Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

Review Article

Chloroplast thioredoxin systems dynamically regulate photosynthesis in plants

Lauri Nikkanen and Eevi Rintamäki

Molecular Plant Biology, Department of Biochemistry, University of Turku, FI-20014 Turku, Finland

Correspondence: Eevi Rintamäki (evirin@utu.fi)

Photosynthesis is a highly regulated process in photoautotrophic cells. The main goal of the regulation is to keep the basic photosynthetic reactions, i.e. capturing light energy, conversion into chemical energy and production of carbohydrates, in balance. The rationale behind the evolution of strong regulation mechanisms is to keep photosynthesis functional under all conditions encountered by sessile plants during their lifetimes. The regulatory mechanisms may, however, also impair photosynthetic efficiency by overriding the photosynthetic reactions in controlled environments like crop fields or bioreactors, where light energy could be used for production of sugars instead of dissipation as heat and down-regulation of carbon fixation. The plant chloroplast has a high number of regulatory proteins called thioredoxins (TRX), which control the function of chloroplasts from biogenesis and assembly of chloroplast machinery to light and carbon fixation reactions as well as photoprotective mechanisms. Here, we review the current knowledge of regulation of photosynthesis by chloroplast TRXs and assess the prospect of improving plant photosynthetic efficiency by modification of chloroplast thioredoxin systems.

Introduction

In nature, light intensity is constantly changing in plant growth habitats, including both seasonal alteration of daily light period and daily fluctuation of light intensity due to cloudiness and other environmental factors. Optimization of photosynthetic production under fluctuating light conditions needs balancing of photosynthetic reactions by induction of regulatory mechanisms. The photosynthetic reactions also produce highly energetic intermediates that are dangerous to cellular infrastructure and function, if they are not consumed/processed correctly by photoprotective mechanisms. The mechanisms to balance light capture and consumption of light energy and to induce protective machinery against oxidative stress include non-photochemical quenching (NPQ), photosynthetic control of electron flow between Photosystem II (PSII) and I (PSI), state transitions (ST), cyclic electron flow (CEF), light activation of photosynthetic enzymes in carbon fixation reactions (Calvin–Benson–Bassham cycle, CBB), and induction of antioxidant systems. Recently, the regulatory proteins called thioredoxins (TRX) have been suggested to control many of these mechanisms balancing photosynthetic reactions in chloroplasts.

A covalent and reversible post-translational modification of thiols in side-chains of cysteine residues (Cys) is a common and evolutionarily ancient mechanism to regulate the structure, interactions, or stability and function of proteins. The most common thiol modification is the reversible formation and cleavage of disulfide bonds between redox-active Cys in proteins. The latter reaction is catalyzed by small dithiol:disulfide oxidoreductases called TRXs that are present in all extant lineages of organisms [1]. The classical TRXs contain a redox-active cysteine pair in a highly conserved amino acid motif WCG/PPC [2,3]. The reaction mechanism of TRXs includes a nucleophilic attack on one of the cysteines in a target protein by the catalytic cysteine in the TRX redox-active motif. This attack forms

Received: 19 December 2018
Revised: 28 March 2019
Accepted: 29 March 2019

Version of Record published:
15 April 2019

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

a mixed disulfide between TRX and target protein. Subsequently, the second, resolving cysteine in the TRX reduces the mixed intermolecular disulfide resulting in the release of reduced target protein(s) and oxidized TRX [4,5]. Oxidized TRX is reactivated through reduction by a specific enzyme called thioredoxin reductase (TR). A TRX and a corresponding TR constitute a thioredoxin system.

Two TRX systems exist in plant chloroplasts with different sources of reducing power. Ferredoxin-thioredoxin reductase (FTR) is involved in the ferredoxin-thioredoxin (Fd-TRX) system and activates the classical chloroplast TRXs by receiving reducing equivalents from photosynthetically reduced ferredoxin [6,7]. Plant chloroplasts contain multiple types of TRXs: two f, four m, and two y isoforms as well as x- and z-type of TRX, and several thioredoxin-like proteins (see recent reviews [3,8,9]). In addition to the Fd-TRX system, chloroplast has the NADPH-dependent chloroplast thioredoxin reductase (NTRC) that is reduced by NADPH [10]. NADPH is produced photosynthetically by light reactions but also in the oxidative pentose phosphate pathway (OPPP) that enables the NTRC system to be active also in darkness and under low irradiance [11,12]. Apart from the diverse content of TRXs, the proteomic studies have expanded the range of TRX-regulated plastid processes [13–17]. Besides the early discovery of the activation of the carbon fixation enzymes, TRXs are also now known to control chloroplast biogenesis, plastid transcription, ATP synthesis, photoprotective mechanisms, carbon metabolism beyond the primary photosynthetic reactions, biosynthesis of starch and chlorophyll, nitrogen and sulfur metabolism, the shikimate and OPPP pathways, as well as oxidative stress responses [8,18–20].

In this review, we concentrate on the novel discoveries of TRX-dependent redox-regulation of photosynthetic reactions, both electron transfer and carbon fixation, and metabolism of reactive oxygen species (ROS) strictly related to photosynthesis. We propose that NTRC is an important redox regulator of photosynthesis during the inductive period of dark–light and low–high light transitions and under light intensities that are lower than what plants are acclimated during the growth (e.g. transient shading of leaves in the plant canopy). NTRC is also involved in balancing chloroplast redox poise by being the primary reductant of 2-Cys peroxiredoxins (2-Cys Prx), which scavenge H_2O_2 in chloroplasts. Control under the Fd-TRX system prevails under constant illumination of growth and higher light intensities.

Chloroplast thioredoxin systems

The Fd-TRX system was originally identified and characterized in the 1970s by Bob Buchanan and co-workers (reviewed by ref. [7]). FTR is a heterodimeric enzyme that consists of a catalytic subunit (FTRc), which includes an iron–sulfur cluster [4Fe–4S] and a redox-active motif that mediates electron transfer from Fd to TRXs, as well as a variable subunit (FTRv) [21]. Two isoforms of FTRv exist in Arabidopsis, but their functional significance is unknown [22]. The abundance of all TRXs and FTR is low in comparison with their target proteins [23]. From the TRXs activated by FTR, TRXm1, m2, m4, and TRXf1 are the highest expressed isoforms in leaves [23,24]. TRXx and TRXy2 are expressed at slightly lower levels [24]. The f2, m3, and y1 isoforms as well as TRXz show very low expression in photosynthetic tissues [24].

The Fd-TRX system is essential for plant development and growth, as knockout-mutations of FTRc are lethal, and virus-induced gene silencing (VIGS) of the *FTRB* gene coding for FTRc causes a severe chlorotic phenotype [25] (Table 1). Yet, however, a low FTRc content is enough to maintain a healthy wild-type (WT) phenotype [26]. Oppositely, knockout (KO) mutants of single FTR-dependent TRXs do not have visible phenotypes, and simultaneous mutation of several isoforms is needed to suppress the redundancy of m-type or f-type TRXs [27–30].

NTRC forms a complete TRX system in a single polypeptide. NTRC consists of an N-terminal thioredoxin reductase domain (NTRd) with binding sites for NADPH and two flavin adenine dinucleotides (FAD), and of a C-terminal TRX domain (TRXd) [10,11]. The expression of the *NTRC* gene in leaf cells is lower than that of the catalytic subunit of FTR [24]. NTRC functions as a homodimer, where the NTRd of one monomer reduces a disulfide in the TRXd of the other monomer [31–33]. The corresponding site on the TRXd of NTRC facing to NTRd is strongly positively charged, facilitating electrostatic interactions with the oppositely charged surface of the NTRd [33]. Among chloroplast TRXs, the surface charge of the TRXf isoforms most closely resembles the surface charge of TRXd of NTRC [33]. Accordingly, NTRC interacted with TRXf1 in bimolecular fluorescence complementation (BiFC) assays and overexpression of the *NTRC* gene in Arabidopsis enhanced the amount of active TRXf in chloroplasts [34], suggesting that NTRC can donate electrons to TRXf. Additionally, it was recently proposed that NTRC can activate TRXz [26].

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

Table 1 Phenotypes of transgenic lines used to study specificity and cross-talk of the NTRC and Fd-TRX systems in the regulation of photosynthesis

Line (genetic background)	Sp.	Protein(s) affected	Phenotype	References
ntrc (WT)	At	NTRC (KO)	Severe impairment of growth and Chl content, high NPQ in low light, impaired reduction in chloroplast enzymes	[10,11,35]
VIGS-*FTRb*	At	FTRc (KD)	Impaired chloroplast development	[25]
ftrb (WT)	At	FTRc (KD)	Slight impairment of growth	[26]
ftra1 or *ftra2* (WT-Ws)	At	FTRv1 (KO) FTRv2 (KO)	Increased sensitivity to oxidative stress	[22]
trxf1 (WT)	At	TRXf1 (KO)	No visible phenotype	[40]
trxf1f2 (WT)	At	TRXf1, TRXf2 (KO)	Slight impairment of growth in short day	[29,119]
trxm1.1 (WT)	At	TRXm1 (KO)	No visible phenotype, decreased activation of NADP+-MDH	[30]
trxm2.1 (WT)	At	TRXm2 (KO)	No visible phenotype	[30]
trxm4 (WT)	At	TRXm4 (KO)	Increased NDH-dependent CEF	[46]
trxz (WT)	At	TRXz (KO)	Impaired plastid transcription	[58]
trxm1m2 (WT)	At	TRXm1,m2 (KO)	No visible phenotype, but improved photosynthetic efficiency in low light phases of fluctuating light	[30]
VIGS-*TRXm2m4/m1* (WT or *ntrc*)	At	TRXm1,2, and 4 (KD)	Impaired leaf development, high NPQ More severe phenotype at *ntrc* background	[27,41]
ntrc trxf1 (WT)	At	NTRC, TRXx (KO)	Very severe impairment of growth and reduction in chloroplast enzymes	[28]
ntrc trxx (WT)	At	NTRC, TRXx (KO)	More severe phenotype than in *ntrc*	[42]
ntrc npq4 (WT)	At	NTRC, PsbS (KO)	Partial recovery of *ntrc* phenotype (lower NPQ)	[69]
ntrc Δ2cp (WT)	At	NTRC (KO), 2-Cys Prx A and B (KD)	Partial recovery of *ntrc* phenotype	[79]
OE-NTRC (*ntrc*)	At	NTRC (OE)	Enhanced leaf growth, increased reductive activation of chloroplast enzymes, increased carbon fixation, decreased NPQ, increased CEF and *pmf*	[12,33,34,70]
OE-NTRC (*ndho*)	At	NTRC (OE) NdhO (KO)	No increase in NDH-dependent CEF in the absence of NDH	[12]
OE-$NTRC_{SAIS}$ (*ntrc*)	At	OE of NTRC with inactive NTRd	Partial recovery of *ntrc* phenotype, but exacerbated impairment of reduction in chloroplast enzymes	[33,34]
OE-$NTRC_{SGPS}$ (*ntrc*)	At	OE of NTRC with inactive TRXd	Partial recovery of *ntrc* phenotype, but exacerbated impairment of reduction in chloroplast enzymes	[33,34]
OE-NTRC (WT)	At	NTRC (OE)	Improved stress tolerance	[44]
OE-NTRC (WT)	At	NTRC (OE)	Reduced growth of rosettes	[43]
OE-TRXm (WT)	Nt	TRXfm (OE)	Inhibition of NDH-dependent CEF	[46]

Abbreviations: At, *Arabidopsis thaliana*; Nt, *Nicotiana tabacum*; OE, overexpression; KD, knockdown; KO, knockout.

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

The *ntrc* KO line of Arabidopsis has a chlorotic and stunted phenotype, which is particularly severe under short photoperiods [11,35,36]. The *ntrc* chloroplasts are morphologically heterogeneous with size variation and a variable amount of thylakoid membranes [35,37]. It is therefore evident that NTRC has an essential role during early leaf and chloroplast development [38].

Thioredoxin-dependent regulation of photosynthesis and oxidative stress in chloroplasts

Both *in vitro* and *in vivo* methods including affinity chromatography, fluorescent gel electrophoresis, co-immunoprecipitation, and BiFC have been used to screen for potential proteins targeted to redox regulation [12,13,26,39]. The effect of TRX on the candidate protein activity is then tested by *in vitro* assays with purified TRX and target proteins [26]. These experiments have demonstrated that NTRC and TRXs of the Fd-TRX system are both able to reduce many redox-regulated chloroplast proteins, albeit by different efficiency [26,29,40], suggesting overlapping function of the two chloroplast TRX systems. But, the KO of either chloroplast TRX systems (NTRC, FTR, and several isoforms of TRXm) seriously compromises the photosynthetic activities of mutant plants [10,25,27,30], implying specific functions of the TRX systems that cannot be compensated by the other system. Recently, specificity and functional overlap of the NTRC and Fd-TRX systems have been studied by construction of various combinations of KO mutants lacking NTRC and a distinct type of FTR-dependent TRXs (f-, m-, or x-type TRX) [28,41,42] (Table 1). Transgenic lines overexpressing (OE) a WT or mutated NTRC gene (Table 1) have also disclosed new relationships between the two TRX systems in control of chloroplast redox homeostasis [12,33,34,43,44].

Based on the experimental approaches listed in the previous chapter, both the NTRC and Fd-TRX systems have been proposed to regulate the electron flow in thylakoid membranes (ATP synthase, CEF, NPQ, and ST), enzymes in carbon fixation and starch synthesis, and antioxidant activity in chloroplasts [45–49]. Originally, the Fd-TRX system and TRXf1 were assigned to activate fructose-1,6-bisphosphatase (FBPase), seduheptulose-1,7-bisphosphatase (SBPase), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and phosphoribulokinase (PRK) in the CBB cycle [6,29,50] as well as the γ-subunit of ATP synthase [51,52]. Recently, the regulation of the CBB cycle also by m-type TRXs has been proposed [53], while initially they were reported to activate the malate dehydrogenase (NADP-MDH) in malate shuttle [54]. TRXx and TRXy2 function in oxidative stress response reactions and in sulfur metabolism [55–57], and TRXz associates with the plastid-encoded RNA polymerase and regulates transcription of plastid genes [58].

NTRC is a primary reductant for 2-Cys Prxs scavenging H_2O_2 in chloroplasts [11,34,59–61]. It has also been assigned to activate ADP-glucose-pyrophosphorylase (AGPase) in starch biosynthesis [36,62,63] and several enzymes in chlorophyll biosynthesis [64–66], although a lesser role of NTRC in the regulation of these enzymes has also been proposed [40,67]. Recently, NTRC was reported to regulate the ATP synthase [34,68], NPQ [69,70], and CEF dependent on chloroplast NADH dehydrogenase-like complex (NDH) in thylakoid membranes [12]. Although NTRC has been found to be an inefficient reductant of TRX targets in the CBB cycle in comparison with TRXf and TRXm *in vitro*, deficiency of NTRC *in vivo* impairs the reduction in the enzymes in CBB cycle and photosynthetic electron transfer as well as leaf growth to much greater extent than deficiency of TRXf or TRXm alone [26,28–30].

NTRC and TRXm may also have antagonistic roles in the regulation of chloroplast electron transfer activities (Figure 1). Plants deficient in m-type TRXs show enhanced PSI yield in low light intensities [30]. This is opposite to *ntrc* KO plants but similar to NTRC-overexpressing plants, whose PSI yield is enhanced in low and fluctuating light [12,34]. Moreover, TRXm4 has been proposed to have an inhibitory effect on NDH-dependent CEF [46], while plants overexpressing NTRC enhance NDH-dependent CEF [12]. Furthermore, NTRC overexpression results in dark phosphorylation of light harvesting complex II (LHCII) proteins by the STN7 kinase and, consequently, to an increase in the relative size of the PSI antenna cross-section in dark-adapted leaves, as well as enhanced efficiency of ST [70]. In contrast, overexpression of TRXm in tobacco inhibited STN7-dependent phosphorylation of LHCII proteins and ST, while overexpression of TRXf had no effect on these processes [71]. The specific targets (single or multiple proteins) of this antagonist regulation by NTRC and TRXm remain to be elucidated in the further studies.

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

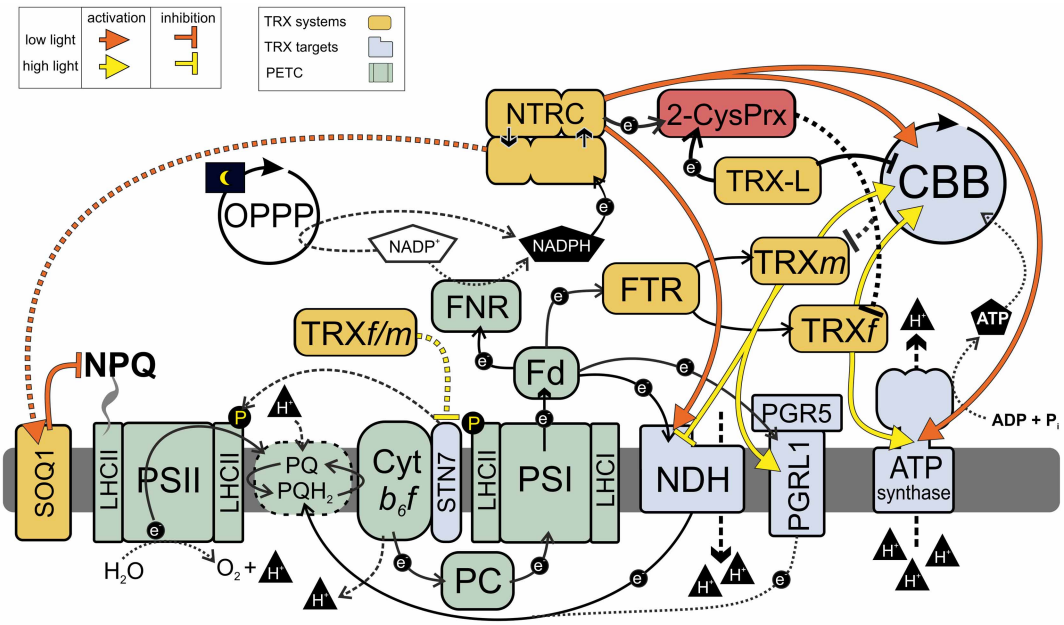

Figure 1. Dynamic regulation of photosynthesis by chloroplast thioredoxin systems.

At dark–light and low–high light transitions as well as under light intensities limiting photosynthesis, NTRC activates NDH complex, ATP synthase, and CBB cycle, which helps to balance the redox poise between light and carbon fixation reactions. NTRC may also mediate the SOQ1-dependent down-regulation of NPQ to prevent heat dissipation under low light. Fd-TRX system keeps the redox-regulated photosynthetic enzymes active under moderate and higher light intensities. In relation to NTRC, TRXm has an antagonist role in the regulation of the electron flow in the thylakoid membrane. 2-CysPRXs are involved in the oxidation of chloroplast TRX systems. For details, see the text. Dotted arrows represent hypothetical or potentially indirect effect on photosynthetic proteins. PETC, photosynthetic electron transfer chain.

NTRC and Fd-TRX systems are differentially activated by light conditions

Instead of strictly distinct tasks with separate target proteins, the two TRX systems may build up a complex redox network in chloroplast with partial specificity but also partially overlapping functions, which facilitates plant growth under repetitively changing environmental conditions.

The Fd-TRX system is tightly coupled to photosynthetic electron transfer activity, and thus, it has been regarded as a primary activator of photosynthetic reactions in light. However, while the Fd-TRX system is activated rapidly under high irradiance [72], it is mostly oxidized in darkness and under low light [29,34,72]. Therefore, initial activation of photosynthetic processes at dark–light transitions and adjustment of plastid redox homeostasis in low light conditions and during sudden changes in irradiance requires a redox regulator capable of being active under these conditions. NTRC is an ideal candidate because it is activated by NADPH present both in dark-adapted and illuminated chloroplasts. Accordingly, it has been demonstrated that the NTRC pool is partially reduced in darkness and the amount of reduced NTRC also stays fairly constant irrespective of light intensity, as well as during dark–light transitions [12]. This unique property of NTRC allows it to function as an effective redox regulator in conditions where light or developmental stage of the chloroplast limits the activation of the Fd-TRX system by the photosynthetic electron transfer chain (PETC) (Figure 1).

How can NTRC act as a dynamic regulator of photosynthetic processes in response to changes in environmental conditions if its own redox state is fairly constant? There are different options to explain this discrepancy. The affinity of the TRX to target proteins may change considerably in different physiological conditions of chloroplasts, such as stromal pH and ion concentrations, which may affect the activation states and target specificities of TRXs [73,74]. For example, NTRC, whose midpoint redox potential has been measured *in vitro* as −275 mV [26], is the primary reductant of 2-Cys Prxs [11,60], whose redox potential *in vitro* is as low as

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

−315 mV [75]. Thus, the reduced NTRC form may only be able to reduce some of its targets in specific circumstances.

Alternatively, the access of NTRC to its substrates may be partly controlled by the redox state of the Fd-TRX system. The f- and m-type TRXs have more negative redox potentials and a higher affinity to common TRX targets *in vitro* than NTRC [26,76,77]. The combined amount f- and m-type TRXs in the chloroplast is about six times higher than that of NTRC [78]. When active, the Fd-TRX system probably competes with NTRC for interaction with common targets. NTRC-mediated reduction would therefore be required when the activation state of the Fd-TRX system is low, such as in darkness and low light conditions [12,34]. This hypothesis would also explain the discrepancies between *in vitro* and *in vivo* experiments of TRX target specificity. NTRC has been found to be an inefficient reductant of TRX targets in the CBB cycle in comparison with TRXf and TRXm *in vitro*, but *in vivo*, deficiency of NTRC impairs the reduction in the enzymes in CBB cycle, photosynthetic electron transfer and leaf growth to much greater extent than deficiency of TRXf or TRXm [26,28–30,34]. These studies demonstrate that NTRC has a specific function in the regulatory network of the chloroplast that cannot be compensated by other TRXs.

Regulation of photosynthetic processes by NTRC allows the maintenance of redox homeostasis at dark–light transitions and during changes in light conditions

We propose that the NTRC system operates as a vital regulatory hub that couples the metabolic state of the stroma to the redox poise of the PETC in low light conditions and during fluctuations in light intensity (Figure 1). This is achieved by simultaneous redox control of the induction of NPQ and the activity of the ATP synthase, cyclic electron transfer around PSI through the NDH complex, and CBB cycle enzymes [12,30,34,68–70]. Under growth and higher light intensities, the Fd-TRX system is fully activated and can take over the redox-regulation of photosynthesis.

Reduction in γ-subunit of the ATP synthase by the f-type TRX of the Fd-TRX system is well established [48,52]. However, the Fd-TRX system can only compensate for a lack of NTRC in moderate to high light conditions, while in low light NTRC deficiency results in impaired reduction in the γ-subunit, with several consequences [34,68]. Firstly, activity of the ATP synthase remains low in *ntrc* plants resulting in the acidification of the lumen that, in turn, induces high NPQ [12,34,69]. Moreover, low activity of the ATP synthase results in low ATP production, which together with impaired reduction in CBB cycle enzymes [26,34,79] contributes to a decreased rate of carbon fixation in low light in NTRC-deficient plants [11,34,35,60].

The rate of CEF also depends on the stromal redox state [49,80]. There are two CEF pathways in plant chloroplasts, one dependent on the proteins proton gradient regulation 5 (PGR5) and PGR5-like 1 (PGRL1) [81,82], and the other dependent on the chloroplast NDH complex [83,84]. PGRL1 has been proposed to form a heterodimer with PGR5 that oxidizes ferredoxin and reduces the plastoquinone (PQ) pool [47]. During dark–light transitions, PGR5/PGRL1-dependent CEF contributes to generation of proton motive force (pmf) that induces photoprotective mechanisms, NPQ, and photosynthetic control [47,81,82,85–87]. PGRL1 contains six conserved Cys residues, which have been proposed to control the conformation of PGRL1 protein and interaction with PGR5 [47,81,82,88]. According to a model postulated by Dario Leister and co-workers, TRXm activates the reduction in the PQ pool by PGR5/PGRL1 proteins [47,89]. PGRL1 protein has, indeed, been shown to be transiently reduced during dark–light transitions, coinciding with an increase in P700 oxidation and NPQ induction [12,47]. Alternatively, it has been suggested that PGR5 alone may down-regulate the proton conductivity of the thylakoid membrane upon sudden increases in light intensity via inhibition of ATP synthase activity [90,91]. This down-regulation is enhanced by NTRC overexpression, but not in the absence of PGR5 [12]. As NTRC was shown to interact with PGR5 [12], it is likely that the function of PGR5 and PGRL1 depends on the stromal thiol redox state.

It has been suggested that NDH-mediated CEF is involved in balancing photosynthetic redox poise and generation of pmf specifically in low light conditions and during increases in light intensity [92–95]. The NDH complex is reduced by Fd [96], which functions as a light-dependent redox hub, controlling the distribution of electrons to several stromal acceptors in addition to CEF pathways [97]. Therefore, strict regulation of NDH activity is likely needed for concerted function of all these processes under fluctuating light conditions, in order to maintain redox balance in the chloroplast. It has been shown by KO and overexpression lines of NTRC that it has a role in activating the NDH complex during dark–light transitions and under low irradiance [12].

NTRC overexpression enhances reduction in the PQ pool in darkness, increases the magnitude of pmf and PSI yield in comparison with WT in low light and upon increases in light intensity, and enhances the acidification of the lumen under all light intensities in an NDH-dependent manner [12]. These observations suggest that in WT plants, NTRC-dependent activation of NDH at dark–light and under fluctuating light releases redox pressure in thylakoid membranes by inducing dissipation of light energy as heat by NPQ until the CBB cycle is ready to exploit the electrons in carbon fixation.

The major, energy-dependent component (qE) of NPQ depends on acidification of the lumen, via protonation of the PSII subunit S (PsbS) protein and association of the xanthophyll cycle enzyme violaxanthin de-epoxidase with the thylakoid membrane [98–100]. As reviewed in the previous chapters, both deficiency and increase in NTRC content *in vivo* cause higher acidification of thylakoid lumen in illuminated leaves, albeit for different reasons (see discussion in ref. [12]). High lumen acidification already at low light intensities correlates with the exceptionally high NPQ reported in *ntrc* plants [68–70]. However, *ntrc* has high NPQ also under growth light and higher intensities, despite the level of ΔpH being comparable to WT in those conditions [70]. But, generation of NPQ is diminished in leaves overexpressing NTRC despite strong acidification of the lumen and higher accumulation of xanthophyll pigments [70]. These observations indicate that an unknown factor independent of trans-thylakoid ΔpH is up-regulating NPQ in *ntrc* and down-regulating it in plants overexpressing NTRC. Inhibition of a slow-relaxing component of NPQ has been shown to depend on SUPPRESSOR OF QUENCHING 1 (SOQ1), an integral thylakoid membrane protein that contains a lumenal thioredoxin-like domain [101,102]. Importantly, this component called qH [102] is ΔpH-independent, as in addition to *soq1* KO plants, a slow-relaxing NPQ component remains elevated in plants lacking both SOQ1 and PsbS [101]. A similar slowly reversible NPQ component was detected also in *ntrc* [70], and SOQ1 was identified as a putative NTRC interactor by co-immunoprecipitation/MS [12]. Thus, the absence of NTRC may impair SOQ1-dependent down-regulation of NPQ, while NTRC overexpression might result in over-activation of this inhibitory mechanism. It has been reported that the mutation of PsbS in the *ntrc* background partially restored the photosynthetic activity of the double mutant by reducing NPQ [69], indicating that the *ntrc* mutant suffers from energy shortage because of the uncontrolled NPQ. Thereby, the task of TRX may be to moderate the induction of NPQ under light intensities limiting photosynthesis.

Impaired and enhanced carbon fixation rates in *ntrc* and plants overexpressing NTRC, respectively [11,34,35], suggested that the activation states of the redox-regulated enzymes in the CBB cycle are either directly or indirectly affected by deficiency and overexpression of NTRC. Indeed, reduction in FBPase and PRK was impaired in low light conditions in *ntrc* [34,79], while the *in vivo* amount of reduced forms of these enzymes was significantly higher than in WT in all light conditions and even in dark-adapted leaves of OE-NTRC plants [34]. As both NTRC and TRXf1 interact with FBPase and PRK in BiFC [34], it is likely that at least these CBB enzymes are directly and cooperatively regulated by both TRX systems.

In summary, the NTRC- and FTR-mediated regulation of photosynthetic redox poise is presented in Figure 1. Upon the onset of illumination of leaves, the NTRC pool is already partially active due to NADPH produced in the OPPP in darkness. It transiently activates CEF that helps to balance redox poise in thylakoid membranes before activation of the CBB cycle. Under light intensities limiting photosynthesis, NTRC activates the ATP synthase by reducing the γ-subunit and induces ATP production. NTRC also contributes to activation of the CBB cycle, which together with activation of NDH-dependent CEF enhances the electron sink capacity of the stroma and alleviates acceptor side limitation of PSI. Under low irradiance, NTRC may also mediate SOQ1-dependent down-regulation of NPQ [70,102]. Under growth light and higher irradiance, the m-type TRXs are involved in the regulation of PGRL1/PGR5-dependent CEF [12,47]. All the events described here contribute to the prevention of over-reduction in the electron transfer chain and allow efficient oxidation of PSI, protecting it from photodamage [103].

Oxidation of chloroplast TRX and redox-regulated proteins

Redox regulation of chloroplast proteins is reversible, including also the oxidation loop of TRX systems and target proteins. Much less, however, is known about the mechanisms oxidizing TRXs and proteins, especially under fluctuating light or at light–dark transitions. Molecular oxygen and ROS have been reported to oxidize protein thiols [6,104], and they are probably involved in adjustment of the redox state of proteins regulated by TRXs. Since the oxygenic photosynthetic organisms evolve oxygen and produce ROS in light, continuous supply of reducing equivalents from TRX systems is needed to keep the photosynthetic enzymes active in light.

Biochemical Journal (2019) 476 1159–1172
https://doi.org/10.1042/BCJ20180707

Accordingly, molecular oxygen and ROS can be involved in oxidation of TRXs and enzyme pools also when light intensity drops.

Recently, involvement of 2-Cys Prxs in oxidation of TRX systems and redox-regulated proteins has been reported (Figure 1) [43,79,105–107]. 2-Cys PrxA and B are highly abundant chloroplast proteins that scavenge peroxides in chloroplasts [23,78]. NTRC is a primary reductant for 2-Cys Prxs, but they can also be reduced by other chloroplast TRXs, including TRXf, TRXx, and TRX-like proteins ACHT1 and ACHT4 [11,77,108]. Danon and his colleagues [105,108] have described an oxidizing loop of TRX system regulating AGPase, a key enzyme in starch synthesis under fluctuating light. ACHT4 is first reduced in light but becomes then oxidized by 2-Cys Prxs. Oxidized ACHT4 inactivates AGPase at light/dark transition or under fluctuating light. Chloroplast TRXs have also been suggested to become oxidized by an oxidized pool of 2-Cys Prxs [79,106]. As highly abundant proteins, 2-Cys Prxs can form a very strong sink for electrons when ROS accumulate in chloroplasts. This strong sink abstracts electrons from TRX systems involved in the activation of redox-regulated chloroplast enzymes [79,106]. Accordingly, it was proposed that the impaired reduction in CBB cycle enzymes in *ntrc* results indirectly from the oxidation of TRXf in light due to high accumulation of oxidized 2-Cys Prxs [79]. An atypical TRX called Thioredoxin-like2 was also suggested to mediate the oxidative effect of 2-Cys Prxs on chloroplast enzymes [107]. However, contrary to [79], reduction in TRXf was not impaired in *ntrc*, or in transgenic lines overexpressing mutated NTRC lacking TRX activity, despite these lines have substantially larger pools of oxidized 2-Cys Prxs in comparison with WT in all tested light conditions [34]. But, higher amounts of active CBB cycle enzymes accumulate in illuminated leaves in mutants with strongly diminished amounts of 2-Cys Prx proteins [79], as well as in illuminated plants overexpressing NTRC, which accumulate low amounts of oxidized 2-Cys Prxs [34]. Both NTRC overexpression [12] and 2-Cys Prx deficiency [106] result in enhanced photosynthetic efficiency in fluctuating light conditions. Thus, 2-Cys Prxs are probably involved in an oxidizing loop of TRX systems in the chloroplast by controlling the amount of redox equivalents available for redox-regulated photosynthetic enzymes (Figure 1). How efficient and dynamic the 2-Cys Prxs-dependent oxidant system is in the control of photosynthetic enzymes remains to be elucidated.

Localization of photosynthetic and regulatory proteins in mesophyll and bundle sheath cells of C4 leaves

Chloroplast TRX systems described above are applied to C_3 plants primarily fixing CO_2 via the CBB cycle. In C_4 plants, two different chloroplast populations exist with the segregation of phosphoenolpyruvate carboxylase (PEPC)-dependent carbon fixation into mesophyll cells (MCs) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)-dependent carbon fixation into bundle sheath cells (BSCs). Three biochemical subtypes of C_4 photosynthesis have been described with variations of C_4 photosynthetic enzymes, transporters, and metabolites transported between MCs and BSCs (see the recent review by Furbank [109]). The proteomic approach has demonstrated remarkable differences in the composition of photosynthetic proteins in MS and BSC chloroplasts of maize, which belongs to the C_4 subtype called NADP malic enzyme (NADP-ME) [110,111]. Maize MC chloroplasts have less CBB cycle enzymes except for the enrichment of GAPDH and triose phosphate isomerase (TPI) catalyzing the reduction phase of 3-phosphoglycerate to triose phosphates, whereas the other CBB cycle enzymes are enriched in BSCs [111]. Subunits of PSII and LHCII are enriched in MCs, while BSC chloroplasts lack the grana structure and accumulate slightly more subunits of PSI, LHCI, cytochrome bf_6 complex ($Cytbf_6$), and ATP synthase than MC chloroplasts [110]. Consistent with the divergent localization of PSI and PSII complexes between MCs and BSCs, the components regulating linear electron transfer (NPQ, ST) and mediating CEF (NDH) are enriched in MCs and BSCs, respectively [110,112]. Not only are the subunits of the NDH complex enriched in BSCs but also the total abundance of NDH is higher in C_4 than in C_3 leaves, suggesting that NDH supports the high CEF activity observed in C_4 leaves [112]. The components of the other CEF pathway involving PRG5/PGRL1 proteins show a different pattern. In maize leaves, PGRL1 is enriched in BSCs, while PGR5 is equally localized to both cells [111], while both proteins are equally present in MCs and BSCs of C4 *Flaveria* species, albeit PGRL1 and PGR5 proteins are more abundant in C_4 than in C_3 *Flaveria* species [112].

Although chloroplast TRX systems are important activators of the CBB cycle in C_3 species, the components of the both chloroplast TRX systems and other TRX-like proteins and glutaredoxins are enriched in maize MCs having only traces of CBB cycle enzymes [111]. Especially both TRs, FTR and NTRC are largely localized to MCs (protein ratio in BSC/MC 0.14 and 0.09, respectively). The m-type TRXs and 2-Cys Prxs are, however, more equally distributed in MCs and BSCs. Only CDSP32, a TRX-like drought-induced stress protein [113] is

highly enriched in maize BSC chloroplasts [111]. The low content of FTR-dependent TRXs in maize BSCs is probably related to low O_2 concentration caused by strong deficiency of PSII and low ROS production due to the almost complete lack of linear electron flow. Accordingly, also the antioxidant proteins are mainly enriched in maize MCs, except for stromal ascorbate peroxidase, which is more abundant in BSCs [111]. This segregation of TRX, antioxidants, linear and CEF, and C_3/C_4 carbon fixation between MCs and BSCs suggests that the demand of TRXs is strongly linked with the presence of molecular oxygen and ROS. Due to the higher oxidizing capacity, continuous relay of electrons from TRX systems to redox-regulated photosynthetic proteins is needed to maintain the active state of the enzymes in illuminated maize MC chloroplasts producing molecular oxygen and ROS. Interestingly, the enzymes catalyzing the electron sinks for linear electron flow in MCs, i.e. reductive phase of CBB cycle (GAPDH and TPI) and the reduction in oxaloacetate to malate in C4 shuttle (NADP-MDH), are both enriched in MCs and potentially (TPI [114], GAPDH [13]), or confirmed (NADP-MDH [115]) to be targets of TRX regulation. In the previous chapters, we proposed that TRXs in C_3 chloroplasts are involved in balancing electron transfer activity in thylakoid membranes with the electron sinks in stroma under fluctuating light conditions. Accordingly, MC chloroplasts probably need to control the redox homeostasis between source and sink of electrons, as observed in C_3 chloroplasts.

How are the redox-regulated enzymes of the CBB cycle activated in BSCs, when both the NTRC and Fd-TRX systems are present as very low concentrations? BSC chloroplasts contain m-type TRXs, and probably this is enough to keep the enzymes active in light, when the production of oxidants that inactivate redox-regulated enzymes is low. Furthermore, the high CO_2 concentration in BSCs makes a strong sink for ATP and NADPH, further lowering the probability of oxidant production in light reactions. The role of CDSP32 and ascorbate peroxidase in BSC remains to be elucidated by further studies.

Can we improve plant photosynthesis by overexpressing NTRC?

Chloroplast TRX systems are engaged in the complex redox network that balances photosynthetic redox poise and consumption of electrons under fluctuating light. This regulatory network helps sessile plants to survive in nature under ever-changing environmental conditions. The novel information about the structure, function, and regulation of photosynthesis has now revealed potential routes to increase photosynthetic efficiency and thus productivity of photosynthetic organisms by manipulating the regulatory network in chloroplasts [116,117]. Crop plants form tight canopies with sun-exposed and shaded leaves that repeatedly face fluctuation of light. The upper canopy exposed to the sun has reported to drive ∼75% of photosynthesis [118], while the photosynthetic efficiency of the rest of the leaves depends largely on sun flecks. The shaded leaves cannot efficiently utilize sunflecks because the increase in light intensity rapidly induces the mechanisms dissipating light energy as heat due to the delay in the activation of CBB cycle enzymes [117]. Thus, the mechanisms that down-regulate photosynthetic activities at light intensity transitions and at low light are potential targets for manipulation in order to improve photosynthetic efficiency of plants.

The effect of an increase (overexpression) or reduction (KO/knockdown) of a single component of the regulatory mechanism depends on how significant the mechanism is for photosynthetic performance and what are the side effects of the modification. So, the regulator that forms a node in the regulatory network and mediates signals to various processes in photosynthesis may be a promising candidate for manipulation, if it has a parallel and positive effect on photosynthetic subreactions. We have proposed that NTRC may fulfill these criteria as a general positive effector of photosynthetic activities. The NTRC system broadly activates photosynthetic reactions including ATP synthesis, CEF, and carbon fixation at low and fluctuating light, as described in the previous chapters. It is also involved in scavenging of H_2O_2 produced in light reactions [11] and in down-regulation of NPQ under conditions where the dissipation of energy as heat would be wasteful [69].

Contradictory reports have been published about the effect of NTRC overexpression on growth of Arabidopsis (Table 1). Arabidopsis lines overexpressing NTRC were constructed by transformation of the *ntrc* mutant with the WT *NTRC* gene under a constitutive 35S-CaMV promoter, and only the transgenic plants with fully-complemented WT phenotype were selected for further studies. The NTRC protein content of these lines was ∼10–20 times higher than in WT Arabidopsis [12,33,34]. In these lines, overexpression of NTRC significantly increases biomass production of Arabidopsis rosettes [33]. Accordingly, Kim et al. [44] have reported on higher tolerance of Arabidopsis plants overexpressing NTRC to oxidative and drought stresses. Neither growth defect was observed in these OE-NTRC lines. However, Ojeda et al. [43] reported reduced growth in two OE-NTRC lines,

Biochemical Journal (2019) 476 1159–1172
https://doi.org/10.1042/BCJ20180707

which were constructed by transformation of WT Arabidopsis with the *NTRC* gene under a 35S-CaMV promoter. The increase in the amount of NTRC was not reported in this paper, but the immunoblot shows substantially higher accumulation of NTRC in these transgenic lines in comparison with previously constructed lines, which may explain the different growth effect of the OE-NTRC lines. The large increase in NTRC content may considerably disturb redox homeostasis in chloroplasts, e.g. by imbalancing the $NADP^+$/NADPH ratio. Secondly, 35S-CaMV promoter causes an ectopic expression of the gene also in non-photosynthetic tissues that endogenously have a low amount of NTRC [24], which may seriously impede the cellular function of these tissues.

In the transgenic lines containing a moderately increased amount of NTRC, photosynthetic carbon fixation was raised ~20% [34]. They have also higher activity of CEF [12] and permanently elevated activation states of the CBB cycle enzymes (FBPase and PRK in ref. [34]) (GAPDH in ref. [43]), which creates a strong sink for electrons from PSI. This allows faster balancing of photosynthetic redox poise and efficient electron transfer upon onset of illumination or under fluctuating light. Hence, the quantum yield of PSI and CO_2 fixation was higher than in WT immediately upon illumination in dark-adapted OE-NTRC leaves and under low light [12,34]. Changes in the NTRC content of leaves also affected a wide variety of chloroplast functions indirectly through cross-talk with the Fd-TRX system and by generally modifying the stromal thiol-redox state [34,43]. The higher accumulation of active TRXs of the Fd-TRX system in plants overexpressing NTRC may be due to the direct reduction in these TRXs by NTRC or to a very small pool of oxidized 2-Cys Prxs in the leaves with elevated NTRC content under all tested light intensities [34]. Better stress tolerance of OE-NTRC plants [44] would also improve the fitness of plants under natural growth conditions. As the redox state of the NTRC pool in leaves moderately overexpressing NTRC was maintained fairly constant in all light conditions [12], these results suggest that overexpression of NTRC is a simple genetic modification that could considerably improve photosynthetic efficiency and biomass yield in shaded leaves and in fluctuating light conditions. To avoid harmful side effects due to the too high NTRC content or ectopic expression of *NTRC* gene in non-photosynthetic tissues, the overexpression construct with leaf- and light-specific promoters should be tested. The potential of TRX overexpression as a bioengineering tool to improve the yields of crop plants or biofuel production seems, however, to be worth considering.

Abbreviations

2-Cys Prx, 2-Cysteine peroxiredoxin; AGPase, ADP-glucose-pyrophosphorylase; At, *Arabidopsis thaliana*; BiFC, bimolecular fluorescence complementation; BSC, bundle sheath cell; CBB cycle, Calvin–Benson–Bassham cycle; CEF, cyclic electron flow; Chl, chlorophyll; Cys, cysteine residue; Cyt b_6f, cytochrome b_6f complex; FAD, flavin adenine dinucleotide; FBPase, fructose-1,6-bisphosphatase; Fd, ferredoxin; Fd-TRX system, ferredoxin-dependent thioredoxin system; FNR, ferredoxin-$NADP^+$ oxidoreductase; FTR, ferredoxin-thioredoxin reductase; FTRc, catalytic subunit of FTR; FTR_v, variable subunit of FTR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; KD, knockdown; KO, knockout; LHCII, light harvesting complex of PSII; MC, mesophyll cell; NADP-MDH, malate dehydrogenase; NDH, chloroplast NADH dehydrogenase-like complex; NPQ, non-photochemical quenching; Nt, *Nicotiana tabacum*; NTRC, chloroplast NADPH-dependent thioredoxin reductase; NTRd, N-terminal thioredoxin reductase domain; OE, overexpression; OPPP, oxidative pentose phosphate pathway; PETC, photosynthetic electron transfer chain; PGR5, proton gradient regulation 5; PGRL1, PGR5-like 1; pmf, proton motive force; PQ, plastoquinone; PRK, phosphoribulokinase; PsbS, PSII subunit S; PSI, photosystem I; PSII, photosystem II; qE, energy-dependent component of non-photochemical quenching; ROS, reactive oxygen species; SBPase, seduheptulose-1,7-bisphosphatase; SOQ1, suppressor of quenching 1; ST, state transitions; TPI, triose phosphate isomerase; TR, thioredoxin reductase; TRX, thioredoxin; VIGS, virus-induced gene silencing; WT, wild type.

Author Contribution

L.N. and E.R. designed and wrote the review article.

Funding

This work was funded by the Academy of Finland Grants 276392 (to E.R.) and 307335 (the Center of Excellence in Molecular Biology of Primary Producers) and by the Doctoral Program in Molecular Life Sciences in the University of Turku Graduate School (to L.N.).

Acknowledgements

We thank Drs Manuel Guinea Diaz and Arjun Tiwari for their fruitful discussions.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Ingles-Prieto, A., Ibarra-Molero, B., Delgado-Delgado, A., Perez-Jimenez, R., Fernandez, J.M., Gaucher, E.A. et al. (2013) Conservation of protein structure over four billion years. *Structure* **21**, 1690–1697 https://doi.org/10.1016/j.str.2013.06.020
2 Balsera, M., Uberegui, E., Susanti, D., Schmitz, R.A., Mukhopadhyay, B., Schürmann, P. et al. (2013) Ferredoxin:thioredoxin reductase (FTR) links the regulation of oxygenic photosynthesis to deeply rooted bacteria. *Planta* **237**, 619–635 https://doi.org/10.1007/s00425-012-1803-y
3 Balsera, M., Uberegui, E., Schürmann, P. and Buchanan, B.B. (2014) Evolutionary development of redox regulation in chloroplasts. *Antioxid. Redox Signal.* **21**, 1327–1355 https://doi.org/10.1089/ars.2013.5817
4 Collet, J. and Messens, J. (2010) Structure, function, and mechanism of thioredoxin proteins. *Antioxid. Redox Signal.* **13**, 1205–1216 https://doi.org/10.1089/ars.2010.3114
5 Meyer, Y., Belin, C., Delorme-Hinoux, V., Reichheld, J. and Riondet, C. (2012) Thioredoxin and glutaredoxin systems in plants: molecular mechanisms, crosstalks, and functional significance. *Antioxid. Redox Signal.* **17**, 1124–1160 https://doi.org/10.1089/ars.2011.4327
6 Schürmann, P. and Buchanan, B.B. (2008) The ferredoxin/thioredoxin system of oxygenic photosynthesis. *Antioxid. Redox Signal.* **10**, 1235–1273 https://doi.org/10.1089/ars.2007.1931
7 Buchanan, B.B. (2016) The path to thioredoxin and redox regulation in chloroplasts. *Annu. Rev. Plant Biol.* **67**, 1–24 https://doi.org/10.1146/annurev-arplant-043015-111949
8 Geigenberger, P., Thormählen, I., Daloso, D.M. and Fernie, A.R. (2017) The unprecedented versatility of the plant thioredoxin system. *Trends Plant Sci.* **22**, 249–262 https://doi.org/10.1016/j.tplants.2016.12.008
9 Nikkanen, L., Toivola, J., Diaz, M.G. and Rintamäki, E. (2017) Chloroplast thioredoxin systems: prospects for improving photosynthesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **372**, 20160474 https://doi.org/10.1098/rstb.2016.0474
10 Serrato, A.J., Pérez-Ruiz, J.M., Spínola, M.C. and Cejudo, F.J. (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 https://doi.org/10.1074/jbc.M404696200
11 Pérez-Ruiz, J.M., Spínola, M.C., Kirchsteiger, K., Moreno, J., Sahrawy, M. and Cejudo, F.J. (2006) Rice NTRC is a high-efficiency redox system for chloroplast protection against oxidative damage. *Plant Cell* **18**, 2356–2368 https://doi.org/10.1105/tpc.106.041541
12 Nikkanen, L., Toivola, J., Trotta, A., Diaz, M.G., Tikkanen, M., Aro, E. et al. (2018) Regulation of cyclic electron flow by chloroplast NADPH-dependent thioredoxin system. *Plant Direct* **2**, 1–24 https://doi.org/10.1002/pld3.93
13 Balmer, Y., Koller, A., del Val, G., Manieri, W., Schurmann, P. and Buchanan, B. (2003) Proteomics gives insight into the regulatory function of chloroplast thioredoxins. *Proc. Natl Acad. Sci. U.S.A.* **100**, 370–375 https://doi.org/10.1073/pnas.232703799
14 Buchanan, B.B. and Balmer, Y. (2005) Redox regulation: a broadening horizon. *Annu. Rev. Plant Biol.* **56**, 187–220 https://doi.org/10.1146/annurev.arplant.56.032604.144246
15 Lindahl, M. and Kieselbach, T. (2009) Disulphide proteomes and interactions with thioredoxin on the track towards understanding redox regulation in chloroplasts and cyanobacteria. *J. Proteomics* **72**, 416–438 https://doi.org/10.1016/j.jprot.2009.01.003
16 Montrichard, F., Alkhalfioui, F., Yano, H., Vensel, W.H., Hurkman, W.J. and Buchanan, B.B. (2009) Thioredoxin targets in plants: the first 30 years. *J. Proteomics* **72**, 452–474 https://doi.org/10.1016/j.jprot.2008.12.002
17 Hall, M., Mata-Cabana, A., Åkerlund, H.E., Florencio, F.J., Schröder, W.P., Lindahl, M. et al. (2010) Thioredoxin targets of the plant chloroplast lumen and their implications for plastid function. *Proteomics* **10**, 987–1001 https://doi.org/10.1002/pmic.200900654
18 Serrato, A.J., Fernández-Trijueque, J., Barajas-López, J.D., Chueca, A. and Sahrawy, M. (2013) Plastid thioredoxins: a 'one-for-all' redox-signaling system in plants. *Front. Plant Sci.* **4**, 463 https://doi.org/10.3389/fpls.2013.00463
19 Nikkanen, L. and Rintamäki, E. (2014) Thioredoxin-dependent regulatory networks in chloroplasts under fluctuating light conditions. *Philos. Trans. R. Soc. B Biol. Sci.* **369**, 20130224 https://doi.org/10.1098/rstb.2013.0224
20 Kang, Z. and Wang, G. (2016) Redox regulation in the thylakoid lumen. *J. Plant Physiol.* **192**, 28–37 https://doi.org/10.1016/j.jplph.2015.12.012
21 Dai, S., Friemann, R., Glauser, D.A., Bourquin, F., Manieri, W., Schürmann, P. et al. (2007) Structural snapshots along the reaction pathway of ferredoxin-thioredoxin reductase. *Nature* **448**, 92–102 https://doi.org/10.1038/nature05937
22 Keryer, E., Collin, V., Lavergne, D., Lemaire, S. and Issakidis-Bourguet, E. (2004) Characterization of *Arabidopsis* mutants for the variable subunit of ferredoxin:thioredoxin reductase. *Photosynthesis Res.* **79**, 265–274 https://doi.org/10.1023/B:PRES.0000017173.46185.3e
23 Peltier, J.B., Cai, Y., Sun, Q., Zabrouskov, V., Giacomelli, L., Rudella, A. et al. (2006) The oligomeric stromal proteome of *Arabidopsis thaliana* chloroplasts. *Mol. Cell Proteomics* **5**, 114–133 https://doi.org/10.1074/mcp.M500180-MCP200
24 Belin, C., Bashandy, T., Cela, J., Delorme-Hinoux, V., Riondet, C. and Reichheld, J.P. (2015) A comprehensive study of thiol reduction gene expression under stress conditions in *Arabidopsis thaliana*. *Plant Cell Environ.* **38**, 299–314 https://doi.org/10.1111/pce.12276
25 Wang, P., Liu, J., Liu, B., Da, Q., Feng, D., Su, J. et al. (2014) Ferredoxin:thioredoxin reductase is required for proper chloroplast development and is involved in the regulation of plastid gene expression in *Arabidopsis thaliana*. *Mol. Plant* **7**, 1586–1590 https://doi.org/10.1093/mp/ssu069
26 Yoshida, K. and Hisabori, T. (2016) Two distinct redox cascades cooperatively regulate chloroplast functions and sustain plant viability. *Proc. Natl Acad. Sci. U.S.A.* **113**, E3967–E3976 https://doi.org/10.1073/pnas.1604101113
27 Wang, P., Liu, J., Liu, B., Feng, D., Da, Q., Wang, P. et al. (2013) Evidence for a role of chloroplastic m-type thioredoxins in the biogenesis of photosystem II in *Arabidopsis*. *Plant Physiol.* **163**, 1710–1728 https://doi.org/10.1104/pp.113.228353
28 Thormählen, I., Meitzel, T., Groysman, J., Öchsner, A.B., von Roepenack-Lahaye, E., Naranjo, B. et al. (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 https://doi.org/10.1104/pp.15.01122
29 Yoshida, K., Hara, S. and Hisabori, T. (2015) Thioredoxin selectivity for thiol-based redox regulation of target proteins in chloroplasts. *J. Biol. Chem.* **290**, 19540 https://doi.org/10.1074/jbc.M115.647545

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

30 Thormählen, I., Zupok, A., Rescher, J., Leger, J., Weissenberger, S., Groysman, J. et al. (2017) Thioredoxins play a crucial role in dynamic acclimation of photosynthesis in fluctuating light. *Mol. Plant* **10**, 168–182 https://doi.org/10.1016/j.molp.2016.11.012
31 Pérez-Ruiz, J.M. and Cejudo, F.J. (2009) A proposed reaction mechanism for rice NADPH thioredoxin reductase C, an enzyme with protein disulfide reductase activity. *FEBS Lett.* **583**, 1399–1402 https://doi.org/10.1016/j.febslet.2009.03.067
32 Pérez-Ruiz, J.M., González, M., Cristina Spínola, M., Maria Sandalio, L. and Javier Cejudo, F. (2009) The quaternary structure of NADPH thioredoxin reductase C is redox-sensitive. *Mol. Plant* **2**, 457–467 https://doi.org/10.1093/mp/ssp011
33 Toivola, J., Nikkanen, L., Dahlström, K.M., Salminen, T.A., Lepistö, A., Vignols, F. et al. (2013) Overexpression of chloroplast NADPH-dependent thioredoxin reductase in *Arabidopsis* enhances leaf growth and elucidates in vivo function of reductase and thioredoxin domains. *Front. Plant Sci.* **4**, 389 https://doi.org/10.3389/fpls.2013.00389
34 Nikkanen, L., Toivola, J. and Rintamäki, E. (2016) Crosstalk between chloroplast thioredoxin systems in regulation of photosynthesis. *Plant Cell Environ.* **39**, 1691–1705 https://doi.org/10.1111/pce.12718
35 Lepistö, A., Kangasjärvi, S., Luomala, E., Brader, G., Sipari, N., Keränen, M. et al. (2009) Chloroplast NADPH-thioredoxin reductase interacts with photoperiodic development in *Arabidopsis*. *Plant Physiol.* **149**, 1261–1276 https://doi.org/10.1104/pp.108.133777
36 Lepistö, A., Pakula, E., Toivola, J., Krieger-Liszkay, A., Vignols, F. and 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 https://doi.org/10.1093/jxb/ert216
37 Lepistö, A., Toivola, J., Nikkanen, L. and Rintamäki, E. (2012) Retrograde signaling from functionally heterogeneous plastids. *Front. Plant Sci.* **3**, 286 https://doi.org/10.3389/fpls.2012.00286
38 Lepistö, A. and Rintamäki, E. (2012) Coordination of plastid and light signaling pathways upon development of *Arabidopsis* leaves under various photoperiods. *Mol. Plant* **5**, 799–816 https://doi.org/10.1093/mp/ssr106
39 Motohashi, K., Kondoh, A., Stumpp, M. and Hisabori, T. (2001) Comprehensive survey of proteins targeted by chloroplast thioredoxin. *Proc. Natl Acad. Sci. U.S.A.* **98**, 11224–11229 https://doi.org/10.1073/pnas.191282098
40 Thormählen, I., Ruber, J., Von Roepenack-Lahaye, E., Ehrlich, S., Massot, V., Hümmer, C. et al. (2013) Inactivation of thioredoxin *f*1 leads to decreased light activation of ADP-glucose pyrophosphorylase and altered diurnal starch turnover in leaves of *Arabidopsis* plants. *Plant Cell Environ.* **36**, 16–29 https://doi.org/10.1111/j.1365-3040.2012.02549.x
41 Da, Q., Sun, T., Wang, M., Jin, H., Li, M., Feng, D. et al. (2017) M-type thioredoxins are involved in the xanthophyll cycle and proton motive force to alter NPQ under low-light conditions in *Arabidopsis*. *Plant Cell Rep.* **37**, 279–291 https://doi.org/10.1007/s00299-017-2229-6
42 Ojeda, V., Pérez-Ruiz, J.M. and Cejudo, F.J. (2018) The NADPH-dependent thioredoxin reductase C-2-cys peroxiredoxin redox system modulates the activity of thioredoxin x in *Arabidopsis* chloroplasts. *Plant Cell Physiol.* **59**, 2155–2164 https://doi.org/10.1093/pcp/pcy134
43 Ojeda, V., Pérez-Ruiz, J.M. and Cejudo, F.J. (2018) 2-cys peroxiredoxins participate in the oxidation of chloroplast enzymes in the dark. *Mol. Plant* **11**, 1377–1388 https://doi.org/10.1016/j.molp.2018.09.005
44 Kim, M.R., Khaleda, L., Jung, I.J., Kim, J.Y., Lee, S.Y., Cha, J. et al. (2017) Overexpression of chloroplast-localized NADPH-dependent thioredoxin reductase C (NTRC) enhances tolerance to photo-oxidative and drought stresses in *Arabidopsis thaliana*. *J. Plant Biol.* **60**, 175–180 https://doi.org/10.1007/s12374-016-0464-y
45 Rintamäki, E., Martinsuo, P., Pursiheimo, S. and Aro, E. (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. *Proc. Natl Acad. Sci. U.S.A.* **97**, 11644–11649 https://doi.org/10.1073/pnas.180054297
46 Courteille, A., Vesa, S., Sanz-Barrio, R., Cazale, A., Becuwe-Linka, N., Farran, I. et al. (2013) Thioredoxin m4 controls photosynthetic alternative electron pathways in *Arabidopsis*. *Plant Physiol.* **161**, 508–520 https://doi.org/10.1104/pp.112.207019
47 Hertle, A.P., Blunder, T., Wunder, T., Pesaresi, P., Pribil, M., Armbruster, U. et al. (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Mol. Cell* **49**, 511–523 https://doi.org/10.1016/j.molcel.2012.11.030
48 Hisabori, T., Sunamura, E., Kim, Y. and Konno, H. (2013) The chloroplast ATP synthase features the characteristic redox regulation machinery. *Antioxid. Redox Signal.* **19**, 1846–1854 https://doi.org/10.1089/ars.2012.5044
49 Strand, D.D., Fisher, N., Davis, G.A. and Kramer, D.M. (2016) Redox regulation of the Antimycin A sensitive pathway of cyclic electron flow around photosystem I in higher plant thylakoids. *Biochim. Biophys. Acta Bioenerg.* **1857**, 1–6 https://doi.org/10.1016/j.bbabio.2015.07.012
50 Schürmann, P., Wolosiuk, R.A., Breazale, V.D. and Buchanan, B.B. (1976) Two proteins function in the regulation of photosynthetic CO_2 assimilation in chloroplasts. *Nature* **263**, 257–258 https://doi.org/10.1038/263257a0
51 McKinney, D., Buchanan, B. and Wolosiuk, R. (1978) Activation of chloroplast ATPase by reduced thioredoxin. *Phytochemistry* **17**, 794–795 https://doi.org/10.1016/S0031-9422(00)94230-4
52 Schwarz, O., Schürmann, P. and Strotmann, H. (1997) Kinetics and thioredoxin specificity of thiol modulation of the chloroplast H^+-ATPase. *J. Biol. Chem.* **272**, 16924–16927 https://doi.org/10.1074/jbc.272.27.16924
53 Okegawa, Y. and Motohashi, K. (2015) Chloroplastic thioredoxin *m* functions as a major regulator of Calvin cycle enzymes during photosynthesis *in vivo*. *Plant J.* **84**, 900–913 https://doi.org/10.1111/tpj.13049
54 Wolosiuk, R., Crawford, N., Yee, B. and Buchanan, B. (1979) Isolation of 3 thioredoxins from spinach leaves. *J. Biol. Chem.* **254**, 1627–1632 PMID:216700
55 Broin, M., Cuine, S., Eymery, F. and Rey, P. (2002) The plastidic 2-cysteine peroxiredoxin is a target for a thioredoxin involved in the protection of the photosynthetic apparatus against oxidative damage. *Plant Cell* **14**, 1417–1432 https://doi.org/10.1105/tpc.001644
56 Collin, V., Lamkemeyer, P., Miginiac-Maslow, M., Hirasawa, M., Knaff, D.B., Dietz, K.J. et al. (2004) Characterization of plastidial thioredoxins from *Arabidopsis* belonging to the new y-type. *Plant Physiol.* **136**, 4088–4095 https://doi.org/10.1104/pp.104.052233
57 Laugier, E., Tarrago, L., Courteille, A., Innocenti, G., Eymery, F., Rumeau, D. et al. (2013) Involvement of thioredoxin y2 in the preservation of leaf methionine sulfoxide reductase capacity and growth under high light. *Plant Cell Environ.* **36**, 670–682 https://doi.org/10.1111/pce.12005
58 Arsova, B., Hoja, U., Wimmelbacher, M., Greiner, E., Ustun, S., Melzer, M. et al. (2010) Plastidial thioredoxin z interacts with two fructokinase-like proteins in a thiol-dependent manner: evidence for an essential role in chloroplast development in *Arabidopsis* and *Nicotiana benthamiana*. *Plant Cell* **22**, 1498–1515 https://doi.org/10.1105/tpc.109.071001
59 Kirchsteiger, K., Pulido, P., Gonzalez, M. and Javier Cejudo, F. (2009) NADPH thioredoxin reductase C controls the redox status of chloroplast 2-Cys peroxiredoxins in *Arabidopsis thaliana*. *Mol. Plant* **2**, 298–307 https://doi.org/10.1093/mp/ssn082

60 Pulido, P., Spínola, M.C., Kirchsteiger, K., Guinea, M., Belen Pascual, M., Sahrawy, M. et al. (2010) Functional analysis of the pathways for 2-Cys peroxiredoxin reduction in *Arabidopsis thaliana* chloroplasts. *J. Exp. Bot.* **61**, 4043–4054 https://doi.org/10.1093/jxb/erq218
61 Bernal-Bayard, P., Ojeda, V., Hervás, M., Cejudo, F.J., Navarro, J.A., Velázquez-Campoy, A. et al. (2014) Molecular recognition in the interaction of chloroplast 2-Cys peroxiredoxin with NADPH-thioredoxin reductase C (NTRC) and thioredoxin *x*. *FEBS Lett.* **588**, 4342–4347 https://doi.org/10.1016/j.febslet.2014.09.044
62 Michalska, J., Zauber, H., Buchanan, B.B., Cejudo, F.J. and 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 https://doi.org/10.1073/pnas.0903559106
63 Skryhan, K., Cuesta-Seijo, J.A., Nielsen, M.M., Marri, L., Mellor, S.B., Glaring, M.A. et al. (2015) The role of cysteine residues in redox regulation and protein stability of *Arabidopsis thaliana* starch synthase 1. *PLoS ONE* **10**, e0136997 https://doi.org/10.1371/journal.pone.0136997
64 Stenbaek, A., Hansson, A., Wulff, R.P., Hansson, M., Dietz, K. and Jensen, P.E. (2008) NADPH-dependent thioredoxin reductase and 2-Cys peroxiredoxins are needed for the protection of Mg-protoporphyrin monomethyl ester cyclase. *FEBS Lett.* **582**, 2773–2778 https://doi.org/10.1016/j.febslet.2008.07.006
65 Richter, A.S., Peter, E., Rothbart, M., Schlicke, H., Toivola, J., Rintamäki, E. et al. (2013) Posttranslational influence of NADPH-dependent thioredoxin reductase C on enzymes in tetrapyrrole synthesis. *Plant Physiol.* **162**, 63–73 https://doi.org/10.1104/pp.113.217141
66 Pérez-Ruiz, J.M., Guinea, M., Puerto-Galán, L. and Javier Cejudo, F. (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 https://doi.org/10.1093/mp/ssu032
67 Richter, A.S., Pérez-Ruiz, J.M., Cejudo, F.J. and Grimm, B. (2018) Redox-control of chlorophyll biosynthesis mainly depends on thioredoxins. *FEBS Lett.* **592**, 3111–3115 https://doi.org/10.1002/1873-3468.13216
68 Carrillo, L.R., Froehlich, J.E., Cruz, J.A., Savage, L.J. and Kramer, D.M. (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 https://doi.org/10.1111/tpj.13226
69 Naranjo, B., Mignée, C., Krieger-Liszkay, A., Hornero-Méndez, D., Gallardo-Guerrero, L., Cejudo, F.J. et al. (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 https://doi.org/10.1111/pce.12652
70 Nikkanen, L., Guinea Diaz, M., Toivola, J., Tiwari, A. and Rintamäki, E. (2019) Multilevel regulation of non-photochemical quenching and state transitions by chloroplast NADPH-dependent thioredoxin reductase. *Physiol. Plant.* https://doi.org/ 10.1111/ppl.12914
71 Ancin, M., Fernández-Sán Millan, A., Larraya, L., Morales, F., Veramendi, J., Aranjuelo, I. et al. (2018) Thioredoxin m overexpression in tobacco chloroplasts inhibits the protein kinase STN7 and alters photosynthetic performance. *J. Exp. Bot.* **70**, 1005–1016 https://doi.org/10.1093/jxb/ery415
72 Yoshida, K. and Hisabori, T. (2018) Determining the rate-limiting step for light-responsive redox regulation in chloroplasts. *Antioxidants* **7**, 153 https://doi.org/10.3390/antiox7110153
73 Setterdahl, A., Chivers, P., Hirasawa, M., Lemaire, S., Keryer, E., Miginiac-Maslow, M. et al. (2003) Effect of pH on the oxidation-reduction properties of thioredoxins. *Biochemistry* **42**, 14877–14884 https://doi.org/10.1021/bi0302088
74 Hochmal, A.K., Zinzius, K., Charoenwattanasatien, R., Gäbelein, P., Mutoh, R., Tanaka, H. et al. (2016) Calredoxin represents a novel type of calcium-dependent sensor-responder connected to redox regulation in the chloroplast. *Nat. Commun.* **7**, 11847 https://doi.org/10.1038/ncomms11847
75 König, J., Baier, M., Horling, F., Kahmann, U., Harris, G., Schürmann, P. et al. (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**, 5738–5743 https://doi.org/10.1073/pnas.072644999
76 Hirasawa, M., Schürmann, P., Jacquot, J., Manieri, W., Jacquot, P., Keryer, E. et al. (1999) Oxidation-reduction properties of chloroplast thioredoxins, ferredoxin:thioredoxin reductase, and thioredoxin *f*-regulated enzymes. *Biochemistry* **38**, 5200–5205 https://doi.org/10.1021/bi982783v
77 Collin, V., Issakidis-Bourguet, E., Marchand, C., Hirasawa, M., Lancelin, J., Knaff, D. et al. (2003) The *Arabidopsis* plastidial thioredoxins — new functions and new insights into specificity. *J. Biol. Chem.* **278**, 23747–23752 https://doi.org/10.1074/jbc.M302077200
78 König, J., Muthuramalingam, M. and Dietz, K. (2012) Mechanisms and dynamics in the thiol/disulfide redox regulatory network: transmitters, sensors and targets. *Curr. Opin. Plant Biol.* **15**, 261–268 https://doi.org/10.1016/j.pbi.2011.12.002
79 Pérez-Ruiz, J.M., Naranjo, B., Ojeda, V., Guinea, M. and Cejudo, F.J. (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 https://doi.org/10.1073/pnas.1706003114
80 Breyton, C., Nandha, B., Johnson, G.N., Joliot, P. and Finazzi, G. (2006) Redox modulation of cyclic electron flow around photosystem I in C3 plants. *Biochemistry* **45**, 13465–13475 https://doi.org/10.1021/bi061439s
81 Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M. and Shikanai, T. (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. *Cell* **110**, 361–371 https://doi.org/10.1016/S0092-8674(02)00867-X
82 DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schünemann, D., Finazzi, G. et al. (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*. *Cell* **132**, 273–285 https://doi.org/10.1016/j.cell.2007.12.028
83 Burrows, P., Sazanov, L., Svab, Z., Maliga, P. and Nixon, P. (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid NDH genes. *EMBO J.* **17**, 868–876 https://doi.org/10.1093/emboj/17.4.868
84 Peltier, G., Aro, E. and Shikanai, T. (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. *Annu. Rev. Plant Biol.* **67**, 55–80 https://doi.org/10.1146/annurev-arplant-043014-114752
85 Suorsa, M., Rossi, F., Tadini, L., Labs, M., Colombo, M., Jahns, P. et al. (2016) PGR5-PGRL1-dependent cyclic electron transport modulates linear electron transport rate in *Arabidopsis thaliana*. *Mol. Plant* **9**, 271–288 https://doi.org/10.1016/j.molp.2015.12.001
86 Shikanai, T. and 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 https://doi.org/10.1016/j.molp.2016.08.004
87 Yamamoto, H. and Shikanai, T. (2019) PGR5-dependent cyclic electron flow protects photosystem I under fluctuating light at donor and acceptor sides. *Plant Physiol.* **179**, 588–600 https://doi.org/10.1104/pp.18.01343
88 Petroutsos, D., Terauchi, A.M., Busch, A., Hirschmann, I., Merchant, S.S., Finazzi, G. et al. (2009) PGRL1 participates in iron-induced remodeling of the photosynthetic apparatus and in energy metabolism in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **284**, 32770–32781 https://doi.org/10.1074/jbc.M109.050468

Biochemical Journal (2019) **476** 1159–1172
https://doi.org/10.1042/BCJ20180707

89 Leister, D. and Shikanai, T. (2013) Complexities and protein complexes in the Antimycin A-sensitive pathway of cyclic electron flow in plants. *Front. Plant Sci.* **4**, 161 https://doi.org/10.3389/fpls.2013.00161

90 Tikkanen, M., Rantala, S. and Aro, E. (2015) Electron flow from PSII to PSI under high light is controlled by PGR5 but not by PSBS. *Front. Plant Sci.* **6**, 521 https://doi.org/10.3389/fpls.2015.00521

91 Kanazawa, A., Ostendorf, E., Kohzuma, K., Hoh, D., Strand, D.D., Sato-Cruz, M. et al. (2017) Chloroplast ATP synthase modulation of the thylakoid proton motive force: implications for photosystem I and photosystem II photoprotection. *Front. Plant Sci.* **8**, 719 https://doi.org/10.3389/fpls.2017.00719

92 Martin, M., Noarbe, D.M., Serrot, P.H. and Sabater, B. (2015) The rise of the photosynthetic rate when light intensity increases is delayed in NDH gene-defective tobacco at high but not at low CO_2 concentrations. *Front. Plant Sci.* **6**, 34 https://doi.org/10.3389/fpls.2015.00034

93 Yamori, W., Shikanai, T. and Makino, A. (2015) Photosystem I cyclic electron flow via chloroplast NADH dehydrogenase-like complex performs a physiological role for photosynthesis at low light. *Sci. Rep.* **5**, 15593 https://doi.org/10.1038/srep15593

94 Yamori, W., Makino, A. and Shikanai, T. (2016) A physiological role of cyclic electron transport around photosystem I in sustaining photosynthesis under fluctuating light in rice. *Sci. Rep.* **6**, 20147 https://doi.org/10.1038/srep20147

95 Shimakawa, G. and Miyake, C. (2018) Changing frequency of fluctuating light reveals the molecular mechanism for P700 oxidation in plant leaves. *Plant Direct* **2**, e00073 https://doi.org/10.1002/pld3.73

96 Yamamoto, H., Peng, L., Fukao, Y. and Shikanai, T. (2011) An SRC homology 3 domain-like fold protein forms a ferredoxin binding site for the chloroplast NADH dehydrogenase-like complex in *Arabidopsis*. *Plant Cell* **23**, 1480–1493 https://doi.org/10.1105/tpc.110.080291

97 Hanke, G. and Mulo, P. (2013) Plant type ferredoxins and ferredoxin-dependent metabolism. *Plant Cell Environ.* **36**, 1071–1084 https://doi.org/10.1111/pce.12046

98 Holt, N., Fleming, G. and Niyogi, K. (2004) Toward an understanding of the mechanism of non-photochemical quenching in green plants. *Biochemistry* **43**, 8281–8289 https://doi.org/10.1021/bi0494020

99 Takizawa, K., Cruz, J.A., Kanazawa, A. and Kramer, D.M. (2007) The thylakoid proton motive force in vivo. Quantitative, non-invasive probes, energetics, and regulatory consequences of light-induced pmf. *Biochim. Biophys. Acta Bioenerg.* **1767**, 1233–1244 https://doi.org/10.1016/j.bbabio.2007.07.006

100 Arnoux, P., Morosinotto, T., Saga, G., Bassi, R. and Pignol, D. (2009) A structural basis for the pH-dependent xanthophyll cycle in *Arabidopsis thaliana*. *Plant Cell* **21**, 2036–2044 https://doi.org/10.1105/tpc.109.068007

101 Brooks, M.D., Sylak-Glassman, E.J., Fleming, G.R. and Niyogi, K.K. (2013) A thioredoxin-like/beta-propeller protein maintains the efficiency of light harvesting in *Arabidopsis*. *Proc. Natl Acad. Sci. U.S.A.* **110**, E2733–E2740 https://doi.org/10.1073/pnas.1305443110

102 Malnoë, A., Schultink, A., Shahrasbi, S., Rumeau, D., Havaux, M. and Niyogi, K.K. (2018) The plastid lipocalin LCNP is required for sustained photoprotective energy dissipation in *Arabidopsis*. *Plant Cell* **30**, 196–208 https://doi.org/ 10.1105/tpc.17.00536

103 Tiwari, A., Mamedov, F., Grieco, M., Suorsa, M., Jajoo, A., Styring, S. et al. (2016) Photodamage of iron-sulphur clusters in photosystem I induces non-photochemical energy dissipation. *Nat. Plants* **2**, 16035 https://doi.org/10.1038/NPLANTS.2016.35

104 Puerto-Galán, L., Pérez-Ruiz, J.M., Guinea, M. and Cejudo, F.J. (2015) The contribution of NADPH thioredoxin reductase C (NTRC) and sulfiredoxin to 2-Cys peroxiredoxin overoxidation in *Arabidopsis thaliana* chloroplasts. *J. Exp. Bot.* **66**, 2957–2966 https://doi.org/10.1093/jxb/eru512

105 Eliyahu, E., Rog, I., Inbal, D. and Danon, A. (2015) ACHT4-driven oxidation of APS1 attenuates starch synthesis under low light intensity in *Arabidopsis* plants. *Proc. Natl Acad. Sci. U.S.A.* **112**, 12876–12881 https://doi.org/10.1073/pnas.1515513112

106 Vaseghi, M., Chibani, K., Telman, W., Liebthal, M.F., Gerken, M., Schnitzer, H. et al. (2018) The chloroplast 2-Cysteine peroxiredoxin functions as thioredoxin oxidase in redox regulation of chloroplast metabolism. *eLife* **7**, e38194 https://doi.org/10.7554/eLife.38194

107 Yoshida, K., Hara, A., Sugiura, K., Fukaya, Y. and Hisabori, T. (2018) Thioredoxin-like2/2-Cys peroxiredoxin redox cascade supports oxidative thiol modulation in chloroplasts. *Proc. Natl Acad. Sci. U.S.A.* **115**, E8296–E8304 https://doi.org/10.1073/pnas.1808284115

108 Dangoor, I., Peled-Zehavi, H., Wittenberg, G. and Danon, A. (2012) A chloroplast light-regulated oxidative sensor for moderate light intensity in *Arabidopsis*. *Plant Cell* **24**, 1894–1906 https://doi.org/10.1105/tpc.112.097139

109 Furbank, R.T. (2016) Walking the C_4 pathway: past, present, and future. *J. Exp. Bot.* **67**, 4057–4066 https://doi.org/10.1093/jxb/erw161

110 Majeran, W., Zybailov, B., Ytterberg, A.J., Dunsmore, J., Sun, Q. and van Wijk, K.J. (2008) Consequences of C_4 differentiation for chloroplast membrane proteomes in maize mesophyll and bundle sheath cells. *Mol. Cell Proteomics* **7**, 1609–1638 https://doi.org/10.1074/mcp.M800016-MCP200

111 Friso, G., Majeran, W., Huang, M., Sun, Q. and van Wijk, K.J. (2010) Reconstruction of metabolic pathways, protein expression, and homeostasis machineries across maize bundle sheath and mesophyll chloroplasts: large-scale quantitative proteomics using the first maize genome assembly. *Plant Physiol.* **152**, 1219–1250 https://doi.org/10.1104/pp.109.152694

112 Nakamura, N., Iwano, M., Havaux, M., Yokota, A. and Munekage, Y.N. (2013) Promotion of cyclic electron transport around photosystem I during the evolution of NADP-malic enzyme-type C4 photosynthesis in the genus *Flaveria*. *New Phytol.* **199**, 832–842 https://doi.org/10.1111/nph.12296

113 Rey, P., Pruvot, G., Becuwe, N., Eymery, F., Rumeau, D. and Peltier, G. (1998) A novel thioredoxin-like protein located in the chloroplast is induced by water deficit in *Solanum tuberosum* L. plants. *Plant J.* **13**, 97–107 https://doi.org/10.1046/j.1365-313X.1998.00015.x

114 López-Castillo, L.M., Jiménez-Sandoval, P., Baruch-Torres, N., Trasviña-Arenas, C.H., Díaz-Quezada, C., Lara-González, S. et al. (2016) Structural basis for redox regulation of cytoplasmic and chloroplastic triosephosphate isomerases from *Arabidopsis thaliana*. *Front. Plant Sci.* **7**, 1817 https://doi.org/10.3389/fpls.2016.01817

115 Decottignies, P., Schmitter, J., Miginiac-Maslow, M., Lemarechal, P., Jacquot, J. and Gadal, P. (1988) Primary structure of the light-dependent regulatory site of corn NADP-malate dehydrogenase. *J. Biol. Chem.* **263**, 11780–11785 PMID:3403553

116 Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K. et al. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science* **354**, 857–861 https://doi.org/10.1126/science.aai8878

117 Slattery, R.A., Walker, B.J., Weber, A.P.M. and Ort, D.R. (2018) The impacts of fluctuating light on crop performance. *Plant Physiol.* **176**, 990–1003 https://doi.org/10.1104/pp.17.01234

118 Long, S., Zhu, X., Naidu, S. and Ort, D. (2006) Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* **29**, 315–330 https://doi.org/10.1111/j.1365-3040.2005.01493.x

119 Naranjo, B., Diaz-Espejo, A., Lindahl, M. and Cejudo, F.J. (2016) Type-*f* thioredoxins have a role in the short-term activation of carbon metabolism and their loss affects growth under short-day conditions in *Arabidopsis thaliana*. *J. Exp. Bot.* **67**, 1951–1964 https://doi.org/10.1093/jxb/erw017