



HAL
open science

A complex and dynamic redox network regulates oxygen reduction at photosystem I in *Arabidopsis*

Umama Hani, Belen Naranjo, Ginga Shimakawa, Christophe Espinasse,
Hélène Vanacker, Pierre Sétif, Eevi Rintamäki, Emmanuelle
Issakidis-Bourguet, Anja Krieger-Liszkay

► To cite this version:

Umama Hani, Belen Naranjo, Ginga Shimakawa, Christophe Espinasse, Hélène Vanacker, et al.. A complex and dynamic redox network regulates oxygen reduction at photosystem I in *Arabidopsis*. Plant Physiology, 2024, 2024, pp.kiae501. 10.1093/plphys/kiae501 . hal-04791971

HAL Id: hal-04791971

<https://hal.science/hal-04791971v1>

Submitted on 19 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

A complex and dynamic redox network regulates oxygen reduction at photosystem I in Arabidopsis

Umama Hani¹, Belen Naranjo², Ginga Shimakawa¹, Christophe Espinasse³, H el ene Vanacker³, Pierre S etif¹, Eevi Rintam aki⁴, Emmanuelle Issakidis-Bourguet^{3,*}, Anja Krieger-Liszkay^{1,*}

¹Universit  Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, 91198 Gif-sur-Yvette, France

²Instituto de Bioqu mica Vegetal y Fotos ntesis, Departamento de Bioqu mica Vegetal y Biolog a Molecular, Universidad de Sevilla, 41092 Sevilla, Spain

³Universit  Paris-Saclay, CNRS, INRAE, Universit  Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif-sur-Yvette, France

⁴ Molecular Plant Biology, Department of Life Technologies, University of Turku, 20014 Turku, Finland

*Corresponding authors: Anja Krieger-Liszkay (anja.liszkay@i2bc.paris-saclay.fr) and Emmanuelle Issakidis-Bourguet (emmanuelle.issakidis-bourguet@universite-paris-saclay.fr)

Short title: Regulation of oxygen reduction at photosystem I

One-sentence summary: A system of redox enzymes facilitates a series of reactions that regulate oxygen reduction at photosystem I in plants.

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 (<https://academic.oup.com/plphys/pages/General-Instructions>) is Anja Krieger-Liszkay.

Abstract

Thiol-dependent redox regulation of enzyme activities plays a central role in regulating photosynthesis. Beside the regulation of metabolic pathways, alternative electron transport is subjected to thiol-dependent regulation. We investigated the regulation of O₂ reduction at photosystem I. The level of O₂ reduction in leaves and isolated thylakoid membranes depends on the photoperiod in which plants are grown. We used a set of Arabidopsis (*Arabidopsis thaliana*) mutant plants affected in the stromal, membrane and lumenal thiol network to study the redox protein partners involved in regulating O₂ reduction. Light-dependent O₂ reduction was determined in leaves and in thylakoids of plants grown in short day and long day conditions using a spin-trapping electron paramagnetic resonance (EPR) assay. In wild type samples from short day conditions, reactive oxygen species (ROS) generation was double that of samples from long day conditions, while this difference was abolished in several redoxin mutants. An *in vitro* reconstitution assay showed that thioredoxin m, NADPH-dependent reductase C and NADPH are required for high O₂ reduction levels in thylakoids from plants grown in long day conditions. Using isolated photosystem I, we also showed that reduction of a photosystem I protein is responsible for the increase in O₂ reduction. Furthermore, differences in the membrane localization of m-type thioredoxins and 2-Cys peroxiredoxin were

detected between thylakoids of short day and long day plants. Overall, we propose a model of redox regulation of O_2 reduction according to the reduction power of the stroma and the ability of different thiol-containing proteins to form a network of redox interactions.

Introduction

Thiol-dependent redox regulation of enzyme activities play a central role in regulating photosynthesis. It has been well established that key enzymes of the Calvin-Benson-Basham cycle are redox-regulated via the thioredoxin (Trx) system (Buchanan, 2016). In the chloroplast Trxs are reduced in the light by ferredoxin in a reaction catalyzed by the Ferredoxin Thioredoxin Reductase (FTR). NADPH-dependent reductase C (NTRC), a protein with both a NADPH-thioredoxin reductase and a Trx domain, has also been found in chloroplasts (Serrato et al., 2004). NTRC has been shown to be involved in controlling the level of H₂O₂ via its interaction with 2-cys peroxiredoxin (2-Cys-PRX), it participates in redox regulation of a key enzyme in starch biosynthesis (Michalska et al., 2009; Lepistö et al., 2013), of enzymes of the chlorophyll biosynthesis pathway (Richter et al., 2013; Perez-Ruiz et al., 2014) and in regulating the activity of the chloroplast ATP-synthase (Naranjo et al., 2016; Carrillo et al., 2016; Nikkanen et al., 2016; for a recent review see Cejudo et al., 2019). A severe growth inhibition phenotype has been reported for a mutant lacking NTRC and Trx f1 (Thormählen et al., 2015) and in the triple mutant *ntrc-trxf1f2* and the double mutant *ntrc-trxx* (Ojeda et al., 2017a). Both, *ntrc-trxf1f2* and *ntrc-trxx* mutants showed a high mortality at the seedling stage (Ojeda et al., 2017b) indicating that NTRC is important for chloroplast redox regulation, for controlling the redox state of several thioredoxins and showing that both redox regulation systems, FTR and the NTRC, are linked via 2-Cys-Prxs (Perez-Ruiz et al., 2017). Interaction between NTRC and Trx m1, Trx m3 and Trx y1 have been shown by bifluorescence complementation assays in vivo (Nikkanen et al., 2016). Trx m4 has been suggested to regulate negatively cyclic electron flow around photosystem I (PSI) (Courteille et al., 2013). Recently, evidence has been provided that m-type thioredoxins form a complex with PROTON GRADIENT REGULATION 5-LIKE 1 (PGRL1), a protein that is supposed to participate together with PGR5 in cyclic electron flow (Okegawa and Motohasi, 2020; Wolf et al., 2020). Trx m1, Trx m2 and Trx m4 have also been reported to be implicated in the biogenesis of PSII (Wang et al., 2013), showing a broad implication of m-type thioredoxins in the photosynthetic light reactions. Compared to the other isoforms, Trx m3 seems not to be relevant for controlling these processes. A mutant of Trx m3, the less abundant protein of the m-type thioredoxins in chloroplast stroma (Okegawa and Motohasi, 2015), shows unaltered chloroplast performance (Benítez-Alfonso et al., 2009). *Nicotiana tabacum* plants overexpressing Trx m were shown to be impaired in photosynthesis but being more tolerant to oxidative stress conditions (Rey et al., 2013). Furthermore, Trx m1, Trx m2 and NTRC have been shown to be indispensable for acclimation of photosynthesis under fluctuating light conditions (Thormählen et al., 2017). Taken together, these reports provide experimental evidence for an important role played by NTRC and Trx m isoforms for the rapid acclimation of plants to changes in the light regime, for controlling alternative photosynthetic electron flow and for coping with oxidative stress. Another important protein in the thiol-based chloroplast redox regulatory network is 2-Cys PRX, the most abundant peroxiredoxin in the chloroplast (Muthuramalingam et al., 2009). 2-Cys PRXs are reduced by NTRC and by Trxs and detoxify H₂O₂. Most importantly, they also act as a system to reoxidize reduced thioredoxins (Telman et al., 2020).

In the thylakoid lumen, candidates for controlling the redox state of protein disulphide bridges are the LUMEN THIOL OXIDOREDUCTASE 1 (LTO1) and the atypical cytochrome *c*_{6A} (Marceida et al., 2006). The LTO1 protein is a transmembrane protein carrying a C-terminal thioredoxin-like domain typical of oxidoreductases belonging to the protein disulphide isomerase family. The *lto1* mutant has been shown previously to be affected in the assembly of active PSII while PSI electron transport was

unaltered upon excitation with far-red light (Karamoko et al., 2011). Since the cysteine residues of LTO1 are at the lumen side of the thylakoid membrane, the redox state of the stroma has to be transmitted to these cysteines. Possible candidates are the transmembrane proteins CCDA and HCF 164 (Karamoko et al., 2013; Kang and Wang, 2016; Motohasi and Hisabori, 2010).

There is a strong link between the level of reactive oxygen species (ROS) and the thiol system. Superoxide anion radicals ($O_2^{\bullet-}$) are mainly generated by the photosynthetic electron transport at photosystem I (PSI) by the classical “Mehler reaction” or pseudocyclic electron flow. It has been reported that leaves of *Arabidopsis thaliana* plants grown under short day conditions (SD, 8 h light, 16 h dark) generate the double amount of superoxide compared with plants grown under long day conditions (LD, 16 h light, 8 h dark) (Michelet and Krieger-Liszkay, 2012). This extra electron transport in SD plants is used to generate a higher proton gradient and more ATP than found in thylakoids from LD plants. In the presence of an uncoupler, the difference in ROS generation was abolished between the two different thylakoid preparations. Addition of NADPH but not of NADH increased the level of ROS generation in LD thylakoids to the same amount as observed in SD thylakoids. Addition of NADPH to SD thylakoids had no important effect. In thylakoids from plants lacking NTRC the ROS production was like in SD wild type (wt) thylakoids and the difference between SD and LD thylakoids was abolished (Lepistö et al., 2013). These results point to a redox regulation of ROS generation at the level of PSI.

It remains an open question whether NTRC interacts with a protein of PSI at the thylakoid membrane, whether a Trx is involved in the redox regulation of ROS generation at PSI and how the redox state of the stroma is transmitted to the thylakoid lumen. In this study we aimed to establish the interaction between different players of the chloroplast thioredoxin network and their ability to alter the capacity of O_2 reduction at the PSI acceptor side. We measured light-dependent ROS generation on leaves and isolated thylakoids of *A. thaliana* grown under SD or LD conditions in wt, in single mutants: *ntrc*, *trxm4*, *ccda*, *lto1*, *cyt c_{6A}*, in double mutants: *trxm1trxm2* and *2cpab*, and in plants overexpressing NTRC (oeNTRC). The sub-chloroplast localization of NTRC and Trx m was studied using immunoblots. In vitro reconstitution experiments were performed using thylakoids and purified recombinant Trx m and/or NTRC protein in order to directly test their effect on light-induced ROS generation.

Results

Leaves and thylakoid membranes isolated from *A. thaliana* wild-type (wt) plants grown in short day (SD) conditions generate in the light about twice the amount of ROS compared to those from plants grown in long day (LD) conditions (Fig. 1) as has been previously reported (Michelet and Krieger-Liszkay, 2012). ROS production was measured using an indirect spin-trapping assay. In this assay hydroxyl radicals are detected which derive from superoxide anion radicals and hydrogen peroxide in a Haber-Weiss reaction catalyzed by Fe(II) (Ramos et al., 1992). In the light, the Mehler reaction generates superoxide that dismutates to hydrogen peroxide. Using the spin trap 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEMPO) that forms specific and distinguishable adducts with superoxide and with hydroxyl radicals, we have shown previously that it is indeed the level of superoxide that is twice higher in thylakoids isolated from SD plants compared with those from LD plants (Michelet and Krieger-Liszkay, 2012). A comparison between ROS production in leaf disks and isolated thylakoids shows that the same differences in ROS production were found in both types of samples (Fig. 1B, C). This demonstrates that in the light the majority of superoxide/hydrogen peroxide is generated by the photosynthetic electron transport chain. Mutants affected in Trx m isoforms or in NTRC lost the difference between SD and LD. The single mutant *trxm4* and the double mutant *trxm1m2* generated similar amounts of ROS like LD plants, while *ntrc* generated high amounts of ROS like SD plants, independently of the photoperiod during their growth. Overexpression of NTRC increased the difference between SD and LD compared to wt. In a similar manner the mutant devoid of 2-Cys PRX A and B showed an overall increase in the ROS production, however, the difference between SD and LD was maintained in *2cpab* (Fig. 1). There was no significant difference in protein levels of Trxm isoforms, NTRC and 2-Cys PRX in leaf extracts from wild type grown in SD or LD conditions (Sup. Fig. 1)

Furthermore, we found that 2-Cys PRX was slightly more abundant in leaf protein samples from plants grown in LD than in SD when prepared in presence of SDS but not in absence of detergent (Fig. 2A and B), suggesting that 2-Cys PRX is mainly stromal and the difference between SD and LD samples would be attributable to a differential association to thylakoid membranes. Indeed, thylakoids from LD grown plants showed a marked higher amount of 2-Cys PRX (recovered from thylakoid membranes in the presence of SDS) compared to SD, confirming that the difference of global (stromal plus thylakoid-associated) abundance in leaf extracts between photoperiods was attributable to the fraction of membrane-bound protein. In LD, the protein was present mainly in its dimeric form, most probably corresponding to the oxidized form as evidenced by the shift of the signal from an apparent mass of about 40 kDa to about 20 kDa produced by reduction with DTT giving the monomeric form. In PSI preparations, we could not immune-detect 2-Cys PRX (Fig. 2C) suggesting that the protein was not directly associated to PSI complexes, or lost upon sample preparation due to a loosening of the association to the photosystem. This latter possibility is strongly suggested by the detection of a faint signal in the supernatant of LD thylakoids resuspended in buffer devoid of detergent.

To see whether the localization of Trx m and/or NTRC is altered in plants grown in the two different light regimes, immunoblots were performed using leaf extracts and isolated thylakoids. As shown in Fig. 3, no difference in the total amount of Trx m4 and NTRC was found in leaf extracts. However, the attachment of Trx m differed in SD and LD thylakoids. Trx m4 was found in the thylakoid fraction in SD thylakoids but not in LD thylakoids. A similar membrane localization like for Trx m4 was observed for Trx m2 (Sup. Fig. 2).

Attachment of Trx m seems to be required for allowing electron transport to oxygen at PSI. To test whether the lack of membrane-associated Trx was indeed responsible for the lower ROS generation in LD thylakoids, we reconstituted LD thylakoids with purified Trx m4 and/or NTRC proteins in the presence of NADPH (Fig. 4). Addition of NADPH alone stimulated slightly the ROS production in LD thylakoids. A further increase in signal size was observed when Trxm4 or NTRC were added together with NADPH. However, these differences were statistically not significant. When Trxm4 was added together with NADPH and NTRC, LD thylakoids generated three-fold more ROS than without any protein addition. In the absence of NADPH, addition of TRxm4 and NTRC had no effect. The different additives had only a small effect on SD thylakoids. This result points to a redox regulation of O₂ reduction at the level of PSI. To investigate whether there is a direct effect of thiol reduction on the O₂-reduction capacity of PSI, we incubated isolated PSI with the reducing agent TCEP and followed light-dependent O₂ consumption using an O₂ electrode. As shown in Fig. 5, O₂ consumption was two times higher in the presence of TCEP. These data show that it is redox regulation of PSI itself that is crucial for the level of O₂ reduction at the acceptor side of PSI.

The question arises which subunit of PSI may be redox-regulated. Several subunits of PSI contain cysteines, however most of them are ligands of the iron-sulphur clusters F_x, F_A and F_B and can therefore be excluded as candidates undergoing reversible regulatory redox modifications. In addition, there are two proteins, PsaN and PsaF that may be candidates for disulfide bridge formation. It has been shown previously that the PSI subunit PsaN contains four cysteine residues that can form two disulfide bridges (Motohashi and Hisabori, 2006). PsaF contains at its luminal site 2 cysteine residues that are close enough to form a disulfide bridge. PsaF is a transmembrane protein that forms the docking site for plastocyanin at the donor side of PSI and at the acceptor side its C-terminus is in direct neighborhood with PsaE. PsaE forms together with PsaC and PsaD the docking site for ferredoxin. O₂ reduction is supposed to take place at this site. Since PsaF is the most likely candidate for redox regulation, and we did not observe any significant quantitative difference of this protein when comparing SD and LD thylakoid and PSI protein preparations (Fig. 2), we performed redox immunoblot assays to explore the influence of light regime on its redox state. Thylakoid membranes were treated with 4'-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid (AMS) that reacts with thiol groups (alkylation reaction), we separated the proteins by SDS-PAGE and analysed the apparent mass of PsaF by immunodetection. Supplementary Fig. 3 shows that two bands were detected when the thylakoids had been chemically reduced with tris(2-carboxyethyl) phosphine prior to the AMS treatment. This indicates that PsaF exists in an oxidized form with cysteines potentially forming a disulfide bridge and, upon addition of a reductant, in a reduced form with AMS accessible thiols. However, without exogenous reductant, in isolated thylakoids from both SD and LD plants only the oxidized form was found, probably due to spontaneous oxidation during membrane sample preparation. Redox immunoblots were performed on the mutants used for detection of the ROS levels in Fig. 1. In leaf extracts prepared in presence of SDS and m-PEG-mal (Fig. 6, Sup. Figs 3, 4) PsaF was immuno-detected as two well distinguishable redox variants that could be quantified by densitometry. We found that relative abundance of PsaF oxidized and reduced forms strongly varied between light and dark samples. In LD, PsaF was reduced equally in all genotypes with a reduction percentage ranging from 24.6 to 32.4 % in the dark, and from 1.5 to 2.9 % in the light. However, we did not observe significant differences in the redox state of PsaF in the two light regimes or between the different mutant lines, with the exception of *ntrc* where PsaF was significantly more reduced in SD (39.7 % in the dark and 8.4 % in the light) than in LD (24.6 % in the dark and 2.4 % in the light), and in comparison to wt (28.4 % in the dark and 2.2 % in the light) and the other mutants analyzed.

However, there was no correspondance with the ROS levels found in *ntrc*. We concluded that the reduction of PsaF may be not stable enough to catch it during the alkylation treatment procedure.

The redox state of the stroma has to be transmitted to the thylakoid lumen to be able to act on thiol/disulphide groups of PsaF. The transmembrane proteins CCDA and HCF164 are likely candidates for the transmission of the redox state from the stromal site to the luminal site of the thylakoid membrane (Motohashi and Hisabori, 2010). LTO1 (Karamoko et al., 2011) and the atypical cytochrome c_{6A} (Marceida et al., 2006) may act as candidates for redox modifications inside the lumen. Fig. 7 shows that the mutants of CCDA (*ccda3* and *ccda4*) and of LTO1 have indeed lost the difference in superoxide production between SD and LD, while lack of cyt c_{6A} has no effect.

Discussion

Redox regulation of linear photosynthetic electron transport and alternative pathways has been reported previously (Johnson, 2003). Courteille and coworkers (2013) showed that cyclic electron transport involving the NDH complex was altered in Trx m4 mutants, Nikkanen et al. (2018) reported the involvement of NTRC in the control of NDH complex-dependent and Naranjo et al. (2021) in PGR5-dependent cyclic flow. Both alternative electron transport pathways, cyclic and pseudocyclic flow, are in competition and down-regulation of cyclic flow is therefore supposed to stimulate pseudocyclic flow. We have shown previously (Michelet and Krieger-Liszkay, 2012) that O_2 reduction at PSI is higher in SD thylakoids than in LD thylakoids and that this difference is abolished in the presence of uncouplers. This observation points to a pH- or redox-regulated process taking place at the luminal side of the thylakoid membrane and controlling O_2 reduction at PSI. Indeed, O_2 reduction of isolated PSI is stimulated by the thiol-reducing agent TCEP (Fig. 5). At low light intensities, such as those used for the measurements shown in Fig. 5, O_2 is reduced by the terminal electron acceptors, the 4Fe4S clusters F_A and F_B , while at higher light intensities it is reduced by the acceptor A_1 , a phylloquinone (Kozuleva et al., 2021). PsaN and PsaF are the only constitutive protein subunits of PSI containing cysteine residues that can form a disulphide bridge. In the structure published by Pan et al. (2018) for maize (*Zea mays*) PSI, PsaN forms close contacts with the N-terminal extension of PsaF. In a spin-trapping EPR experiment on leaves of the *psan* mutant, the same difference between SD and LD was observed as in wt, ruling out PsaN as a candidate for the redox regulation of O_2 reduction (Sup. Fig. 5). The most likely candidate for the redox modification affecting O_2 reduction is PsaF for the following reasons: 1. PsaF was found in PSI x-ray structures from pea either in the reduced state (Mazor et al., 2015) or with a disulfide bond (Qin et al., 2015), and 2. PsaF has been identified by a proteomics approach as a redox-affected protein (Ströher and Dietz, 2008). PsaF is a transmembrane protein, and we hypothesize here that, upon a modification of the redox state of its cysteines, a long-range structural change affects the neighboring subunit PsaE and thereby the ferredoxin docking site at the PSI acceptor side. Accordingly, O_2 reduction is favored when the disulfide bridge in PsaF is reduced while Fd reduction becomes less efficient. The subunit PsaE seems to be crucial for O_2 reduction at PSI in Arabidopsis (Krieger-Liszkay et al., 2020). Unfortunately, we were not able to detect significant differences in the reduction level of PsaF in the two light regimes and in the mutant lines analyzed for which distinct O_2 reduction levels were clearly found (Fig. 1).

We also addressed the question how the redox state of PsaF is controlled in the lumen. The *Lto1* mutant has lost the difference between SD and LD (Fig. 7), implying a role of this protein in redox regulation of PSI. The difference between SD and LD is also lost in the mutants *ccda3* and

ccda4 (Fig. 7), demonstrating the importance of CCDA in transmitting the stromal redox state to the lumen *in vivo*. The CCDA and LTO1 mutants show high ROS levels independent of the growth photoperiod. We hypothesize that LTO1 keeps PsaF oxidized under LD conditions, while CCDA keeps LTO1 oxidized, leading to low O₂ reduction in LD. In SD conditions, where the redox state of CCDA and LTO1 is highly reduced, the situation changes. Under these circumstances, reduction of PsaF seems feasible (Fig. 8).

Trx m has been shown to be the electron donor to CCDA *in vitro* (Motohashi and Hisabori, 2010). We propose here that Trx m association to the thylakoid membrane is required for an efficient electron donation to CCDA and onwards to redox regulated proteins in the thylakoid lumen, reduction of PsaF and increase in O₂ reduction (Fig. 7). Trx m association to the membrane in SD conditions (Fig. 3 and Sup. Fig. 2) may be facilitated by the higher proton motive force generated in thylakoids from SD plants (Michelet and Krieger-Liszkay, 2012). Such a mechanism has been shown to play a role for the attachment of the plastid terminal oxidase to the thylakoid membrane (Bolte et al., 2020). Future experiments using leaves harvested towards the end of the dark period compared with leaves harvested from plants exposed to high light or using leaves infiltrated with uncouplers could answer whether Trx m association is controlled by the proton motive force.

The question arises how to integrate 2-Cys-PRX and NTRC into this model. According to a previous report (Bohrer et al., 2012) and our measurements of DTNB reduction (Sup. Fig. 6), NTRC is not able to reduce m-type Trxs in a direct manner when tested at physiologically relevant concentrations. Furthermore, 2-Cys-PRXs are mainly reduced by NTRC and act as an oxidizing system towards reduced Trxs (Nikkanen et al., 2016; Perez-Ruiz et al., 2017; Telman et al., 2020) as well as they are responsible for H₂O₂ detoxification in the stroma (König et al., 2002). We suggest that membrane association of 2-Cys-PRX in LD is important to keep CCDA oxidized. As shown in Fig. 2, 2-Cys PRX is associated to the membrane only in LD conditions, and it is found mostly in its dimeric form. H₂O₂ detoxification may be more efficient if 2-Cys PRX is attached to the membrane close to the site of superoxide production and conversion into H₂O₂ by membrane-associated SOD. In the absence of NTRC or 2-Cys PRX, however, the redox state of the chloroplast is strongly disturbed, which complicates the interpretation of the experiments with these mutants (Fig. 1).

In conclusion, our hypothesis for the redox regulation of pseudocyclic electron flow is based on three mechanisms: 1. The availability of electron acceptors other than O₂ for photosynthetic electron transport, 2. Redox regulation of the different players according to the redox state of the stroma and the capabilities of the different players to form a network of redox-driven interactions and 3. The reversible membrane attachment of Trx m and 2-Cys-PRX that may depend on changes in pH and ion concentration controlled by the proton motive force. Attachment of Trx m seems to be necessary to achieve a high reduction state of CCDA and PsaF in SD resulting in high O₂-reduction levels at PSI, while detachment of Trx m and attachment of 2-Cys-PRX in LD seems to favor oxidation of CCDA, while PsaF is oxidized by LTO1, resulting in low levels of O₂-reduction. However, the connection of redox regulation of PsaF *in vivo* with the photoperiod remains to be demonstrated. Besides redox regulation at the PSI complex itself, other redox-regulated proteins are likely to play a role in regulating the partitioning between linear, cyclic flow and the Mehler reaction. Trxm has been shown to regulate negatively cyclic flow (Courteille et al. 2013), and LTO1 may also play a more indirect role by inhibiting cyclic electron flow via the NDH complex (P. Hamel, personal communication).

Under SD conditions, plants show an alteration in the partitioning of assimilated CO₂ in soluble sugars and starch. An upregulation of the Mehler reaction may help the plants to counteract the reduced

hours for performing photosynthesis. Thanks to the increased Mehler reaction SD thylakoids build up a larger proton gradient and generate more ATP at a given time and light intensity compared with LD thylakoids (Michelet and Krieger-Liszkay, 2012) showing the physiological importance of a redox control of the Mehler reaction. A higher photosynthetic rate, and a redistribution of photosynthates between soluble sugars and starch may be advantageous to generate the necessary reserve for the prolonged dark period. Furthermore, higher ROS levels in SD may play an important role as signaling compounds affecting the expression of ROS-responsive genes (Dietz et al., 2016).

Reduction of O₂ in pseudocyclic electron flow is in competition with cyclic electron flow. Both pathways lead to the formation of a proton gradient without generating NADPH. According to Takahashi et al. (2013), the redox state of the chloroplast controls the formation of supercomplexes composed of PSI, Cyt *b₆f*, LHCs, PGRL1 and FNR. Such supercomplexes are thought to be required for cyclic electron flow (Iwai et al., 2010). The formation of a complex between reduced Trx *m* and PGRL1 may inhibit cyclic electron flow by preventing the supercomplex formation required for cyclic flow. This suggestion is supported by the recent reports on the interaction between Trx *m* and PGRL1 (Wolf et al., 2020; Okegawa and Motohashi, 2020). As shown recently, Trx *x* and Trx *y* play an important role in the acceptor-side regulation of PSI and protection of PSI against photoinhibition under fluctuating light conditions (Okegawa et al., 2023). Future work on the extent of cyclic flow and superoxide production in mutants of the different components of the Trx system will show if both, pseudocyclic and cyclic flow are controlled by the same proteins but in the opposite way. Furthermore, it would be interesting to investigate redoxin mutants under fluctuating light condition to demonstrate the importance of the Mehler reaction under abiotic stress conditions. It has been shown previously that growth of *ntrc* is highly susceptible to fluctuating light conditions (Thormählen et al., 2017).

Materials and Methods

Plant Material

Arabidopsis (*Arabidopsis thaliana*) wt (Col-0) and mutants were grown for 6 weeks in soil either under short day conditions (8 h continuous white light - 160 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$, 21°C/16 h dark, 18°C) or long day conditions (16 h continuous white light - 160 $\mu\text{mol quanta m}^{-2}\text{s}^{-1}$, 21°C /8 h dark, 18°C). All mutants and over-expressing plants used were already described in previous studies: the *trxm4* T-DNA mutant (Courteille et al., 2007) and the *trxm1m2* mutant (Thormählen et al., 2017); the T-DNA insertion mutant of NTRC (*ntrc*) (Lepistö et al., 2009) and transgenic plants overexpressing wild type NTRC protein in *ntrc* background (Toivola et al., 2013); the double T-DNA mutant lacking the two 2-Cys Prxs, A and B (*2cpab*) (Ojeda et al., 2018); the T-DNA mutants lacking CCDA isoforms (Page et al., 2004); the T-DNA mutant lacking Cytcochrome *c_{6A}* (Pesaresi et al., 2009).

Extraction of proteins from leaves

Arabidopsis shoots were harvested 1 h after onset of light (light samples) or 1h before onset of light (dark samples) and ground in liquid nitrogen before homogenization in lysis buffer. The lysis buffer contained 100 mM Tris-HCl pH 6.8, 2% (w/v) sodium dodecyl sulphate (SDS), 20 mM EDTA and protease inhibitor cocktail (for plant cell and tissue extracts, 1 X final concentration, Sigma-Aldrich, St. Louis, MI, USA).

Extraction of thylakoids from A. thaliana

Young fully expanded leaves were ground in 0.33 M sorbitol, 50 mM KCl, 10 mM EDTA, 1 mM MgCl₂, 25 mM Mes pH 6.1. After centrifugation, the pellet was washed twice with 0.33 M sorbitol, 60 mM KCl, 2 mM EDTA, 1 mM MgCl₂, 25 mM HEPES pH 6.7. After centrifugation, the pellet was resuspended in 0.3 M sorbitol, 50 mM KCl, 1 mM MgCl₂, 20 mM HEPES pH 7.6. This procedure was repeated once, and the pellet was resuspended to a final concentration of about 1 mg of chlorophyll (chl) per ml of thylakoids. All centrifugations were performed at 3,000xg for 3 min at 4°C.

Isolation of Photosystem I

Isolated thylakoids Solubilization buffer (10 mM HEPES pH 7.5) containing 1.2% (w/v) beta-dodecyl maltoside (β -DM) was added to the thylakoid suspension (final chl concentration: 0.5 mg ml⁻¹, β -DM: 0.6% (w/v)). The sample was mixed for 1 min, followed by centrifugation to remove unsolubilized membranes. The supernatant was then loaded onto sucrose gradients (0.1-1 M sucrose, 10 mM HEPES pH 7.5, 0.03% (w/v) β -DM) and centrifuged for 145 min at 57,000 rpm using a fixed angle rotor (70.1 Ti). The lower band containing PSI was collected, frozen in liquid nitrogen and stored at -80°C.

Room-temperature spin-trapping EPR Measurements

Thylakoid membranes generate superoxide in the light, which dismutates either spontaneously or catalyzed by SOD to hydrogen peroxide. To detect O₂^{•-}/H₂O₂-derived hydroxyl radicals an assay with the spin trap α -(4-pyridyl-1-oxide)-N-tert-butyl nitron (4-POBN, Sigma-Aldrich) was used. Spin-trapping assays were carried out using leaf disks or freshly isolated thylakoid membranes at a concentration of 20 μ g Chl ml⁻¹. Leaf disks were vacuum-infiltrated with the buffer containing the spin trap reagents, 50 mM 4-POBN, 4% (v/v) ethanol, 50 μ M Fe-EDTA, prior to the illumination and then floating on the same buffer during illumination. Samples were illuminated for a given time with white light (200 μ mol quanta m⁻² s⁻¹ in case of leaf disks and 500 μ mol quanta m⁻² s⁻¹ in case of thylakoids in the presence of 50 mM 4-POBN, 4% (v/v) ethanol, 50 μ M Fe-EDTA, and buffer (25 mM HEPES, pH 7.5, 5 mM MgCl₂, 0.3 M sorbitol). When indicated, 200 μ M NADPH, 0.3 μ M Trxm4 and 0.3 μ M NTRC were added to the assay before starting the illumination.

EPR spectra were recorded at room temperature in a quartz flat cell using an ESP-300 X-band (9.73 GHz) spectrometer (Bruker, Rheinstetten, Germany). The following parameters were used: microwave frequency 9.73 GHz, modulation frequency 100 kHz, modulation amplitude: 1G, microwave power: 6.3 milliwatt in 4-POBN assays, receiver gain: 2x10⁴, time constant: 40.96 ms; number of scans: 4.

O₂ consumption

Measurements of O₂-consumption were performed in a Liquid-Phase Oxygen Electrode Chamber (Hansatech Instruments, Norfolk, England) using isolated PSI (10 μ g Chl ml⁻¹) in Tricine 20 mM pH 8.0, in the presence of 5 mM MgCl₂, 30 mM NaCl and 5 mM ascorbate, 30 μ M 2,6-dichlorophenolindophenol (DCPIP) as exogenous electron donors to P700⁺.

SDS-PAGE and Immunoblotting

SDS-PAGE was performed using 8% or 4-20 % (w/v) polyacrylamide gels. Proteins were blotted onto a nitrocellulose or a PVDF membrane. Labelling of the membranes with polyclonal antibodies, produced in the lab (anti-Trxm; anti-NTRC; anti-2-Cys PRX) or commercially available (PsaF and β -subunit of ATP synthase; Agrisera, Vännäs, Sweden), was carried out at room temperature in 50 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% (v/v) Tween-20 and 5% (w/v) non-fat milk powder. After washing, bound antibodies were revealed with a peroxidase-conjugated secondary anti-rabbit antibody (Agrisera, Vännäs, Sweden) and visualized by enhanced chemiluminescence.

Redox state of PsaF

Total leaf protein samples were prepared as described above with 2 mM AMS (4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid, ThermoFisher Scientific, MW 536.44 Da) or mPEG-mal(24) (MM(PEG)24 Methyl-PEG-Maleimide, ThermoFisher Scientific, MW 1239.44 Da) added to the extraction buffer. 50 μ g protein samples were precipitated (addition of 7 vol. cold ethanol, overnight incubation at -20°C), after spinning (11,000 g 10 min at RT) protein pellets were dried and resuspended in non-reducing electrophoresis loading buffer. After SDS-PAGE (4-20% (w/v) polyacrylamide gel), PsaF was immunodetected (PVDF membrane / Chemiluminescence) using a Chemidoc ISV0025 (BioRad) and signals corresponding to reduced (pegylated) and oxidized forms were quantified using ImageLab software (BioRad).

Statistical analyses

All statistical analyses were conducted as indicated in the figure legends using Tukey or Student's t-test. The number of replicates are given in the legends. A difference was considered significant when its adjusted P-value was <0.05 .

Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers_.

Supplementary Data

Supplementary Figure S1. Immunoblot analysis of NTRC, Trx-m and 2-Cys Prx proteins under long- and short-day conditions.

Supplementary Figure S2. Attachment of Trx m2 and m4 to thylakoid membranes. Supplementary Figure S3. PsaF immunodetection in thylakoid membranes.

Supplementary Figure S4. Immunodetection and redox state of PsaF in leaf extracts.

Supplementary Figure S5. Light-induced hydroxyl radical formation in SD and LD leaf disks of *psan*.

Supplementary Figure S6. 6 Reduction tests of Trxs m by NTRC *in vitro*.

Funding

This work was supported by the Labex Saclay Plant Sciences-SPS (ANR-17-EUR-0007) and the platform of Biophysics of the I2BC supported by the French Infrastructure for Integrated Structural Biology (FRISBI; grant number ANR-10-INSB-05) and the Infrastructures en Biologie Santé et Agronomie (IBISA). U.H. is supported by a CNRS PhD fellowship. B. N. acknowledges funding by MCIN/AEI/10.13039/501100011033, grant IJC2019-040972-I and E. Rintamäki by the Jane and Aatos Erkkö Foundation (Finland).

Acknowledgements

We would like to thank Patrice Hamel (Ohio State University, USA) for scientific discussions and for sending us the seeds of the *lto1* mutants.

Author Contributions

E.I-B. and A.K-L. designed the project. U.H., B.N., G.S., C.E., H.V., E.I-B. and A.K-L. performed the experiments and analysed the data. P.S. and E.R. participated in discussions. A.K-L. wrote the initial version of the manuscript that was read and revised by all authors.

Figure Legends

Figure 1

Light-induced hydroxyl radical formation in SD and LD leaf disks and washed thylakoid membranes in wild type and redoxin mutants. Generation of hydroxyl radicals originating from $O_2^{\cdot-}/H_2O_2$ was detected by spin trapping with 4-POBN. A: Typical EPR spectra of the 4-POBN/ α -hydroxyethyl adduct are shown. After infiltration with assay medium containing 4-POBN/ethanol/FeEDTA, leaf disks were incubated in the same medium for 30 min in light ($200 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$) before detection of the radicals in the medium. Relative EPR intensities in leaves (B) and thylakoid membranes (C). Thylakoid membranes ($20 \mu\text{g ml}^{-1}$) were illuminated for 2 min ($500 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$) in the presence of the spin trapping assay before detection of the radical. Grey bars: SD, white bars: LD. All EPR signals were normalized to the signal of SD thylakoids without addition (100%). Mean values with SE are shown ($n \geq 6$, biological replicates; *, $P < 0.05$ (comparison between SD and LD growth conditions for each genotype) according to Tukey test.

Figure 2

Association of 2-Cys PRX with thylakoid membranes depends on the photoperiod. Immuno-detection of 2-Cys PRX in (A) soluble leaf extracts ($50 \mu\text{g protein per well}$), (B) thylakoid membranes ($5 \mu\text{g chl per well}$) and (C) PSI complexes ($0.5 \mu\text{g chl per well}$) prepared from plants grown in short day (SD) and long day (LD) conditions. Immuno-detection of chloroplastic NADP-malate dehydrogenase and PsaF were taken as controls as stromal and membrane-associated protein, respectively. Coomassie staining of the blot (input) was taken for gel loading control. For thylakoid and PSI membrane samples, SDS (2% (w/v)) and DTT (10 mM) treatments were performed for 10 min at RT. After centrifugation, the supernatant was collected and analysed. All samples were supplemented with non-reducing loading blue prior heat treatment and gel loading.

Figure 3

Membrane association of Trx m and 2-Cys PRX. Immuno-detection of NTRC and Trx m4 in leaf extracts and thylakoid membranes prepared from plants grown in short day (SD) and long day (LD) conditions. Samples corresponding to 5 μg chlorophyll were loaded in each well. The results shown are representative of three biologically independent experiments.

Figure 4

Reconstitution of ROS generation in LD thylakoids by Trx m, NTRC and NADPH. Light-induced hydroxyl radical formation in SD and LD thylakoids is shown by spin trapping with 4-POBN. Thylakoid membranes ($20 \mu\text{g Chl ml}^{-1}$) were illuminated for 2 min ($500 \mu\text{mol quanta m}^{-2}\text{s}^{-1}$) before detection of the radical. When indicated, 200 μM NADPH, 0.3 μM Trx m4 and 0.3 μM NTRC were added to the assay before starting the illumination. All EPR signals were normalized to the signal of SD thylakoids without addition (100%). Grey bars: SD, white bars: LD. Mean values with SE are shown ($n=3$; *, $P < 0.05$ (comparison with LD no protein added) according to the Tukey test.

Figure 5

Dependence of O_2 consumption by isolated PSI on the redox state. O_2 consumption was measured with an O_2 electrode using DCPIP/ascorbate as electron donor. Samples were illuminated with green actinic light. When indicated, isolated PSI had been incubated with 1 mM TCEP for 15 min prior to the measurement. Mean values with SE are shown ($n=4$).

Figure 6

Redox state of PsaF in dark- and light-adapted plants. Total leaf protein extracts from WT (Col-0) and redoxins (*ntrc*, *trxm4*, *trxm1m2* and *2cpab*) mutant plants grown in short day (SD) or long day (LD) conditions were prepared in the presence of the thiol alkylating agent mPEG-maleimide as described in methods. After SDS-PAGE, proteins (50 μg per well) were electro-transferred onto a PVDF membrane for PsaF immuno-detection (chemi-luminescence). See Sup. Figs. 3 and 4 for details about the quantification method used and an example of redox immunoblot signals. (A) and (B) PsaF redox state in leaves in the dark and in the light, respectively. Values correspond to the mean of 5-6 experiments. Error bars correspond to standard deviation. * and # designate significant differences between mutant and wt genotypes, and between SD and LD (for each genotype), respectively; according to Student's t-test, $P < 0.05$.

Figure 7

Light-induced hydroxyl radical formation in SD and LD leaf disks of mutants affected in LTO1, CCDA (*ccda3* and *ccda4*) and Cyt c_{6A} . Generation of hydroxyl radicals originating from $\text{O}_2^{\bullet-}/\text{H}_2\text{O}_2$ was detected in leaves by spin trapping with 4-POBN. Grey bars: SD, white bars: LD. All EPR signals were normalized to the signal of SD thylakoids without addition (100%). Mean values with SE are shown ($n=8$, 2-3 biological replicates; *, $P < 0.05$ (comparison between growth conditions for each genotype) according to Tukey test.

Figure 8

Model of redox regulation of O_2 reduction at photosystem I. Red arrows indicate also photosynthetic electron transport. Black arrow shows strong reduction of O_2 , while the dashed black arrows shows weak reduction of O_2 . Reduced cysteine residues in PsaF favor higher O_2 reduction

activity. The redox state of PsaF is modified by two thiol modulating redox systems. The first one is required for reduction of the disulfide bond (red arrows), the second one for their oxidation (blue arrows). Trx m is a central player for controlling the reduction state of PsaF. Trx m is reduced by FTR. Under SD conditions, Trx m associates to the thylakoid membrane and reduces CCDA which reduces via HCF164 finally PsaF. In LD conditions, reduced PsaF is oxidized by LTO1, which itself is oxidized by CCDA (blue arrows. 2-Cys-PRX attaches to the membrane, allowing the oxidation of CCDA. In addition, it can oxidize Trx m (blue arrows).

Data availability

Data will be made available on demand.

References

Benitez-Alfonso Y, Cilia M, San RA, Thomas C, Maule A, Hearn S, Jackson D (2009) Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 3615–3620.

Bolte S, Marcon E, Jaunario M, Moyet L, Paternostre M, Kuntz M, Krieger-Liszka A (2020) Dynamics of the localization of the plastid terminal oxidase inside the chloroplast. *J Exp Bot.* **71**: 2661-2669.

Buchanan BB (2016) The path to thioredoxin and redox regulation in chloroplasts. *Annu. Rev. Plant Biol.* **67**: 1–24.

Carrillo LR, Froehlich JE, Cruz JA, Savage LJ, Kramer DM (2016) Multi-level regulation of the chloroplast ATP synthase: the chloroplast NADPH thioredoxin reductase C (NTRC) is required for redox modulation specifically under low irradiance. *Plant J.* **87**: 654–663.

Cejudo FJ, Ojeda V, Delgado-Requerey V, González M, Pérez-Ruiz JM (2019) Chloroplast Redox Regulatory Mechanisms in Plant Adaptation to Light and Darkness. *Front Plant Sci.* **10**: 380.

Courteille A, Vesa S, Sanz-Barrio R, Cazale AC, Becuwe-Linka N, Farran I, Havaux M, Rey P, Rumeau D (2013) Thioredoxin m4 controls photosynthetic alternative electron pathways in Arabidopsis. *Plant Physiol.* **161**: 508–520.

Iwai M, Takizawa K, Tokutsu R, Okamuro A, Takahashi Y, Minagawa J (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. *Nature* **464**: 1210-3.

Johnson GN (2003) Thiol regulation of the thylakoid electron transport chain--a missing link in the regulation of photosynthesis? *Biochemistry* **42**: 3040-4.

Kang ZH, Wang GX (2016) Redox regulation in the thylakoid lumen. *J. Plant Physiol.* **192**: 28–37.

Karamoko M, Cline S, Redding K, Ruiz N, Hamel PP (2011) Lumen Thiol Oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in Arabidopsis. *Plant Cell* **23**: 4462-75.

Karamoko M, Gabilly ST, Hamel PP (2013) Operation of trans-thylakoidthiol-metabolizing pathways in photosynthesis. *Front. Plant Sci.* **4**: 476.

König J, Baier M, Horling F, Kahmann U, Harris G, Schürmann P, Dietz KJ (2002) The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. *Proc Natl Acad Sci U S A* **99**: 5738-43.

Krieger-Liszkay A, Shimakawa G, Sétif P (2020) Role of the two PsaE isoforms on O₂ reduction at photosystem I in Arabidopsis thaliana. *Biochim Biophys Acta Bioenerg.* **1861**: 148089

Lepistö A, Kangasjärvi S, Luomala EM, Brader G, Sipari N, Keränen M, Keinänen M, Rintamäki E (2009) Chloroplast NADPH-thioredoxin reductase interacts with photoperiodic development in Arabidopsis. *Plant Physiol.* **149**: 1261-76.

Lepistö A, Pakula E, Toivola J, Krieger-Liszkay A, Vignols F, Rintamäki E (2013) Deletion of chloroplast NADPH-dependent thioredoxin reductase results in inability to regulate starch synthesis and causes stunted growth under short-day photoperiods. *J. Exp. Bot.* **64**: 3843–3854.

Marcaida MJ, Schlarb-Ridley BG, Worrall JA, Wastl J, Evans TJ, Bendall DS, Luisi BF, Howe CJ (2006) Structure of cytochrome c6A, a novel dithio-cytochrome of Arabidopsis thaliana, and its reactivity with plastocyanin: implications for function. *J Mol Biol* **360**: 968-77.

Mazor Y, Borovikova A, Nelson N (2015) The structure of plant photosystem I super-complex at 2.8 Å resolution. *Elife*: 4:e07433. doi: 10.7554/eLife.07433

Michalska J, Zauber H, Buchanan BB, Cejudo FJ, Geigenberger P (2009) NTRC links built-in thioredoxin to light and sucrose in regulating starch synthesis in chloroplasts and amyloplasts. *Proc. Natl. Acad. Sci. U. S. A.* **106**: 9908–9913.

Michelet L, Krieger-Liszkay A (2012) Reactive oxygen intermediates produced by photosynthetic electron transport are enhanced in short-day grown plants. *Biochim Biophys Acta* **1817**: 1306-13.

Motohashi K, Hisabori T (2006) HCF164 receives reducing equivalents from stromal thioredoxin across the thylakoid membrane and mediates reduction of target proteins in the thylakoid lumen. *J. Biol. Chem.* **281**: 35039–35047.

Motohashi K, Hisabori T (2010) CcdA is a thylakoid membrane protein required for the transfer of reducing equivalents from stroma to thylakoid lumen in the higher plant chloroplast. *Antioxid. Redox Signal.* **13**: 1169–1176.

Muthuramalingam M, Seidel T, Laxa M, Nunes de Miranda SM, Gärtner F, Ströher E, Kandlbinder A, Dietz KJ (2009) Multiple redox and non-redox interactions define 2-Cys peroxiredoxin as a regulatory hub in the chloroplast. *Mol Plant* **2**: 1273-88.

- Naranjo, B, Mignee C, Krieger-Liszky A, Hornero-Mendez D, Gallardo-Guerrero L, Cejudo FJ, Lindahl, M (2016)** The chloroplast NADPH thioredoxin reductase C, NTRC, controls non-photochemical quenching of light energy and photosynthetic electron transport in Arabidopsis. *Plant Cell Environ.* **39**: 804–822.
- Naranjo B, Penzler J-F, Rühle T, Leister D (2021)** NTRC Effects on Non-Photochemical Quenching Depends on PGR5. *Antioxidants* **10**:900.
- Nikkanen L, Toivola J, Rintamäki E (2016)** Crosstalk between chloroplast thioredoxin systems in regulation of photosynthesis. *Plant Cell Environ.* **39**: 1691–1705.
- Nikkanen L, Toivola J, Trotta A, Diaz MG, Tikkanen M, Aro EM, Rintamäki E (2018)** Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system. *Plant Direct.* 2: e00093. doi: 10.1002/pld3.93.
- Ojeda V, Perez-Ruiz JM, Gonzalez M, Najera VA, Sahrawy M, Serrato AJ, Geigenberger P, Cejudo FJ (2017a)** NADPH thioredoxin reductase C and thioredoxins act concertedly in seedling development. *Plant Physiol.* **174**: 1436–1448.
- Ojeda V, Perez-Ruiz JM, Cejudo FJ (2018)** 2-Cys peroxiredoxins participate in the oxidation of chloroplast enzymes in the dark. *Mol. Plant* **11**: 1377–1388.
- Ojeda V, Pérez-Ruiz JM, González M, Nájera VA, Sahrawy M, Serrato AJ, Geigenberger P, Cejudo FJ (2017b)** NADPH Thioredoxin Reductase C and Thioredoxins Act Concertedly in Seedling Development. *Plant Physiol* **174**: 1436-1448.
- Okegawa Y, Motohashi K (2015)** Chloroplastic thioredoxin m functions as a major regulator of Calvin cycle enzymes during photosynthesis in vivo. *Plant J* **84**: 900-13.
- Okegawa Y, Motohashi K (2020)** M-Type Thioredoxins Regulate the PGR5/PGRL1-Dependent Pathway by Forming a Disulfide-Linked Complex with PGRL1. *Plant Cell* **32**: 3866-3883.
- Okegawa Y, Sato N, Nakakura R, Murai R, Sakamoto W, Motohashi K (2023)** x- and y-type thioredoxins maintain redox homeostasis on photosystem I acceptor side under fluctuating light. *Plant Physiol* :kiad466. doi: 10.1093/plphys/kiad466.
- Page ML, Hamel PP, Gabilly ST, Zegzouti H, Perea JV, Alonso JM, Ecker JR, Theg SM, Christensen SK, Merchant S (2004)** A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b6f complex in Arabidopsis chloroplasts. *J Biol Chem.* **279**: 32474-82.
- Pan X, Ma J, Su X, Cao P, Chang W, Liu Z, Zhang X, Li M (2018)** Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II. *Science* **360**: 1109-1113.
- Pesaresi P, Scharfenberg M, Weigel M, Granlund I, Schröder WP, Finazzi G, Rappaport F, Masiero S, Furini A, Jahns P, Leister D (2009)** Mutants, overexpressors, and interactors of Arabidopsis plastocyanin isoforms: revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. *Mol Plant* **2**: 236-48.

Perez-Ruiz JM, Guinea M, Puerto-Galan L, Cejudo FJ (2014) NADPH thioredoxin reductase C is involved in redox regulation of the Mg-chelatase I subunit in *Arabidopsis thaliana* chloroplasts. *Mol. Plant* **7**: 1252–1255.

Perez-Ruiz JM, Naranjo B, Ojeda V, Guinea M, Cejudo FJ (2017) NTRC-dependent redox balance of 2-Cys peroxiredoxins is needed for optimal function of the photosynthetic apparatus. *Proc. Natl. Acad. Sci. U. S. A.* **114**: 12069–12074.

Qin X, Suga M, Kuang T, Shen JR (2015) Photosynthesis. Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. *Science* **348**: 989-95.

Ramos CL, Pou S, Britigan BE, Cohen MS, Rosen GM (1992) Spin trapping evidence for myeloperoxidase-dependent hydroxyl radical formation by human neutrophils and monocytes. *The Journal of Biological Chemistry* **267**: 8307–8312.

Rey P, Sanz-Barrio R, Innocenti G, Ksas B, Courteille A, Rumeau D, Issakidis-Bourguet E, Farran I (2013) Overexpression of plastidial thioredoxins f and m differentially alters photosynthetic activity and response to oxidative stress in tobacco plants. *Front Plant Sci* **4**: 390.

Richter AS, Peter E, Rothbart M, Schlicke H, Toivola J, Rintamaki E, Grimm B (2013) Posttranslational influence of NADPH-dependent thioredoxin reductase C on enzymes in tetrapyrrole synthesis. *Plant Physiol.* **162**: 63–73.

Serrato AJ, Perez-Ruiz JM, Spinola MC, Cejudo FJ (2004) A novel NADPH thioredoxin reductase, localized in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in *Arabidopsis thaliana*. *J. Biol. Chem.* **279**: 43821–43827.

Ströher E, Dietz KJ (2008) The dynamic thiol-disulphide redox proteome of the *Arabidopsis thaliana* chloroplast as revealed by differential electrophoretic mobility. *Physiol Plant.* **133**: 566-83

Takahashi H, Clowez S, Wollman FA, Vallon O, Rappaport F (2013) Cyclic electron flow is redox-controlled but independent of state transition. *Nat. Commun.* **4**: 1954.

Telman W, Liebthal M, Dietz KJ (2020) Redox regulation by peroxiredoxins is linked to their thioredoxin-dependent oxidase function, *Photosynth. Res.* **145**: 31–41.

Thormählen, Meitzel T, Groysman J, Ochsner AB, von Roepenack-Lahaye E, Naranjo B, Cejudo FJ, Geigenberger P (2015) Thioredoxin f1 and NADPH-dependent thioredoxin reductase C have overlapping functions in regulating photosynthetic metabolism and plant growth in response to varying light conditions. *Plant Physiol* **169**: 1766–1786.

Thormählen I, Zupok A, Rescher J, Leger J, Weissenberger S, Groysman J, Orwat A, Chatel-Innocenti G, Issakidis-Bourguet E, Armbruster U, Geigenberger P (2017) Thioredoxins play a crucial role in dynamic acclimation of photosynthesis in fluctuating light. *Mol. Plant* **10**: 168-182.

Vaseghi MJ, Chibani K, Telman W, Liebthal MF, Gerken M, Schnitzer H, Mueller SM, Dietz KJ (2018) The chloroplast 2-cysteine peroxiredoxin functions as thioredoxin oxidase in redox regulation of chloroplast metabolism. *ELife* **7**: e38194. DOI: <https://doi.org/10.7554/eLife.3819>

Wang, P, Liu J, Liu B, Feng D, Da Q, Wang P, Shu S, Su J, Zhang Y, Wang J, Wang HB (2013) Evidence for a role of chloroplastic m-type thioredoxins in the biogenesis of photosystem II in Arabidopsis. *Plant Physiol.* **163**: 1710–1728.

Wolf BC, Isaacson T, Tiwari V, Dangoor I, Mufkadi S, Danon A (2020) Redox regulation of PGRL1 at the onset of low light intensity. *Plant J* **103**: 715-725