Short title:

Proton conductivity of the pgr5 mutant

Article title:

Does the Arabidopsis proton gradient regulation 5 mutant leak protons from the thylakoid membrane?¹

Hiroshi Yamamoto² and Toshiharu Shikanai³

²Department of Botany, Graduate School of Science, Kyoto University, Kyoto, 606-8502 Japan
ORCID IDs: 0000-0002-1739-1226 (H.Y); 0000-0002-6154-4728 (T.S.)

One sentence summary:

High $g_{H^+}$ of the pgr5 mutant is compensated to the WT level by introduction of flavodiiron protein and strong down-regulation of the cytochrome $b_{6}f$ complex, objecting to the proton leakage from the thylakoid membrane.

Footnotes

¹This work was supported by the Japanese Society for the Promotion of Science KAKENHI (16H06553 and 19H00992).

Responsibilities of the Author for Contact:

²Senior author.
³Author for contact: shikanai@pmg.bot.kyoto-u.ac.jp

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Toshiharu Shikanai (shikanai@pmg.bot.kyoto-u.ac.jp).

Author contributions:

H.Y. and T.S. designed the research; H.Y. performed the experiments; H.Y. analyzed the data; H.Y. and T.S. wrote the article.

Both authors contributed equally to this work.
Research area:
Biochemistry and Metabolism
Membranes, Transport, and Bioenergetics

Article types: Research article
Abstract

Despite generating an obvious mutant phenotype, whether the Arabidopsis thaliana proton gradient regulation 5 (pgr5) mutation influences cyclic electron transport (CET) around photosystem I is a topic of debate. Based on a parameter of electrochromic shift analysis ($g^{+}_H$), the proton conductivity of the thylakoid membrane in the pgr5 mutant is enhanced at high light intensity. Given this observation, PGR5 was proposed to regulate ATP synthase activity rather than mediating CET. The originally reported pgr5 phenotype reflects a smaller proton motive force (pmf) and could be explained by this H$^+$ leakage model. In this study, we genetically reexamined the high $g^{+}_H$ phenotype of the pgr5 mutant. Transgenic lines in which flavodiiron protein-dependent pseudo-CET replaced PGR5-dependent CET had wild-type levels of $g^{+}_H$, suggesting that the high $g^{+}_H$ phenotype in pgr5 plants is caused secondarily by the low pmf. The pgr1 mutant shows a similar reduction in pmf because of enhanced sensitivity of its cytochrome b$_6$f complex to lumenal acidification. In contrast to the pgr5 mutant, $g^{+}_H$ was lower in the pgr1 mutant than in the WT. In the pgr1 pgr5 double mutants, $g^{+}_H$ was intermediate to those of the respective single mutants. It is unlikely that the $g^{+}_H$ is upregulated simply in response to a low pmf, as we did not observe uncoupling of the thylakoid membrane in the pgr5 mutant upon monitoring the quenching of 9-aminoacridine fluorescence. We conclude that the $g^{+}_H$ parameter may be influenced by other factors not related to the H$^+$ leakage through ATP synthase. It is unlikely that the pgr5 mutant leaks protons from the thylakoid membrane.

Keywords: ATP synthase, flavodiiron protein, PGR5, photosynthetic control, PSI cyclic electron transport, proton conductivity
The light reactions of photosynthesis are driven by two photosystems, photosystem II and I (PSII and PSI), functioning in that order. They mediate electron transport from water to NADP⁺. This linear electron transport does not satisfy the ATP/NADPH production ratio required by the Calvin-Benson cycle (Allen, 2002). Additional ATP requirement is satisfied by cyclic electron transport (CET) around PSI (Yamori and Shikanai, 2016). In angiosperms, PSI CET consists of at least two pathways (Fig. 1). The main route of electron transport is sensitive to antimycin A and depends on PROTON GRADIENT REGULATION 5 (PGR5) and PGR5-LIKE PHOTOSYNTHETIC PHENOTYPE (PGRL1) (Munekage et al., 2002; DalCorso et al., 2008). The minor pathway is mediated by the NADH dehydrogenase-like (NDH) complex (Peltier et al., 2016). The mutants defective in both pathways showed severe mutant phenotypes in photosynthesis and plant growth (Munekage et al., 2004).

The Arabidopsis (*Arabidopsis thaliana*) pgr5 mutant was isolated on the basis of its high chlorophyll fluorescence phenotype at high light intensity (Munekage et al., 2002). Because of the reduced size of ΔpH across the thylakoid membrane, the mutant cannot induce the energy-dependent (qE) component of non-photochemical quenching (NPQ) of chlorophyll fluorescence. In addition to the qE induction, lumenal acidification also down-regulates electron transport through the cytochrome (Cyt) b₆f complex (Fig. 1). This regulatory process, called photosynthetic control, is important to protect PSI from photodamage, especially in fluctuating light intensities (Suorsa et al., 2012; Kono et al., 2014). The pgr5 mutant cannot exert photosynthetic control due to the reduced size of ΔpH, and consequently PSI is hypersensitive to fluctuating light (Yamamoto and Shikanai, 2019).

Mainly on the basis of the reduced activity of the ferredoxin-dependent plastoquinone reduction in ruptured chloroplasts, we proposed that the Arabidopsis pgr5 mutant was defective in PSI CET (Munekage et al., 2002). A similar phenotype but to a lesser extent was observed in Arabidopsis mutants defective in the chloroplast NDH complex, and the activity was completely absent in double mutants defective in both pathways (Munekage et al., 2004). PGRL1 was discovered in the Arabidopsis mutant with a similar photosynthetic phenotype to the pgr5 mutant, and is essential for the accumulation of PGR5 (DalCorso et al., 2008). PGR5 and PGRL1 proteins were discovered in the CET supercomplex including the Cyt b₆f complex, PSI and ferredoxin-dependent NADP⁺ reductase (FNR) in *Chlamydomonas reinhardtii* (Iwai et al., 2010; Johnson et al., 2014). Because a single amino acid alteration in PGR5 confers resistance of photosynthesis to antimycin A in Arabidopsis (Sugimoto et al., 2013) and PGRL1-dependent quinone reduction is sensitive to antimycin A *in vitro* (Hertle et al., 2013), we proposed that the...
PGR5/PGRL1 pathway corresponds to the cyclic phosphorylation discovered by Arnon’s group in the 1950s (Arnon et al., 1954).

PGR5 is a small protein without any known motif, and it is still unclear how it is involved in PSI CET. Despite the strong mutant phenotype, it remains a topic of debate as to whether PGR5 is involved in PSI CET. The problem is related to the lack of a definitive method of monitoring the operation of PSI CET, especially in vivo (Johnson, 2005). It was proposed that PSI CET is still operating in the pgr5 mutant and PGR5 is a regulator of PSI CET (Nandha et al., 2007). In contrast, Kou et al. (2015) reported that antimycin A-sensitive CET was almost completely abolished in the pgr5 mutant in steady-state photosynthesis. One of the mysterious phenotypes of the Arabidopsis pgr5 mutant is the enhanced proton (H⁺) conductivity of the thylakoid membrane (Avenson et al., 2005; Wang et al., 2015). It was monitored as the $g_{H^+}$ parameter of electrochromic shift (ECS) analysis (Kanazawa and Kramer, 2002). The $g_{H^+}$ parameter is calculated as an inverse of the time constant of the ECS decay from a first-order exponential fit. A similar phenotype has also been observed in rice (Oryza sativa) PGR5 knock-down lines (Nishikawa et al., 2012). In the pgr5 mutant, H⁺ leakage from the thylakoid membrane may be upregulated and PGR5 may be involved in the regulation of ATP synthase activity. The model for the direct interaction of PGR5 with ATP synthase was proposed (Rantala et al., 2020). The original pgr5 mutant phenotype, depending on the reduced size of ΔpH, can be explained by this idea. In fact, unusual H⁺ leakage from the thylakoid membrane in the overexpressers of the mutant version of the putative H⁺/K⁺ antiporter K⁺ efflux antiporter 3 (KEA3) mimicked the pgr5 mutant phenotype (Wang et al., 2019). In this study, we genetically reexamined the high $g_{H^+}$ phenotype of the pgr5 mutant.

RESULTS

The pgr5 mutation is not directly linked to H⁺ leakage from the thylakoid membrane

The pgr5 mutant is defective in PSI CET and the high $g_{H^+}$ may be secondarily caused by the reduced size of the proton motive force (pmf). If this hypothesis were true, the artificial remodeling of the pmf generation system may compensate $g_{H^+}$ to the WT level in the pgr5 mutant background. To test this possibility, we selected flavodiiron protein (Flv)-dependent pseudo-CET (Fig. 1). Because Flv is not conserved in angiosperms, including Arabidopsis, we cloned the FlvA and FlvB genes from Physcomitrella patens and introduced them into Arabidopsis WT and pgr5-1 mutant plants (Yamamoto et al., 2016). Because we also used a weak allele of pgr5-2 in this study, we call the original strong allele pgr5-1 (Yamamoto and Shikanai, 2019). Flv reduces O₂ to water, probably by accepting electrons from NADPH or

Downloaded on November 5, 2020. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.
ferredoxin (Vicente et al., 2002). Consequently, Flv-dependent pseudo-CET from water to water generates pmf without any net accumulation of NADPH, like PGR5-dependent PSI CET.

In electrochromic shift analysis, one parameter, ECSt, represents the light-dark difference in absorbance at 515 nm (ECS signal), which corresponds to the total size of pmf formed under illumination (Bailleul et al., 2010). The pmf consists of ΔpH and membrane potential (∆ψ) formed across the thylakoid membrane (Shikanai and Yamamoto, 2017). Although both components contribute to pmf, only ΔpH down-regulates electron transport by inducing qE and photosynthetic control. The ECSt level was standardized by the ECS signal by a single turnover flash (ECSST). In wild-type (WT) plants, the size of pmf was saturated at 252 µmol photons m⁻² s⁻¹ (Fig. 2A). In the pgr5 mutant, pmf was saturated at a lower light intensity at a lower level. As reported previously, introduction of FlvA/B complemented the size of pmf to the WT level in the pgr5 mutant, indicating that Flv-dependent pseudo-CET contributed to pmf formation instead of the PGR5-dependent PSI CET (Fig. 2A). In contrast, Flv did not function during steady-state photosynthesis in a WT background.

Figure 2B shows the light intensity-dependence of H⁺ conductivity of the thylakoid membrane monitored by gH⁺. Consistent with a previous report (Wang et al., 2015), gH⁺ was constant (~30 s⁻¹) independent of light intensity but was enhanced at high light intensities of up to 50 s⁻¹ in the pgr5-1 mutant (Fig. 2B). Introduction of Flv into the pgr5-1 mutant suppressed gH⁺ to the WT level but did not affect the gH⁺ level in the WT background. Even under high light intensity in the pgr5-1 mutant background, gH⁺ was maintained at the WT level because of the active operation of Flv-dependent pseudo-CET under high light intensity (Yamamoto et al., 2016). This result suggests that the PGR5 function is not directly related to the regulation of H⁺ leakage through ATP synthase. Instead, it is likely that the pgr5 mutation indirectly affects the gH⁺ parameter, possibly via the reduced size of pmf.

The pgr1 mutation in the Cyt b₆f complex alleviates the gH⁺ phenotype in the pgr5 mutant background

If the ATP level limited the rate of the Calvin-Benson cycle in the pgr5 mutant, it would be reasonable to conclude that ATP synthase activity was enhanced to supplement ATP production. PGR5 is unlikely to regulate ATP synthase directly, but the pgr5 mutant phenotype observed in gH⁺ may reflect the general plant response to reduced pmf. To test this possibility, we analyzed the pgr1 mutant, in which the induction of NPQ was severely impaired due to reduced ΔpH formation, as in the pgr5 mutant (Munekage et al., 2001). The pgr1 mutant has a single amino acid alteration in the Rieske subunit of the Cyt b₆f complex. This missense mutation does not affect the stability of the Rieske subunit, but makes the Cyt b₆f complex hypersensitive to luminal acidification (Fig. 1; Jahns et al., 2002). Even in WT plants, the Cyt b₆f complex
restricts the rate of electron transport when the thylakoid lumen is acidified. This photosynthetic control is essential for protecting PSI, especially under conditions of fluctuating light intensity (Allahverdiyeva et al., 2015). In the pgr1 mutant, photosynthetic control down-regulates electron transport through the Cyt b6f complex even when the thylakoid lumen is neutral at relatively low light intensity.

Figure 3A shows the light intensity-dependence of pmf. In this analysis, we also characterized a weak allele of pgr5-2, in which PGR5-dependent PSI CET was partially impaired (Yamamoto and Shikanai, 2019). Consistent with a previous report (Nakano et al., 2019), the size of pmf was between the WT and the pgr5-1 mutant in the pgr5-2 mutant (Fig. 3A). In the pgr1 single mutant, the size of pmf was more severely affected by the strong down-regulation of electron transport at the Cyt b6f complex, and was not reduced further in the pgr1 pgr5-1 or pgr1 pgr5-2 double mutants. Consistent with the observation in Figure 2B, gH+ was enhanced in the pgr5-1 mutant at high light intensities. A similar trend, but to a lesser degree, was observed in the pgr5-2 mutant. Unexpectedly, the size of gH+ was more reduced in the pgr1 mutant than in the WT plants (Fig. 3B). The high gH+ was not simply related to the reduced size of pmf. In the double mutants with two alleles of pgr5, the size of gH+ was between the pgr1 and pgr5 mutant alleles. Consequently, gH+ was similar to the WT level, especially at 663 µmol photons m⁻² s⁻¹ (Fig. 3B). To explain all these results, it is probably necessary to consider the different reasons for enhanced and reduced gH+ in the pgr5 and pgr1 mutant, respectively (see Discussion).

Relaxation of ΔpH is unaffected in the ruptured chloroplasts isolated from the pgr5-1 mutant

Because our assay solely depended on the ECS signal, we also analyzed the formation of ΔpH in the light and its relaxation in the dark by monitoring the quenching of 9-aminoacridine (9-AA) fluorescence in ruptured chloroplasts (Fig. 4). In the assay, ΔpH was formed solely depending on linear electron transport because 100 µM methyl viologen was applied as a terminal electron acceptor. Figure 4A shows representative trances of the quenching in ruptured chloroplasts isolated from WT and pgr5-1 plants. Upon the onset of actinic light, quenching of the 9-AA fluorescence was observed, which reflected the acidification of the thylakoid lumen. The dark-light differences in the amplitude of quenching represented the size of ΔpH formed in the light and rose upon the increase in the light intensity up to 661 µmol photons m⁻² s⁻¹, where the quenching levels were saturated (Fig. 4B). The size of ΔpH formed in the light was not different between the WT and pgr5-1 plants at any light intensities. To monitor the H⁺ leakage from the thylakoid lumen via ATP synthase, we monitored the recovery kinetics of the 9-AA fluorescence in the dark in the presence of ADP. We did not detect any difference in the t₁/₂
value of the relaxation between the WT and pgr5-1 plants (Fig. 4C). We did not monitor any uncoupling of the thylakoid membrane in the pgr5-1 mutant.

DISCUSSION

Why is the $g_{H^+}$ parameter enhanced in the pgr5 mutants? The $g_{H^+}$ was compensated to the WT level by the introduction of Flv into the pgr5 mutant background (Fig. 2B). If PGR5 directly involved the down-regulation of ATP synthase activity and $H^+$ was unusually leaked through ATP synthase in the pgr5 mutant, how did the artificial Flv system complement the regulatory function of PGR5? It is unlikely that PGR5 directly interacts with ATP synthase to down-regulate the activity ($H^+$ conductivity). The 9-AA quenching analysis also did not suggest any uncoupling of the thylakoid membrane in ruptured chloroplasts (Fig. 4). The idea is consistent with several lines of evidence: 1) PGR5 was detected in the supercomplex for PSI CET in C. reinhardtii (Iwai et al., 2010; Johnson et al., 2014); 2) PGR5 and PGRL1 interact with PSI in Arabidopsis (DalCorso et al., 2008); 3) ferredoxin-dependent pmf formation and ATP synthesis were impaired in ruptured chloroplasts isolated from the pgr5 mutant plants (Wang et al., 2018); and 4) the variegated leaf phenotype of the immutans (im) mutant was alleviated by the pgr5 mutation, suggesting collaboration between PGR5 and plastid terminal oxidase (PTOX) to maintain the correct redox state of the plastoquinone pool during early chloroplast development (Okegawa et al., 2010). The Flv-dependent pseudo-CET likely adjusted $g_{H^+}$ to the WT level by enhancing the pmf level. The Y(I)/Y(II) represents the ratio of yields of both photosystems and remained at ~1 in the pgr5 mutant accumulating Flv, reflecting the absence of CET in the pgr5 mutant background (Yamamoto et al., 2016). However, Y(I) was double that of Y(II) in the WT plants, suggesting the efficient operation of PSI CET. To optimize the pmf level, PGR5 depends solely on PSI (CET), resulting in a larger Y(I) than Y(II); but Flv requires both photosystems (pseudo-CET).

The high $g_{H^+}$ phenotype of the pgr5 mutants may reflect the general response of ATP synthase to the reduced size of pmf. However, the size of $g_{H^+}$ was lower in the pgrl mutant than in the WT (Fig. 3B). The high $g_{H^+}$ is not simply explained by the response to low pmf. It is possible that $g_{H^+}$ was reduced because of the lower pmf size in the pgrl mutant. However, $g_{H^+}$ tended to be constant in the WT plants, although the size of pmf depended on the light intensity from low (33 µmol photons m^{-2} s^{-1}) to moderate (100 - 252 µmol photons m^{-2} s^{-1}) light intensities (Fig. 3). In the transgenic plants accumulating the mutant version (dpgr-type) of KEA3, $H^+$ was unusually leaked from the thylakoid membrane, resulting in reduced plant growth (Wang et al., 2019). It is probably necessary to maintain a certain level of pmf in

Downloaded on November 5, 2020. - Published by https://plantphysiol.org
Copyright (c) 2020 American Society of Plant Biologists. All rights reserved.
chloroplasts, and it would be dangerous to drive ATP synthase more when the size of pmf is low.

How can we explain the discrepancy in \( g_{H^+} \) observed in the \( pgr1 \) and \( pgr5 \) mutants? The question is related to what \( g_{H^+} \) indicates. The steady-state ECS signal peaking at 515 nm is contaminated with an absorbance at 505 nm caused by the synthesis of zeaxanthin and also by a qE-related 535-nm change (Johnson and Ruban, 2014). We have to be careful on the evaluation of the steady-state ECS parameters. We focused on the rapid decay kinetics of the ECS signal and observed the opposite response of \( g_{H^+} \) between the \( pgr1 \) and \( pgr5 \) mutants, both of which are defective in the qE induction (Fig. 3). Most probably, \( g_{H^+} \) mainly reflects the H\(^+\) conductivity of ATP synthase during steady-state photosynthesis (Kanazawa and Kramer, 2002). The idea is supported by an early study (Schönfeld and Neumann, 1977). But we observed that over-accumulation of the \( dpgr \)-type KEA3 also contributed to increased \( g_{H^+} \) (Wang et al., 2019).

Ion movement across the thylakoid membrane contributes to \( g_{H^+} \), although the extent depends on the conditions. In addition to the movement of H\(^+\) or ions, charge separation in both photosystems also induces the ECS signal. The post-illumination electron flux from plastocyanin (PC) to P700\(^+\) is used to estimate the rate of electron flow before cessation of illumination (Fan et al., 2016) and may affect the ECS decay. At high light intensities, Y(ND) was extremely low in the \( pgr5 \) mutant but was higher than that in the WT in the \( pgr1 \) mutant (Yamamoto and Shikanai, 2019). The result suggests the PC pool to be more reduced and more oxidized than in the WT in the \( pgr5 \) mutant and the \( pgr1 \) mutant, respectively. We are unsure whether the fast electron transport from PC to P700\(^+\) affects the ECS decay. The Q cycle in the Cyt \( b_{\gamma}f \) complex also continues until all the reducing equivalents available for plastoquinone reduction are consumed, and this is coupled with H\(^+\) translocation across the thylakoid membrane. Notably, the down-regulation of the Q cycle is more sensitive to lumenal acidification, and the plastoquinone pool is more reduced in the \( pgr1 \) mutant (Munekage et al., 2001; Jahns et al., 2002). The Q cycle may operate for a longer period in the dark in the \( pgr1 \) mutant, contributing to the lower \( g_{H^+} \). In the \( pgr5 \) mutant, however, the Q cycle may operate more efficiently in the dark because of the higher lumenal pH than in the WT and the \( pgr5 \) mutant accumulating Flv. Alternatively, absence of a main route for electron donation from ferredoxin to plastoquinone (PGR5-dependent PSI CET) may limit the electron pool consumed by the Q cycle in the dark. The Q cycle may be also restricted by the reduction of the PC pool because of the severe acceptor limitation from PSI in the \( pgr5 \) mutant (Takagi and Miyake, 2018). Any hypothesis must be consistent with the fact that the size of \( g_{H^+} \) in the \( pgr1 pgr5-1 \) and \( pgr1 pgr5-2 \) double mutants was between that of the single mutants. We are still unsure as to precisely why \( g_{H^+} \) was enhanced in the \( pgr5 \) mutant. Clearly, more research is needed for
characterizing the impact of the Q cycle on $g_{H^+}$. However, it is necessary to amend the $H^+$ leakage model of the pgr5 mutant simply depending on its high $g_{H^+}$ phenotype.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana (accession Columbia gll) WT, mutants and transgenic plants accumulating Physcomitrella patens Flvs in chloroplasts were grown in soil in a growth chamber (50 - 60 µmol photons m$^{-2}$ s$^{-1}$, 9-h photoperiod, 23 °C, 55% humidity) for 6 - 8 weeks after germination. Fully expanded leaves were used for the experiments.

In vivo measurements of the ECS signal

The ECS signal was monitored as an absorption change at 515 - 550 nm using a Dual-PAM 100 equipped with a P515/535 module (Walz). Detached leaves of plants adapted to the dark for 30 min were used for the analysis. The ECS signal was detected after 3-min illumination at different actinic light intensities (33, 100, 252 and 663 µmol photons m$^{-2}$ s$^{-1}$) using the same leaf and then, to record ECSt, the actinic light was turned off for 1 minute. ECSt represents the size of the light-induced $pmf$ and was estimated from the total amplitude of the rapid decay of the ECS signal in the dark, as described previously (Wang et al., 2015). ECSt levels were standardized against a 515-nm absorbance change induced by a single turnover flash (ECS$_{ST}$), as measured in dark-adapted leaves before recording. This normalization took account of variations in leaf thickness and the chloroplast density between the leaves (Takizawa et al., 2008). $g_{H^+}$ was estimated by fitting the first 300 ms of the decay curve with a first-order exponential decay kinetic as the inverse of the decay time constant (Avenson et al., 2005).

Measurement of 9-AA fluorescence in ruptured chloroplasts

Intact chloroplasts were prepared by homogenizing fresh leaves in ice cold medium (330 mM sorbitol, 5 mM MgCl$_2$, 10 mM Na$_4$P$_2$O$_7$, 20 mM D-isoascorbate, and 2.5 mM EDTA (pH = 6.5)) with a polytron. The homogenate was filtered through two layers of Miracloth. The filtrate was centrifuged for 3 min at 4,200 g. The chloroplast-enriched pellet was resuspended in a small volume of resuspension medium (330 mM sorbitol, 10 mM KCl, 5 mM MgCl$_2$, 2.5 mM EDTA, and 50 mM HEPES (pH = 7.6)) and kept on ice until use.

The measurement of 9-AA fluorescence was performed using the Dual-ENADPH and Dual-DNADPH modules (Walz) for the Dual-PAM 100. Isolated chloroplasts with 35 µg chlorophylls were added to 700 µL buffer (7 mM MgCl$_2$, 30 mM KCl, 2 mM KH$_2$PO$_4$, and 50 mM HEPES-KOH (pH 8.0)) in a reaction cuvette. After a 1-min incubation to osmotically burst...
chloroplasts, 700 μL double concentrated reaction buffer (14 mM MgCl₂, 60 mM KCl, 4 mM KH₂PO₄, 660 mM sorbitol, 100 mM HEPES-KOH (pH 8.0)), 100 μM methyl viologen, 2 mM ADP, 105 units of superoxide dismutase and 385 units of catalase were added to the reaction cuvette. Finally, 1.8 μM 9-AA was added to the reaction and the 9-AA fluorescence was monitored. Excitation was provided by 365 nm LEDs and fluorescence emission was detected between 420 and 580 nm. The ruptured chloroplasts were illuminated using four consecutive cycles of 2-min illumination (96, 236, 661 and 1143 μmol photons m⁻² s⁻¹) followed by 2-min dark recovery. The 9-AA fluorescence traces were normalized to the initial dark fluorescence levels at time 0.

The baseline drift of fluorescence was observed during the measurement as shown in Fig. 4A. After the baseline correction, 9-AA quenching upon AL illumination and recovery kinetics upon turning off AL was calculated. The rate constant (κ) for the dark recovery of 9-AA quenching was estimated by fitting the first 1-min recovery curve with first-order exponential rise kinetics. The half-life (t₁/₂) of the 9-AA fluorescence recovery was calculated as ln2/κ.

Accession Numbers

The sequence data from this article can be found in The Arabidopsis Information Resource database (https://www.arabidopsis.org/) under the following accession numbers: PGR5 (At2g05620) and PGR1/petC (At4g03280).

ACKNOWLEDGMENTS

We thank Shinji Masuda (Tokyo Institute of Technology) for the use of Dual-ENADPH and Dual-DNADPH modules.

FIGURE LEGENDS

Figure 1. Regulatory model of photosynthetic electron transport via ΔpH induced by operation of alternative electron transport. PSI CET and Flv-dependent pseudo-CET are indicated by red and blue arrows, respectively. In angiosperms, CET consists of PGR5/PGR1- and NDH-dependent pathways. Both protein complexes mediate the backflow of electrons from PSI to the plastoquinone (PQ) pool via ferredoxin (Fd) as an electron donor. CET contributes to the ΔpH formation across the thylakoid membrane via the Q-cycle in the Cyt b₆f complex. In Flv-dependent pseudo-CET, Flv directly reduces O₂ to water using photoreductant X (NADPH or Fd) from PSI. Pseudo-CET contributes to ΔpH formation via water oxidation in PSII and the Q-cycle. Lumenal acidification slows down plastoquinol oxidation at the Cyt b₆f complex to prevent excess electron flow toward PSI (photosynthetic control). Lumenal acidification also
induces qE quenching in the PSII antennae to discard excess photon energy as heat. The pmf composed of ΔpH and ΔΨ drives ATP synthesis via ATP synthase. The pgr5 mutants are defective in PGR5/PGRL1-dependent ΔpH formation. In the pgr1 mutant, photosynthetic control is more sensitive to lumenal acidification. In the H⁺ leakage model, PGR5 functions to down-regulate ATP synthase instead of PSI CET. PC represents plastocyanin.

**Figure 2.** Enhanced pseudo-CET by Flv suppresses the high gH⁺ phenotype in the pgr5 mutants. The light intensity-dependence of pmf formation (A) and gH⁺ (B) was monitored in the WT, pgr5-1, WT+35S;PpFlv no. 13, and pgr5-1+35S;PpFlv no. 13 (biological replicates n = 6 ± sd). Symbols with the same letters are not significantly different between genotypes at 252 and 663 µmol photons m⁻² s⁻¹ (Tukey-Kramer test, P < 0.05).

**Figure 3.** Enhanced photosynthetic control induced by the pgr1 mutation suppresses the high gH⁺ phenotype of the pgr5 mutants. The light intensity-dependence of pmf formation (A) and gH⁺ (B) was monitored in the WT, pgr1, mutants of the pgr5 alleles, and pgr1 pgr5 double mutants (biological replicates n = 8 - 10 ± sd). Symbols with the same letters are not significantly different between genotypes at 252 and 663 µmol photons m⁻² s⁻¹ (Tukey-Kramer test, P < 0.05).

**Figure 4.** Kinetics of 9-aminoacridine (9-AA) quenching in ruptured chloroplasts during the four consecutive cycles of illumination (2 min) followed by the dark recovery (2 min). Ruptured chloroplasts were illuminated with an actinic light using the Dual-ENADPH and Dual-DNADPH modules in the presence of 2 mM ADP and 100 µM methyl viologen as a terminal electron acceptor. (A) Representative 9-AA quenching traces measured on WT and pgr5-1 ruptured chloroplasts were normalized to the initial dark levels. Arrows indicate actinic light on/off cycles. The intensity of actinic light is indicated in white boxes in the top bar. (B) The light intensity-dependence of 9-AA quenching upon illumination. (C) Half-time (t₁/₂) of the 9-AA fluorescence recovery upon transition from illumination to darkness (C) were monitored in the WT and pgr5-1 ruptured chloroplasts (biological replicates n = 4 ± sd). In (B) and (C), there are no significant differences between WT and pgr5-1 plants at each light intensity (Welch’s t-test, P < 0.05).
LITERATURE CITED

Allahverdiyeva Y, Suorsa M, Tikkanen M, Aro E-M (2015) Photoprotection of photosystems in fluctuating light intensities. J Exp Bot 66: 2427–2436

Allen J (2002) Photosynthesis of ATP-electrons, proton pumps, rotors, and poise. Cell 110: 273–726

Arnon DI, Allen MB, Whatley FR (1954) Photosynthesis by isolated chloroplasts. Nature 174: 394–396

Avenson TJ, Cruz JA, Kanazawa A, Kramer DM (2005) Regulating the proton budget of higher plant photosynthesis. Proc Natl Acad Sci USA 102: 9709–9713

Bailleul B, Cardol P, Breyton C, Finazzi G (2010) Electrochromism: a useful probe to study algal photosynthesis. Photosynth Res 106: 179–189

DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schünemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. Cell 132: 273–285

Fan D-Y, Fitzpatrick D, Oguchi R, Ma W, Kou J, Chow WS (2016) Obstacles in the quantification of the cyclic electron flux around Photosystem I in leaves of C3 plants. Photosynth Res 129: 239–251

Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. Mol Cell 49: 511–523

Iwai M, Takizawa K, Tokutsu R, Okamura O, Takahashi Y, Minagawa J (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature 464: 1210–1213

Jahns P, Graf M, Munekage Y, Shikanai T (2002) Single point mutation in the Rieske iron-sulfur subunit of cytochrome b6/f leads to an altered pH dependence of plastoquinol oxidation in Arabidopsis. FEBS Lett 519: 99–102

Johnson GN (2005) Cyclic electron transport in C3 plants: fact or artefact? J Exp Bot 56: 407–416

Johnson MP, Ruban AV (2014) Rethinking the existence of a steady-state Δψ component of the proton motive force across plant thylakoid membranes. Photosynth Res 119: 233–242.

Johnson X, Steinbeck J, Dent RM, Takahashi H, Richaud P, Ozawa S, Houille-Vernes L, Petroutsos D, Rappaport F, Grossman AR, Niyogi KK, Hippler M, Alric J (2014) Proton gradient regulation 5-mediated cyclic electron flow under ATP- or redox-limited conditions: a study of ΔATPase pgr5 and ΔrbcL pgr5 mutants in the green alga Chlamydomonas reinhardtii. Plant Physiol 165: 438–452
Kanazawa A, Kramer DM (2002) In vivo modulation of nonphotochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase. Proc Natl Acad Sci USA 99: 12789–12794

Kono M, Noguchi K, Terashima I (2014) Roles of the cyclic electron flow around PSI (CEF-PSI) and O2-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in Arabidopsis thaliana. Plant Cell Physiol 55: 990–1004

Kou J, Takahashi S, Fan D-Y, Badger MR, Chow WS (2015) Partially dissecting the steady-state electron fluxes in Photosystem I in wild-type and pgr5 and ndh mutants of Arabidopsis. Front Plant Sci 6: 758

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 429: 579–582

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell 110: 361–371

Munekage Y, Takeda S, Endo T, Jahns P, Hashimoto T, Shikanai T (2001) Cytochrome b6f mutation specifically affects thermal dissipation of absorbed light energy in Arabidopsis. Plant J 28: 351–359

Nakano H, Yamamoto H, Shikanai T (2019) Contribution of NDH-dependent cyclic electron transport around photosystem I to the generation of proton motive force in the weak mutant allele of pgr5. Biochim Biophys Acta Bioenergetics 1860: 369–374

Nandha B, Finazzi G, Joliot P, Hald S and Johnson GN (2007) The role of PGR5 in the redox poising of photosynthetic electron transport. Biochim Biophys Acta Bioenergetics 1767: 1252-1259

Nishikawa Y, Yamamoto H, Okegawa Y, Wada S, Sato N, Taira Y, Sugimoto K, Makino A, Shikanai T (2012) PGR5-dependent cyclic electron transport around PSI contributes to the redox homeostasis in chloroplasts rather than CO2 fixation and biomass production in rice. Plant Cell Physiol 53: 2117–2126

Okegawa Y, Kobayashi Y, Shikanai T (2010) Physiological links among alternative electron transport pathways that reduce and oxidize plastoquinone in Arabidopsis. Plant J 63: 458–468

Peltier G, Aro E-M, Shikanai T (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. Annu Rev Plant Biol 67: 55–80
Rantala S, Lempääinen T, Gerotto C, Tiwari A, Aro EM, Tikkanen (2020) PGR5 and
NDH-1 systems do not function as protective electron acceptors but mitigate the
consequences of PSI inhibition. Biochim Biophys Acta Bioenerg 1861: 148154

Schönfeld M, Neumann J (1977) Proton conductance of the thylakoid membrane: Modulation
by light. FEBS Lett 73: 51–54

Shikanai T, Yamamoto H (2017) Contribution of cyclic and pseudo-cyclic electron transport
to the formation of proton motive force in chloroplasts. Mol Plant 10: 20–29

Sugimoto K, Okegawa Y, Tohri A, Long TA, Sarah FS, Hisabori T, Shikanai T (2013) A
single amino acid alteration in PGR5 confers resistance to antimycin A in cyclic electron
transport around PSI. Plant Cell Physiol 54: 1525–1534

Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzykowska M, Rantala M, Kangasjärvi S,
Paakkarinen V, Tikkanen M, Jansson S, Aro E-M (2012) PROTON GRADIENT
REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to
naturally and artificially fluctuating light conditions. Plant Cell 24: 2934–2948

Takagi D, Miyake C (2018) PROTON GRADIENT REGULATION 5 supports linear electron
flow to oxidize photosystem I. Physiol Plant 164: 337–348

Takizawa K, Kanazawa A, Kramer DM (2008) Depletion of stromal P_i induces high
“energy-dependent” antenna exciton quenching (q_E) by decreasing proton conductivity at
CF_o-CF_1 ATP synthase. Plant Cell Environ 31: 235–243

Vicente JB, Gomes CM, Wasserfallen A., Teixeira M (2002) Module fusion in an A-type
flavoprotein from the cyanobacterium Synechocystis condenses a multiple-component
pathway in a single polypeptide chain. Biochem Biophys Res Commun 294: 82–87

Wang C, Shikanai T (2019) Modification of activity of the thylakoid H+/K⁺ antiporter KEA3
disturbs ΔpH-dependent regulation of photosynthesis. Plant Physiol 181: 762–773

Wang C, Yamamoto H, Shikanai T (2015) Role of cyclic electron transport around
photosystem I in regulating proton motive force. Biochim Biophys Acta Bioenergetics 1847:
931–938

Wang C, Takahashi H, Shikanai T (2018) PROTON GRADIENT REGULATION 5
contributes to ferredoxin-dependent cyclic phosphorylation in ruptured chloroplasts.
Biochim Biophys Acta Bioenergetics 1859: 1173–1179

Yamamoto H, Shikanai T (2019) PGR5-dependent cyclic electron flow protects photosystem I
under fluctuating light at donor and acceptor sides. Plant Physiol 179: 588–600

Yamamoto H, Takahashi S, Badger MR, Shikanai T (2016) Artificial remodelling of
alternative electron flow by flavodiiron proteins in Arabidopsis. Nat Plants 2: 16012
Yamori W, Shikanai T (2016) Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. Annu Rev Plant Biol 67: 81–106
Figure 1. Regulatory model of photosynthetic electron transport via ΔpH induced by operation of alternative electron transport. PSI CET and Flv-dependent pseudo-CET are indicated by red and blue arrows, respectively. In angiosperms, CET consists of PGR5/PGRL1- and NDH-dependent pathways. Both protein complexes mediate the backflow of electrons from PSI to the plastoquinone (PQ) pool via ferredoxin (Fd) as an electron donor. CET contributes to the ΔpH formation across the thylakoid membrane via the Q-cycle in the Cyt b_{6}f complex. In Flv-dependent pseudo-CET, Flv directly reduces O_{2} to water using photoreductant X (NADPH or Fd) from PSI. Pseudo-CET contributes to ΔpH formation via water oxidation in PSII and the Q-cycle. Lumenal acidification slows down plastoquinol oxidation at the Cyt b_{6}f complex to prevent excess electron flow toward PSI (photosynthetic control). Lumenal acidification also induces qE quenching in the PSII antennae to discard excess photon energy as heat. The pmf composed of ΔpH and ΔΨ drives ATP synthesis via ATP synthase. The pgr5 mutants are defective in PGR5/PGRL1-dependent ΔpH formation. In the pgr1 mutant, photosynthetic control is more sensitive to lumenal acidification. In the H+ leakage model, PGR5 functions to down-regulate ATP synthase instead of PSI CET. PC represents plastocyanin.
Figure 2. Enhanced pseudo-CET by Flv suppresses the high $g_{H^+}$ phenotype in the pgr5 mutants. The light intensity-dependence of pmf formation (A) and $g_{H^+}$ (B) was monitored in the WT, pgr5-1, WT+35S;PpFlv no. 13, and pgr5-1+35S;PpFlv no. 13 (biological replicates $n = 6 \pm \text{sd}$). Symbols with the same letters are not significantly different between genotypes at 252 and 663 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Tukey-Kramer test, $P < 0.05$).
**Figure 3.** Enhanced photosynthetic control induced by the pgr1 mutation suppresses the high $g_{H}^{+}$ phenotype of the pgr5 mutants. The light intensity-dependence of $pmf$ formation (A) and $g_{H}^{+}$ (B) was monitored in the WT, pgr1, pgr5 alleles, and pgr1 pgr5 alleles (biological replicates $n = 8 - 10 \pm sd$). Symbols with the same letters are not significantly different between genotypes at 252 and 663 $\mu$mol photons m$^{-2}$ s$^{-1}$ (Tukey-Kramer test, $P < 0.05$).
Figure 4. Kinetics of 9-aminoacridine (9-AA) quenching in ruptured chloroplasts during the four consecutive cycles of illumination (2 min) followed by the dark recovery (2 min). Ruptured chloroplasts were illuminated with an actinic light using the Dual-ENADPH and Dual-DNADPH modules in the presence of 2 mM ADP and 100 μM methyl viologen as a terminal electron acceptor. (A) Representative 9-AA quenching traces measured on WT and pgr5-1 ruptured chloroplasts were normalized to the initial dark levels. Arrows indicate actinic light on/off cycles. The intensity of actinic light was indicated in white boxes in the top bar. (B) The light intensity-dependence of 9-AA quenching upon illumination. (C) Half-time ($t_{1/2}$) of the 9-AA fluorescence recovery upon transition from illumination to darkness (C) were monitored in the WT and pgr5-1 ruptured chloroplasts (biological replicates $n = 4 \pm \text{sd}$). In (B) and (C), there are no significant differences between WT and pgr5-1 plants at each light intensity (Welch’s t-test, $P < 0.05$).
Ahmed A, Suorsa M, Tikkanen M, Arro E-M (2015) Photoprotection of photosystems in fluctuating light intensities. J Exp Bot 66: 2427–2436

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Allen J (2002) Photosynthesis of ATP-electrons, proton pumps, rotors, and poise. Cell 110: 273–276

Pubmed: Author and Title
Google Scholar: Author Only

Arnon DI, Allen MB, Whatley FR (1954) Photosynthesis by isolated chloroplasts. Nature 174: 394–396

Pubmed: Author and Title
Google Scholar: Author Only

Avenson TJ, Cruz JA, Kanazawa A, Kramer DM (2005) Regulating the proton budget of higher plant photosynthesis. Proc Natl Acad Sci USA 102: 9709–9713

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bailleul B, Cardol P, Breyton C, Finazzi G (2010) Electrochromism: a useful probe to study algal photosynthesis. Photosynth Res 106: 179–189

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schünemann D, Finazzi G, Joliot P, Barbato R, Leister D (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. Cell 132: 273–285

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Fan D-Y, Fitzpatrick D, Oguchi R, Ma W, Kou J, Chow WS (2016) Obstacles in the quantification of the cyclic electron flux around Photosystem I in leaves of C3 plants. Photosynth Res 129: 239–251

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hertle A P, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U, Leister D (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. Mol Cell 49: 511–523

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Iwai M, Takizawa K, Tokutsu R, Okamura A, Takahashi Y, Minagawa J (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature 464: 1210–1213

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Jahns P, Graf M, Munekage Y, Shikanai T (2002) Single point mutation in the Rieske iron-sulfur subunit of cytochrome b6/f leads to an altered pH dependence of plastoquinol oxidation in Arabidopsis. FEBS Lett 519: 99–102

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Johnson GN (2005) Cyclic electron transport in C3 plants: fact or artefact? J Exp Bot 56: 407–416

Johnson MP, Ruban AV (2014) Rethinking the existence of a steady-state Δψ component of the proton motive force across plant thylakoid membranes. Photosynth Res 119: 233–242.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Johnson X, Steinbeck J, Dent RM, Takahashi H, Richaud P, Ozawa S, Houille-Vernes L, Petroutsos D, Rappaport F, Grossman AR, Niyogi KK, Hippler M, Alric J (2014) Proton gradient regulation 5-mediated cyclic electron flow under ATP- or redox-limited conditions: a study of ΔAATPase pgr5 and ΔrbcL pgr5 mutants in the green alga Chlamydomonas reinhardtii. Plant Physiol 165: 438–452

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kanazawa A, Kramer DM (2002) In vivo modulation of nonphotochemical exciton quenching (NPQ) by regulation of the chloroplast ATP synthase. Proc Natl Acad Sci USA 99: 12789–12794

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kono M, Noguchi K, Terashima I (2014) Roles of the cyclic electron flow around PSI (CEF, PSI), and O2-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in Arabidopsis thaliana. Plant Cell Physiol 55: 990–1004

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kou J, Takahashi S, Fan D-Y, Badger MR, Chow WS (2015) Partially dissecting the steady-state electron fluxes in Photosystem I in wild-
type and pgr5 and ndh mutants of Arabidopsis. Front Plant Sci 6: 758

Munekage Y, Hashimoto M, Miyake C, Tomizawa K, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 429: 579–582

Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell 110: 361–371

Munekage Y, Takeda S, Endo T, Jahns P, Hashimoto T, Shikanai T (2001) Cytochrome b6f mutation specifically affects thermal dissipation of absorbed light energy in Arabidopsis. Plant J 28: 351–359

Nakano H, Yamamoto H, Shikanai T (2019) Contribution of NDH-dependent cyclic electron transport around photosystem I to the generation of proton motive force in the weak mutant allele of pgr5. Biochim Biophys Acta Bioenergetics 1860: 369–374

Nandha B, Finazzi G, Joliot P, Hald S and Johnson GN (2007) The role of PGR5 in the redox poising of photosynthetic electron transport. Biochim Biophys Acta Bioenergetics 1767: 1252-1259

Nishikawa Y, Yamamoto H, Okegawa Y, Wada S, Sato N, Taira Y, Sugimoto K, Makino A, Shikanai T (2012) PGR5-dependent cyclic electron transport around PSI contributes to the redox homeostasis in chloroplasts rather than CO2 fixation and biomass production in rice. Plant Cell Physiol 53: 2117–2126

Okegawa Y, Kobayashi Y, Shikanai T (2010) Physiological links among alternative electron transport pathways that reduce and oxidize plastoquinone in Arabidopsis. Plant J 63: 458–468

Peltier G, Aro E-M, Shikanai T (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. Annu Rev Plant Biol 67: 55–80

Rantala S, Lempiäinen T, Gerotto C, Tiwari A, Aro EM, Tikkanen (2020) PGR5 and NDH-1 systems do not function as protective electron acceptors but mitigate the consequences of PSI inhibition. Biochim Biophys Acta Bioenerg 1861: 148154

Schönfeld M, Neumann J (1977) Proton conductance of the thylakoid membrane: Modulation by light. FEBS Lett 73: 51–54

Shikanai T, Yamamoto H (2017) Contribution of cyclic and pseudo-cyclic electron transport to the formation of proton motive force in chloroplasts. Mol Plant 10: 20–29

Sugimoto K, Okegawa Y, Tohri A, Long TA, Sarah FS, Hisabori T, Shikanai T (2013) A single amino acid alteration in PGR5 confers resistance to antimycin A in cyclic electron transport around PSI. Plant Cell Physiol 54: 1525–1534

Suorsa M, Järvi S, Grieco M, Nurmi M, Pietrzynowska M, Rantala M, Kangasjärvi S, Paakkarinen V, Tikkanen M, Jansson S, Aro E-M (2012) PROTON GRADIENT REGULATION5 is essential for proper acclimation of Arabidopsis photosystem I to naturally and artificially fluctuating light conditions. Plant Cell 24: 2934–2948

Takagi D, Miyake C (2018) PROTON GRADIENT REGULATION5 supports linear electron flow to oxidize photosystem I. Physiol Plant 164: 337–348
Takizawa K, Kanazawa A, Kramer DM (2008) Depletion of stromal Pi induces high "energy-dependent" antenna exciton quenching \( qE \) by decreasing proton conductivity at CFO-CF1 ATP synthase. Plant Cell Environ 31: 235–243

Vicente JB, Gomes CM, Wassfallen A, Teixeira M (2002) Module fusion in an A-type flavoprotein from the cyanobacterium Synechocystis condenses a multiple-component pathway in a single polypeptide chain. Biochem Biophys Res Commun 294: 82–87

Wang C, Shikanai T (2019) Modification of activity of the thylakoid H+/K+ antiporter KEA3 disturbs \( \Delta pH \)-dependent regulation of photosynthesis. Plant Physiol 181: 762–773

Wang C, Yamamoto H, Shikanai T (2015) Role of cyclic electron transport around photosystem I in regulating proton motive force. Biochim Biophys Acta Bioenergetics 1847: 931–938

Wang C, Takahashi H, Shikanai T (2018) PROTON GRADIENT REGULATION 5 contributes to ferredoxin-dependent cyclic phosphorylation in ruptured chloroplasts. Biochim Biophys Acta Bioenergetics 1859: 1173–1179

Yamamoto H, Shikanai T (2019) PGR5-dependent cyclic electron flow protects photosystem I under fluctuating light at donor and acceptor sides. Plant Physiol 179: 588–600

Yamori W, Shikanai T (2016) Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. Annu Rev Plant Biol 67: 81–106