photophosphorylation. 1) Cyclic electron transport is driven solely by PSI photochemistry. 2) Its activity is almost completely inhibited by antimycin A. 3) It does not require a final electron acceptor, since the electrons are recycled from Fd or NADPH to plastoquinone. 4) It can produce ATP (Tagawa et al. 1963a, 1963b; Arnon et al. 1967; Arnon and Chain 1975). Contribution of PSI cyclic electron transport to gross ΔpH was roughly estimated by 9-amminoacridine fluorescence quenching (Crowther et al. 1979; Miyake et al. 1995).

The involvement of plastoquinone and the cyt b_6f complex in PSI cyclic electron transport was shown by studies using specific inhibitors such as DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), which binds to the Q_B site of PSII; DBMIB (2,5-dibromo-3-methyl-6-isoplophyl-*p*-benzoquinone); HQNO (2-heptyl-4-hydroxyquinolines), and stigmatellin, which binds to the cyt b_6f complex. Although inhibitors of the cyt b_6f complex inhibited PSI cyclic electron transport, DCMU did not (Hauska et al. 1974; Bendall and Manasse 1995). Since antimycin A is a well-characterized inhibitor of the cyt bc_1 complex in the mitochondria, the active site for PSI cyclic electron transport was believed to be in the cyt b_6f complex. However, this was later shown to be not true, since antimycin A does not affect the electron transport activity of the cyt b_6f complex (Hauska et al. 1983; Moss and Bendall 1984). Antimycin A is now generally accepted as a specific inhibitor of PSI cyclic

electron transport in the chloroplast. Nevertheless, the site of the action is still unclear.

Early investigations suggested that cyclic electron transport occurs under certain redox 'poising' conditions (Tagawa et al. 1963a, 1963b; Arnon and Chain 1975; Mills et al. 1979). Investigation of the transition from noncyclic to cyclic mode in broken chloroplasts has shown that cyclic photophosphorylation is activated only after the consumption of pyridine nucleotide by noncyclic electron transport. It suggested that, under physiological conditions, the activation of PSI cyclic electron transport is regulated by the availability of electron acceptors (e.g. $NADP^+$) (Tagawa et al. 1963a). The reduction of O_2 as an alternative electron sink *in vivo* (Asada 1999) also relates to photophosphorylation, but was shown to be independent of the PSI cyclic electron transport pathways (Tagawa et al. 1963a). In broken chloroplasts, electron donation to plastoquinone from the stoma was observed without PSI photochemistry if an exogenous electron donor such as Fd or NADPH was supplied (Mills et al. 1979), suggesting that PSI cyclic electron transport is promoted by reducing power within the chloroplast. PSI cyclic electron transport may be regulated by the redox state of the chloroplast.

A genetic approach; the identification of PGR5

An *Arabidopsis* mutant, *pgr5*, defective in PSI cyclic electron transport, was isolated based on its lack of qE induction (Munekage et al. 2002). A striking phenotype in *pgr5* was the reduction of $P700^+$ (the oxidized reaction center of PSI) level under illumination. While P700 was oxidized by increasing light intensity in the wild type, it was reduced at high light intensities in *pgr5* (Munekage et al. 2002). P700 oxidation was restored by adding methylviologen, an artificial electron acceptor from PSI, a result which indicates that electron transport is limited at the acceptor side of PSI. The rate of linear electron transport was not impaired in isolated thylakoid membranes, indicating that *pgr5* is defective in alternative electron transport. This idea was also supported by the fact that the reduction of P700 is enhanced in CO_2-free air and even in N_2 gas, suggesting that *pgr5* is deficient in O_2-independent alternative electron transport. Finally, *in vitro* plastoquinone reduction assay using ruptured chloroplasts clearly showed that Fd-dependent plastoquinone reduction is impaired in *pgr5* (Munekage et al. 2002). The *pgr5* defect in plastoquinone reduction was mimicked by adding antimycin A (Munekage et al. 2002). Furthermore, antimycin A completely inhibits the plastoquinone reduction activity which remains in *crr2* (*chlororespiratory reduction*) and is completely inhibited in the *crr2 pgr5* double mutant, even in the absence of antimycin A (Munekage et al. 2004). *crr2* lacks the NDH (NAD(P)H dehydrogenase) complex due to its defect in chloroplast gene expression (Hashimoto et al. 2003). The NDH complex is involved in the alternative route of electrons in PSI cyclic electron transport (reviewed in Shikanai and Endo 2000; Peltier and Cournac 2002; see also Figure 1). From these results, we concluded that *pgr5* is impaired in antimycin A-sensitive PSI cyclic electron transport. The mutant is defective in a unique pathway of Fd-dependent PSI cyclic electron transport.

PGR5 gene encodes a 10-kDa protein associated with the thylakoid membrane (Munekage et al. 2002). PGR5 does not contain any putative metal-binding or transmembrane motifs and is stable even in mutant backgrounds lacking PSII, PSI, the cyt b_6f complex or ATPase, suggesting that PGR5 does not directly interact with these major complexes. Although it is unlikely that Fd-dependent PSI cyclic activity is catalyzed solely by PGR5, it is possible that PGR5 directly interacts with the proteins responsible for Fd-dependent plastoquinone reduction activity.

Previous studies on the distribution of photosynthetic components using fragmentation or separation of membranes together with analysis by electron microscopy have shown that PSII is chiefly distributed in the grana-stacking membranes, whereas PSI is located in the marginal region of the grana and stroma lamellae (Albertsson 2001). This structural model of the thylakoid membranes suggests that linear electron transport is performed in the stacked grana, while PSI cyclic electron transport takes place in the stroma lamellae. The NDH complex has been shown to be mainly located in the stroma lamellae (Rumeau et al. 2005). PGR5 was also localized to the stroma thylakoid fraction (unpublished data). Although the proteins directly involved in Fd-dependent plastoquinone reduction are not yet identified, their site of action may be in the stroma lamellae, together with PGR5. Further studies are necessary to clarify the function of PGR5 and the overall molecular mechanism of Fd-dependent cyclic electron transport.

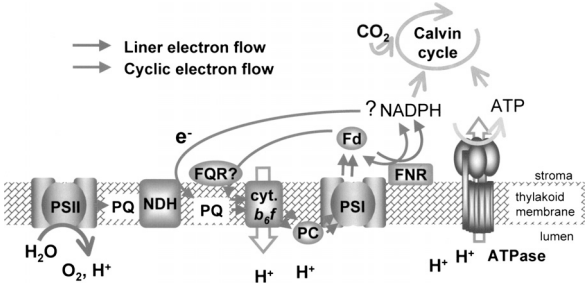

Figure 1. Schematic model of linear and cyclic electron transport. Electron donor to the NDH complex is unclear. PQ, plastoquinone; PC, plastocyanin.

Fd-plastoquinone reductase (FQR)

The activity of Fd-dependent plastoquinone reduction is unique to PSI cyclic electron transport, and with the exception of PGR5, no other molecule involved in this reaction has been identified. However, the enzyme name "Fd-quinone reductase (FQR)" is often used for this unidentified or unsubstantiated protein(s). It has been suggested that low-redox potential cyt b_{559}, as distinct from PSII cyt b_{559}, mediates the Fd-dependent plastoquinone reduction; however, no electron carrier containing cyt b_{559} has been identified (Arnon and Chain 1975; Miyake et al. 1995).

There has been a debate as to whether the Fd-dependent plastoquinone reduction occurs *via* the cyt b_6f complex. The cyt b_6f complex and Fd-$NADP^+$ reductase (FNR) may form a supercomplex (Zhang et al. 2001), in which FNR would be involved in Fd oxidation, while the Q_i site of the cyt b_6f complex would be involved in the plastoquinone reduction. However, there is still disagreement on this model due to antimycin A-insensitivity to flash-induced oxidation of heme b_6 (Moss and Bendall 1984) and a lack of evidence to show that FNR is involved in cyclic activity (Bendall and Manasse 1995).

Recently, crystallography has revealed the existence of an additional heme on the stromal face at the Q_i site of the cyt b_6f complex (Kurisu et al. 2003; Stroebel et al. 2003). This atypical heme is regarded as a possible catalytic reaction center of FQR. Recently, this heme was suggested to be essential to the Q-cycle itself (Zhang et al. 2004). To assess the involvement of the cyt b_6f complex as a direct electron mediator from stroma to plastoquinone, PSI cyclic activity was analyzed in *pgr1*. The cyt b_6f activity in this mutant is sensitive to lumen acidification due to an amino acid alteration in the Rieske subunit (Munekage et al. 2001; Jahns et al. 2002). Neither *in vitro* nor *in vivo* analyses have shown any evidence of the involvement of the cyt b_6f complex in Fd-dependent plastoquinone reduction (Okegawa et al. 2005). However, the *pgr1* mutation may not impair the Fd-dependent plastoquinone reduction, even though it takes place via the stromal-side hemes of cyt b_6. Consequently, the route taken by electrons in cyclic electron transport remains unknown.

In vivo measuring techniques of the rate of PSI cyclic electron transport

It is important to establish a measuring technique of the rate of PSI cyclic electron transport in the leaf. Several techniques, including P700 re-reduction measurement and photoacoustic spectroscopy, have been applied to detect cyclic activity in the leaf (Herbert et al. 1990; Joët et al. 2002). The re-reduction kinetics of P700 in the dark accelerates when electrons coming from the stroma are donated to PSI. This fast re-reduction has been observed in several species of algae and cyanobacteria (Maxwell and Biggins 1976; Fork and Herbert 1993). In cyanobacteria, this measurement can be used to evaluate the activity of NDH-dependent PSI cyclic electron transport (Mi et al. 1994). Fast re-reduction of P700 is also observed in C_4 plants, but it is slower in C_3 plants, probably due to lower NDH activity and the loss of favourable redox poising (Fork and Herbert 1993; Joët et al. 2002). In C_3 plants, fast re-reduction is accelerated in anaerobiosis, drought conditions or after long dark adaptation (Golding et al. 2004), but under anaerobic conditions, fast re-reduction shows little dependency on electron donation by NDH activity (Joët et al. 2002).

Measurement of P700 re-reduction was also applied to *pgr5* to assess whether this type of measurement could monitor PGR5-dependent cyclic electron transport. The results showed that a maximum P700 oxidation level of *pgr5* was strongly reduced due to the charge recombination of P700 in anaerobiosis. To avoid electron input from PSII, leaves were infiltrated with DCMU prior to measurement. Although the maximum P700 oxidation in *pgr5* was restored to wild-type levels, fast kinetics was absent, even in the wild type (unpublished data). A low level of electron input is likely to be required to activate PSI cyclic electron transport. Since the P700 oxidation level is determined from the balance between electron input and output through PSI, it is influenced not only by cyclic electron transport but also by other factors such as electron leakage to O_2 or electron storage in the inter-system chain and stroma. Furthermore, the activity of PSI cyclic electron transport has been suggested to require a high NADPH/$NADP^+$ ratio or reduction of the plastoquinone pool (Arnon and Chain 1975; Fork and Herbert 1993; Mills et al. 1979; Joët et al. 2002). This favourable redox poising may occur in cyanobacteria and green algae via respiratory electron transport and C_4 plants via the malate pool, even under the experimental conditions of dark following far-red illumination, but may not occur in C_3 plants (Fork and Herbert 1993; Joët et al. 2002). Consequently, it would appear to be difficult to evaluate PSI cyclic electron transport by P700 re-reduction measurement in C_3 plants.

Photoacoustic techniques for measuring PSI cyclic electron transport provide more convincing results. This technique depends on the quantification of the conversion of absorbed light energy to heat. If light energy is used for chemical energy storage as a result of photochemistry, heat dissipation decreases, resulting in a decreased photoacoustic signal. Energy storage under modulated far-red light illumination, which is related to PSI cyclic electron transport, has been observed in cyanobacteria, algae, and C_4 plants, but is less evident in

C_3 plants (Herbert et al. 1990; Joët et al. 2002). In *Synechocystis* PCC6803, photoacoustic measurements were applied to a deletion mutant of *ssr2016* that is a homologue of *Arabidopsis* PGR5, M55 (*ndhB* disruptant) and their double mutant, DM55-2016 (Yeremenko et al. 2005). Although NDH-dependent activity is significantly reflected in the photoacoustic signal, there is no difference in energy storage between the wild type and the single mutant SM2016. In contrast, the energy storage in the double mutant DM55-2016 was almost completely lost, demonstrating that both NDH and *ssr2016*-dependent cyclic activity can be detected using this technique. In tobacco, energy storage is observed under anaerobic conditions and the *ndhB* knockout line showed partial loss of energy storage under high-frequency-modulated light (Joët et al. 2002). Energy storage, however, has not been detected in *Arabidopsis*, even in anaerobisis, probably due to inadequate measuring conditions for activating PSI cyclic activity (personal communication with Havaux). Although photoacoustic measurement permits the detection of PSI cyclic activity under anaerobic conditions in tobacco, measurements are again limited under far-red light conditions where redox poising may disappear in *Arabidopsis* and even in tobacco under aerobic conditions. For these technical reasons, it is difficult to apply this measurement to different species of C_3 plants or evaluate PSI cyclic activity under ambient conditions.

The afterglow (AG) luminescence technique has been recently established as a probe of PSI cyclic electron transport. Heat-stimulated electron transport from the stroma to plastoquinone observed as AG emissions peaking at 42°C was investigated in tobacco, *Arabidopsis* and cyanobacteria (Havaux et al. 2005) and maize (Ducruet et al. 2005). AG emission is mostly correlated with antimycin A-sensitive cyclic activity in tobacco, whereas it correlates with NDH activity in *Arabidopsis*. The results are consistent with those of *in vitro* plastoquinone reduction assay, where the contribution of antimycin A-sensitive activity to plastoquinone reduction is higher in tobacco than in *Arabidopsis* (Endo et al. 1998; Munekage et al. 2004). On the other hand, *pgr5* showed a closely similar AG emission level to the wild type, and antimycin A slightly affected AG emission in the wild type. Based on the strong phenotype of *pgr5* on the photosynthetic electron transport relative to that of NDH-deficient mutants under illumination (Munekage et al. 2002; Munekage et al. 2004; Rumeau et al. 2005), the PGR5-dependent pathway is regarded as the main pathway of PSI cyclic electron transport in *Arabidopsis*. Therefore, luminescence measurement seems to be unable to fully reveal PGR5-dependent activity. One of the limitations of this technique is that it needs to be done in the dark, in which PGR5-dependent cyclic electron transport is probably less active. Classical studies have pointed out the requirement of reducing power for activation of the Fd-dependent cyclic electron transport (Tagawa et al. 1963a; Arnon et al. 1967; Mills et al. 1979). At present, though, no method is available to evaluate the full PSI cyclic electron flow under ambient conditions in C_3 plants.

Physiological role of Fd-dependent cyclic electron transport

Regulation of thermal dissipation via ΔpH across the thylakoid membrane

Induction of thermal dissipation occurs not only at high light intensities but also at low light intensities under stressed conditions, including low CO_2 concentration and low temperature. Since the rate of linear electron transport decreases when consumption of NADPH by the Calvin cycle is decreased, the generation of ΔpH needs to be compensated by alternative electron transport. PSI cyclic electron transport contributes to ΔpH generation without accumulation of NADPH, suggesting that the induction of thermal dissipation (Heber and Walker 1992; Shikanai et al. 2000) can be regulated.

The *pgr5* mutant completely lacks induction of ΔpH-dependent NPQ (qE) during steady-state photosynthesis at high light intensities and also significantly lacks qE in CO_2-free air or during the induction phase of photosynthesis (Munekage et al. 2002). These results show that PGR5-dependent PSI cyclic electron transport is essential for qE induction under excessive light conditions. However, how qE induction is regulated under stressed conditions remains controversial. The simplest idea is that ΔpH generation is regulated by proton influx via alternative electron transport activity. It is also possible that ΔpH is regulated by reduced proton efflux via ATP synthase (Kramer et al. 2004). Interestingly, a higher efflux of protons is reported in *pgr5*, which may cause the qE-deficient phenotype of *pgr5* (Avenson et al. 2005). However, the linkage between the impaired PSI cyclic electron transport activity and the higher efflux of protons appears to be indirect. The higher proton efflux may be caused by disturbed regulation of ATPase due to the reduced ATP/ADP ratio, as discussed by Avenson et al. (2005). Up-regulation of PSI cyclic electron transport has also been suggested by several *in vivo* measurements at high light intensity, low temperature, drought, and during the induction phase of photosynthesis (Cornic et al. 2000; Clarke and Johnson 2001; Makino et al. 2002; Golding and Johnson 2003; Miyake et al. 2005). These questions derive from the lack of a reliable method to monitor the activity of PSI cyclic electron transport *in vivo* and the ambiguous form of proton conductivity regulation by ATPase. This topic clearly requires further research.

Contribution to ATP synthesis

Cyclic electron transport was discovered to take the form of cyclic photophosphorylation (Arnon et al. 1954), and its contribution to CO_2 fixation was shown by the effect of antimycin A in chloroplasts (Furbank and Horton 1987) and in leaves (Cornic et al. 2000). Historically, PSI cyclic electron transport has been believed to contribute to the production of the ATP required for CO_2 fixation or photorespiration. Interestingly, a double mutant *crr2 pgr5* affected in both the NDH-dependent and antimycin A-sensitive pathways showed a dramatic reduction in photosynthetic growth (Munekage et al. 2004). Complete inhibition of PSI cyclic electron transport activity influences steady-state photosynthesis, suggesting that PSI cyclic electron transport is required for photosynthesis. How significant is the contribution of the PSI cyclic electron transport to ATP synthesis during steady-state photosynthesis? Although the double mutants show severe growth inhibition, we cannot quantify the contribution of PSI cyclic electron transport to ATP synthesis. Even though the contribution of PSI cyclic electron transport to gross ATP synthesis is small, it may compensate for the ATP/NADPH production balance, a process essential for normal plant growth since it maintains homeostasis of the energy budget in the chloroplast. In fact, in the double mutants, photoinhibition of PSII and reduction of chlorophyll content were observed even under low-light growth conditions (Munekage et al. 2004), and consequently these secondary effects further decrease photosynthetic activity.

The requirement of PSI cyclic electron transport for CO_2 fixation was re-calculated from a recent view of the stoichiometry of ATP/NADPH ratio produced by linear electron transport (Allen 2003). According to this calculation, during the reduction of one molecule of $NADP^+$ by two electrons through the linear electron chain, six protons are translocated to the lumen with the coupling of the Q-cycle in the cyt b_6f complex. Assuming the ATP synthesis model of chloroplast, where 14 protons are used for the synthesis of three molecules of ATP (Seelert et al. 2000), the ATP/NADPH production ratio is 1.29 by linear electron transport. Since the ATP/NADPH requirement for the Calvin cycle is 1.5–1.66 depending on the operation of photorespiration (Osmond 1981), theoretically 14–22% more ATP is required during steady-state photosynthesis.

It is possible to estimate the contribution of PSI cyclic electron transport using *pgr5* and *crr2*. Although the phenotype is much more evident in the double mutant, *crr2 pgr5*, it cannot be used for estimating the *in vivo* rate of electron transport, since the severe defect appears to have secondary effects on photosynthesis. It is therefore preferable to use the single mutants of *pgr5* and *crr2*, since the direct impact of the defect in each cyclic pathway can then be evaluated. In *pgr5*, the rate of linear electron transport is not affected at low light intensities. Taking into account the fact that *pgr5* grew normally at low light intensities, these results suggest that plants can manage the homeostasis of energy budgets without help from PGR5-dependent cyclic electron transport, provided light intensity is low. During this process of maintaining homeostasis, the contribution of NDH-dependent cyclic electron flow is essential, as evident in the phenotype of the double mutants. However, *pgr5* cannot manage the balance of ATP/NADPH production at high light intensities, leading to stromal over-reduction. The use of intermediate light conditions raises the imbalance but does not induce severe photodamage, making it possible to quantify, *in vivo*, the contribution of PGR5-dependent PSI cyclic electron transport.

Simultaneous measurement of CO_2 gas exchange and chlorophyll fluorescence has shown that at high light intensities, the rate of CO_2 assimilation decreases by 14%, whereas electron transport rate is 27% lower than in the wild type (unpublished data). This result is roughly consistent with the required rate of PSI cyclic electron transport calculated from the stoichiometry of the ATP/NADPH production ratio. The result is also consistent with the experimental data obtained by simultaneous determination of the yields of both photosystems ($\Phi_{PSI}/\Phi_{PSII}=1.2$) (Miyake et al. 2004; Okegawa et al. 2005). However, we found that PSI was inactivated in *pgr5* even at moderate light intensities. This may lead to an over-estimation of the PSI cyclic electron transport rate. On the other hand, it should also be taken into account that the *pgr5* defect is compensated by another acclimation strategy, which would lead to the under-estimation of the rate. We calculate that the contribution of PGR5-dependent cyclic electron transport for CO_2 fixation is approximately 14%, although the physiological significance of the PSI cyclic electron transport grows at high photorespiratory rates. Our hypothesis was supported by the preliminary results, which indicate that the growth inhibition of *pgr5* is more pronounced under low or ambient-CO_2 conditions than under high-CO_2 conditions. It is possible that the loss of poising of the ATP/NADPH ratio leads to worse defects in plant growth than a simple reduction of energy production. As seen, the PGR5-dependent cyclic electron transport is important in maintaining the correct ratio of ATP/NADPH under fluctuating light conditions.

Photoprotection

It is generally accepted that PSII is the primary target of photoinhibition (Aro et al. 1993) and that selective PSI photoinhibition occurs under specific environmental conditions such as chilling combined with relatively weak light intensities (Sonoike 1996; Terashima et al. 1998). It was not expected that PSI would be damaged

prior to PSII by high-light treatment in *pgr5* (Munekage et al. 2002). Inactivation of PSI was observed in even moderate light intensities in *pgr5* but not in *npq4* lacking qE (unpublished data), suggesting that PSI photoinhibition is not due to the lack of qE but probably due to over-reduction of the stroma. In low-temperature-sensitive plants like cucumber, the process of PSI photoinhibition has been studied in detail (Sonoike 1996): over-reduction of the stroma decreases the efficiency of electron transfer from PSI, leading to an increase in the lifetime of the reduced electron carriers on the PSI acceptor side, particularly $F_{A/B}^{-}$. This is due to the higher probability of O_2 reduction resulting in production of H_2O_2 and further in the production of hydroxyl radicals that attack iron-sulfur centers such as $F_{A/B}$ and F_X, ultimately causing degradation of PsaB. Unlike PSII, restoration of PSI activity requires several days.

The exact process of PSI photoprotection by PGR5-dependent PSI cyclic electron transport remains unclear. Most probably, the imbalance of ATP and NADPH production due to lack of cyclic electron transport causes over-reduction of the stroma, which directly triggers PSI photoinhibition. We believe that this process is the primary reason for the drastic reduction in photosynthetic growth in the *crr2 pgr5* double mutants. We conclude that PSI cyclic electron transport is required for the maintenance of the correct ATP/NADPH ratio, which is in turn essential to plant survival.

Concluding remarks

Since its discovery, our knowledge of PSI cyclic electron transport has progressed only slightly. It is surprising that such basic processes in photosynthesis have remained unclear for 50 years. Identification of mutants defective in PSI cyclic electron transport indicate that it is essential for both photosynthesis and photoprotection. This means that it is necessary to reconsider the frame of the light reactions of photosynthesis. However, we are still not sure of the routes that electrons take along these pathways. There are still numerous debates on the rate of electron transport *in vivo* and its regulation. "To understand the new, study the old." We cannot afford, though, to wait another 50 years to find the answers.

Acknowledgements

We thank Dr. Michel Havaux for his critical reading of the manuscript and for providing unpublished information. This work was supported by the "Research for the Future" Program (JSPS-RFTF00L01604), and is supported by a Grant-in-aid for Scientific Research on Priority Areas (16085206) and for Creative Scientific Research (17GS0316) from the Ministry of Education, Culture, Sports, Science and Technology, Japan to TS.

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