Signaling and Communication in Plants

Sanjib Kumar Panda
Yoshiharu Y. Yamamoto *Editors*

Redox Homeostasis in Plants

From Signalling to Stress Tolerance

Signaling and Communication in Plants

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Redox Homeostasis in Plants

From Signalling to Stress Tolerance

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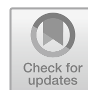
Thiol-based Redox Regulation in Plant Chloroplasts



Keisuke Yoshida and Toru Hisabori

Abstract To cope with fluctuating environmental cues, plants must regulate their own biological systems in a flexible manner. Thiol-based redox regulation is an important strategy to control the activity of target proteins in response to changes in local redox environments. In chloroplasts, this regulatory system is linked to the excitation of photosynthetic electron transport, allowing light-responsive control of chloroplast functions. A simple redox cascade mediated by the thioredoxin (Trx) has been accepted as the molecular basis of chloroplast redox regulation. However, it is becoming increasingly apparent that chloroplasts have a complicated redox network with divergent composition of redox-mediator proteins and their target proteins. Next, major challenges should be directed to comprehensively clarify how the overall system is organized in chloroplasts and works toward environmental fluctuations. This chapter gives an overview of the recent advances in understanding the biochemical basis and physiological significance of redox-based regulatory network in chloroplasts.

1 Introduction

Thiol-based redox regulation is a post-translational mechanism to control enzymatic activity by modifying the Cys residue on the target protein (e.g., formation/cleavage of disulfide bond). This regulatory system transmits environmental signals as a reducing power to targets, ensuring rapid adjustment of cellular functions. A key player for the redox regulation is the thioredoxin (Trx), which is a small protein first discovered in *Escherichia coli* as a hydrogen donor to ribonucleotide reductase (Laurent et al. 1964). Trx possesses a redox-sensitive Cys pair in the active site sequence of WCGPC. By using this Cys pair, Trx catalyzes the dithiol-disulfide exchange reac-

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tion and thereby acts as the redox mediator to its target proteins. Redox regulation system driven by Trx is ubiquitously found in all kingdoms of life.

In plant chloroplasts, redox regulation has a unique property that is associated with the photosynthetic electron transport chain. The canonical regulatory pathway is shown in Fig. 1a. A part of reducing power generated by photochemical reactions is transferred from a mobile electron carrier protein ferredoxin (Fd) to Trx via Fd-Trx reductase (FTR). A reduced form of Trx then transfers reducing power to specific target proteins. Four enzymes in the Calvin-Benson cycle (glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fructose-1,6-bisphosphatase (FBPase), sedoheptulose-1,7-bisphosphatase (SBPase), and phosphoribulokinase (PRK)) are classically well known as representative Trx targets. Because these enzymes are activated upon reduction, a series of reducing power transfer via FTR/Trx redox cascade makes it possible to turn on the carbon fixation metabolism in concert with the excitation of electron transport. This is a classic model for chloroplast redox regulation, established by Buchanan et al. in 1970s (Buchanan et al. 1979; Buchanan 1980; Buchanan and Balmer 2005). Chloroplast redox regulation dynamically works in vivo in response to changes in light conditions (Konno et al. 2012; Yoshida et al. 2014).

Now, we are at a turning point for the field of redox study. Owing to growing availability of omics data, a large number of proteins have been identified as the possible constituent of redox regulation system in chloroplasts (Fig. 1b). This emerging protein catalog indicates that chloroplasts have evolved a complex redox-based regulatory network, controlling a diverse array of functions in a flexible and sophisticated manner. In this regard, it is of great importance to understand how the redox regulation system is organized in chloroplasts and has impacts on plant viability. In this chapter, we review the current knowledge of the biochemical basis and physiological significance of chloroplast redox regulation. Recent other reviews are also available to gain insights into these issues (Michelet et al. 2013; Richter and Grimm 2013; Serrato et al. 2013; Balsera et al. 2014; Nikkanen and Rintamaki 2014; Geigenberger et al. 2017; Nikkanen et al. 2017).

2 Overview of Protein Multiplicity in Chloroplast Redox Regulation

The completion of the *Arabidopsis thaliana* genome sequence (Arabidopsis Genome Initiative 2000) has led to the identification of multiple genes encoding redox-mediator proteins, including Trxs and other related proteins. In *Arabidopsis*, seven subtypes of Trx (*f*-, *m*-, *x*-, *y*-, *z*-, *h*-, and *o*-type) encoded by totally twenty genes have been found in various subcellular compartments. Among them, five (*f*-, *m*-, *x*-, *y*-, and *z*-type) are localized in chloroplasts (Fig. 1b) (Lemaire et al. 2007; Serrato et al. 2013). They have different molecular characteristics, such as the protein surface charge and midpoint redox potential (Collin et al. 2003; Michelet et al. 2005; Toivola et al. 2013; Yoshida et al. 2015; Yoshida and Hisabori 2016), which possibly confers

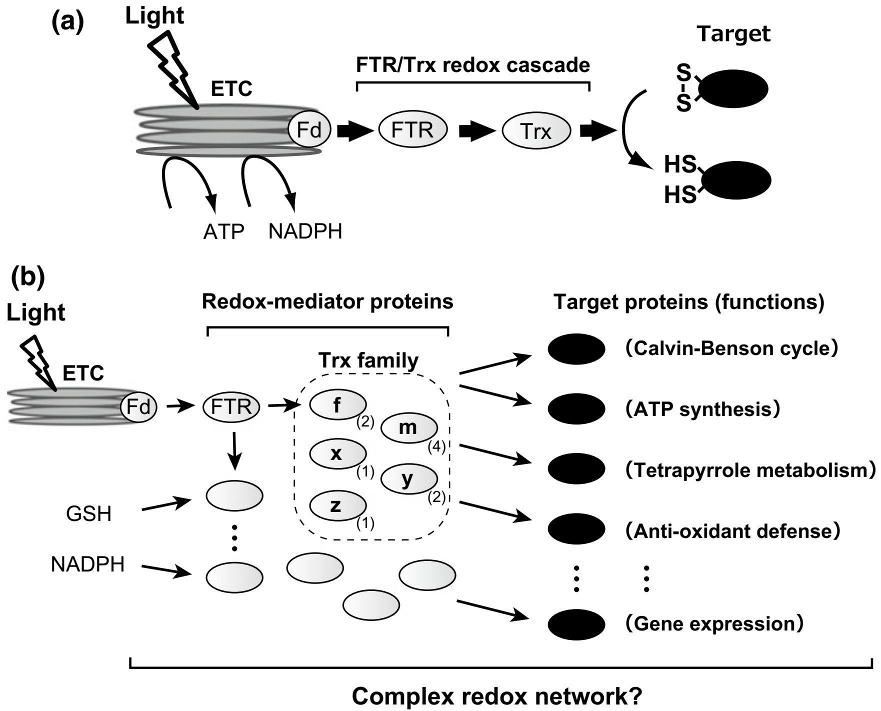

Fig. 1 Overview of thiol-based redox regulation in plant chloroplasts. **a** Classically known regulatory pathway. Reducing power is transferred from ferredoxin (Fd) in the electron transport chain (ETC) to specific target proteins *via* ferredoxin-thioredoxin reductase (FTR)/thioredoxin (Trx) redox cascade, enabling light-responsive control of chloroplast functions. **b** Newly emerging regulatory network, that is organized by multiple redox-mediator proteins and target proteins. This is possibly helpful for flexibly controlling a range of chloroplast functions; however, its whole picture remains undescribed. The number of gene encoding each Trx subtype in *Arabidopsis* is shown in the parenthesis. Abbreviations: GSH, glutathione

the functional versatility on chloroplast Trx family (see below). Besides, some novel redox-mediator proteins are also located in chloroplasts. The best-studied example is the NADPH-Trx reductase C (NTRC), a hybrid protein composed of an NADPH-Trx reductase (NTR) domain and a Trx domain (Serrato et al. 2004). Other proteins containing the Trx-like motif have been also predicted to participate in chloroplast redox regulation, although their functions remain to be characterized in detail.

Before the beginning of this century, a limited number of chloroplast proteins have been reported as the target of redox regulation. They included four Calvin-Benson cycle enzymes (GAPDH, FBPase, SBPase, and PRK), NADP-malate dehydrogenase (NADP-MDH, involved in the malate valve), glucose-6-phosphate dehydrogenase (G6PDH, involved in the oxidative pentose phosphate pathway), acetyl-CoA carboxylase (ACCase, involved in the fatty acid metabolism), Rubisco activase (RCA), and ATP synthase CF_1-γ subunit (Buchanan et al. 1979; Buchanan 1980; Mills et al.

1980; Scheibe and Anderson 1981; Sasaki et al. 1997; Zhang and Portis 1999). The major breakthrough was brought by the development of strategies for systematically screening Trx target candidates (Motohashi et al. 2001; Yano et al. 2001). For example, the Trx affinity chromatography using monocysteinic Trx as a bait allowed identification of previously unrecognized Trx targets in chloroplast stroma as well as classically known ones (Motohashi et al. 2001; Balmer et al. 2003). The newly discovered targets contain numerous proteins involved in nitrogen metabolism, starch synthesis, tetrapyrrole metabolism, antioxidant defense system, and other metabolic pathways in chloroplasts. Furthermore, target screening from the thylakoid membrane or chloroplast inner envelope has been challenged (Balmer et al. 2006; Motohashi and Hisabori 2006; Bartsch et al. 2008; Hall et al. 2010). These studies have indicated that Trx associates with the protein import machineries, proteases, and photosynthetic electron transport complexes. Thus, novel methods of so-called redox proteomics have identified a number of chloroplast proteins as the Trx target candidate (Fig. 1b). The integral lists for these proteins are provided by other reviews (Lindahl and Kieselbach 2009; Montrichard et al. 2009).

3 Functional Diversity of Chloroplast Trx Family: Insights from Biochemical Studies

Protein multiplicity in chloroplast redox regulation has raised a question: How do a variety of redox-mediator proteins and target proteins communicate in chloroplasts? Several biochemical studies have addressed functional specificity and redundancy of chloroplast Trx family. Before the genomic era, only Trx-*f* and Trx-*m* were known to exist in chloroplasts. As named according to their properties, Trx-*f* and Trx-*m* were originally defined as the efficient reductive activator for FBPase and NADP-MDH, respectively (Wolosiuk et al. 1979; Schurmann et al. 1981). High specificity of Trx-*f* for regulating FBPase has been well established by following biochemical studies (Geck et al. 1996; Collin et al. 2003; Yoshida et al. 2015). By contrast, the initial view for NADP-MDH redox regulation should be revised; NADP-MDH was shown to undergo Trx-*f*-dependent reductive activation with the efficiency comparable with or even higher than that of Trx-*m* (Hodges et al. 1994; Geck et al. 1996; Collin et al. 2003). NADP-MDH contains two redox-active Cys pairs at N- and C-terminal extensions (Miginiac-Maslow and Lancelin 2002). Our recent study revealed that Trx-*m* can reduce only C-terminal disulfide bond, whereas Trx-*f* can reduce both N- and C-terminal ones, allowing full activation of NADP-MDH (Yoshida et al. 2015). This highlights a unique aspect of NADP-MDH redox regulation; two Cys pairs in a single polypeptide have different Trx selectivity.

It has been shown that, compared to Trx-*m*, Trx-*f* is more effective in activating other Calvin-Benson cycle enzymes (GAPDH, SBPase, and PRK), RCA, and ATP synthase (Schwarz et al. 1997; Zhang and Portis 1999; Marri et al. 2009; Yoshida et al. 2015). Furthermore, other metabolic enzymes involved in the oxidative pen-

tose phosphate pathway (G6PDH), starch synthesis or degradation (ADP-glucose pyrophosphorylase (AGPase), starch synthase, α- or β-amylase, and starch phosphorylase), and lipid synthesis (ACCase and monogalactosyldiacylglycerol synthase) are redox-regulated by Trx-*f* with higher efficiency than that of Trx-*m* (Sasaki et al. 1997; Ballicora et al. 2000; Mikkelsen et al. 2005; Sparla et al. 2006; Yamaryo et al. 2006; Nee et al. 2009; Seung et al. 2013; Silver et al. 2013; Thormahlen et al. 2013; Skryhan et al. 2015). Taken together, Trx-*f* can be regarded as the major redox regulator for a range of photosynthetic reactions and other metabolisms in chloroplasts (Fig. 2a). By contrast, the specific role of Trx-*m* remains elusive. As a Trx-*m*-specific target, HCF164 was identified; HCF164 is reduced by Trx-*m*, but not Trx-*f* (Motohashi and Hisabori 2006). HCF164 is a Trx-like protein anchored to the thylakoid membrane, and its redox-active site faces the lumenal side. Together with the thylakoid protein CcdA (Motohashi and Hisabori 2010), HCF164 is supposed to act as a reducing power transmitter from stroma to lumenal proteins. Some lumenal proteins involved in the photosynthetic electron transport, xanthophylls cycle, and immunophilins were suggested to be under redox regulation (Gopalan et al. 2004; Motohashi and Hisabori 2006; Hall et al. 2010; Simionato et al. 2015). It is therefore possible that Trx-*m* indirectly controls key reactions in the thylakoid membrane. This may be related to growth inhibition in Trx-*m*-deficient plants (see below).

In 2000s, genomic and phylogenic studies have identified additional three types of chloroplast Trx (Trx-*x*, -*y*, -*z*) (Fig. 2a). Trx-*x* and Trx-*y* are efficient electron donor to 2-Cys peroxiredoxin (Prx) and Prx Q, respectively, both of which are involved in the antioxidant defense system (Collin et al. 2003, 2004; Yoshida et al. 2015). In addition, we recently showed that Trx-*y* can reduce Mg-chelatase I subunit (CHLI, involved in the tetrapyrrole metabolism) with high efficiency (Yoshida and Hisabori 2016). Trx-*z* was also reported to serve to transfer reducing power to several antioxidant enzymes, such as Prx Q and Met sulfoxide reductase (MSR) (Chibani et al. 2011; Yoshida et al. 2015). More importantly, Trx-*z* may play a critical role in regulating plastidial transcription, although its regulatory mechanism is somewhat unclear (Arsova et al. 2010; Wimmelbacher and Bornke 2014). Further biochemical characterization is required for better understanding of Trx-*z*-mediated redox regulation.

Given that each Trx subtype differentially controls diverse target proteins, the electron partitioning from FTR to Trx family is thought to be the critical step for determining the consequence of chloroplast redox regulation. We recently addressed the electron transfer from FTR to ten Trx isoforms in *Arabidopsis* (Yoshida and Hisabori 2017). The results showed that all Trxs can be reduced by FTR, but their reduction kinetics are largely variable (Fig. 2b). In addition to the above-described target selectivity, these data also highlight an aspect of highly organized circuits of chloroplast Trx family.

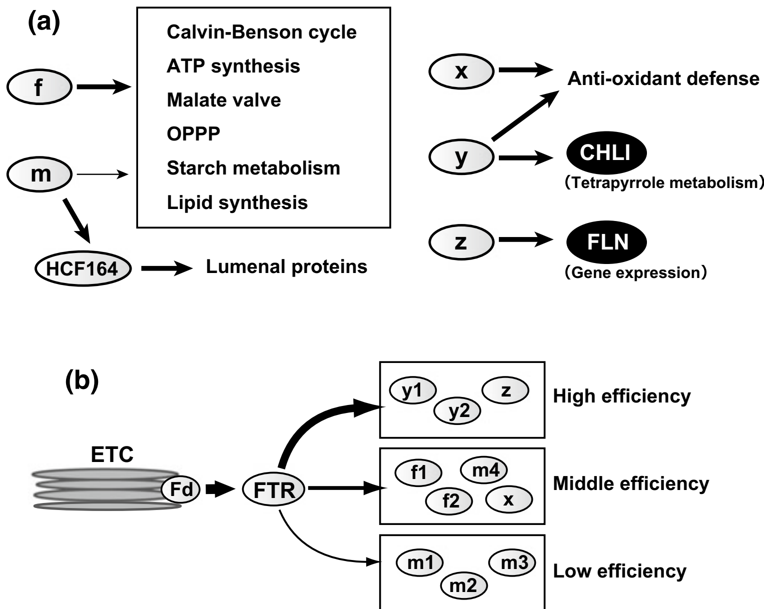

Fig. 2 Functional diversity of chloroplast thioredoxin (Trx) family. **a** Target selectivity of five Trx subtypes (Trx-*f*, -*m*, -*x*, -*y*, and -*z*). Pathways for transferring reducing power from each Trx to target proteins or functions are described. An arrow with a thin line indicates low efficiency in reducing power transfer. See text for details. **b** Distinct reducing power transfer from ferredoxin-thioredoxin reductase (FTR) to ten Trx isoforms in *Arabidopsis*. Ten Trx isoforms can be clustered into three classes based on the efficiencies in FTR-dependent reduction. Modified from Yoshida and Hisabori (2017). Abbreviations: CHLI, Mg-chelatase I subunit; ETC, electron transport chain; Fd, ferredoxin; FLN, fructokinase-like protein; OPPP, oxidative pentose phosphate pathway

4 Physiological Impact of Trx: Insights from Reverse-Genetic Studies

Biochemical data have provided valuable insights into molecular mechanisms of redox regulation, which is, however, still weak to discuss their in vivo relevance. Instead, reverse-genetic studies using *Arabidopsis* mutant plants have been adopted to understand physiological significances of each Trx subtype. Their results are summarized in Table 1.

In the Trx-*f*-deficient mutant (*trxf1* single or *trxf1/trxf2* double mutant), light-dependent reduction of FBPase, RCA, and AGPase was partially impaired, suggesting that Trx-*f* donates reducing power to these target proteins in vivo (Thormahlen et al. 2013, 2015; Yoshida et al. 2015; Naranjo et al. 2016a). Accompanied by the impairment of AGPase reduction, starch accumulation level was lowered in *trxf1* mutant (Thormahlen et al. 2013, 2015). By contrast, Trx-*f*-overexpressed tobacco plants showed an elevated level of starch accumulation (Sanz-Barrio et al. 2013). The *trxf1/trxf2* double mutant showed impaired photosynthesis and growth at high

Table 1 Phenotypes of *Arabidopsis* mutants deficient in each Trx subtype

Arabidopsis mutant	Notable phenotype	References
Trx-f-deficient mutant		
trxf1	Impairment of light-dependent redox regulation of AGPase	Thormahlen et al. (2013)
trxf1/trxf2	Impairment of light-dependent redox regulation of FBPase	Yoshida et al. (2015)
trxf1/trxf2	Growth impairment under high-light and short-day conditions	Naranjo et al. (2016a)
	Impairment of light-dependent redox regulation of FBPase and RCA	
	Decrease in photosynthetic electron transport efficiency	
Trx-m-deficient mutant		
trxm4	Enhancement of NDH-dependent cyclic electron transport	Courteille et al. (2013)
trxm1/trxm2/trxm4	Growth impairment with pale-green leaves	Wang et al. (2013)
	Impaired biogenesis of photosystem II	
trxm1/trxm2/trxm4	Growth impairment with pale-green leaves	Okegawa and Motohashi (2015)
trxm1/trxm2	Impairment of light-dependent activation of NADP-MDH	Thormahlen et al. (2017)
trxm1/trxm2/trxm4	Impaired chlorophyll synthesis	Da et al. (2017)
trxm1/trxm2/trxm4	Elevated NPQ concomitant with disturbance of xanthophyll cycle	Da et al. (2018)
Trx-x-deficient mutant		
trxx	Not observed	Pulido et al. (2010)
trxx	Not observed	Ojeda et al. (2017)
Trx-y-deficient mutant		
trxy1/trxy2	Lowered capacity of MSR	Laugier et al. (2013)
Trx-z-deficient mutant		
trxz	Albino phenotype	Arsova et al. (2010)
	Modified expression of chloroplast-encoded genes	

Abbreviations *AGPase* ADP-glucose pyrophosphorylase; *FBPase* fructose-1,6-bisphosphatase; *MSR* Met sulfoxide reductase; *NADP-MDH* NADP-malate dehydrogenase; *NDH* NADH dehydrogenase-like complex; *NPQ* non-photochemical quenching; *RCA* Rubisco activase; *Trx* thioredoxin

light intensity (Naranjo et al. 2016a). These all data suggest that Trx-*f* has a significant impact on the adjustment of photosynthetic metabolism in illuminated leaves. On the other hand, the Trx-*x*-deficient mutant did not show any notable phenotype, possibly due to functional redundancy with other redox systems (Pulido et al. 2010; Ojeda et al. 2017). As well, the deficiency of Trx-*y* did not cause drastic alteration of growth, but MSR capacity was slightly lowered (Laugier et al. 2013).

In *Arabidopsis*, suppressed expression of three Trx-*m* isoforms (Trx-*m*1, Trx-*m*2, and Trx-*m*4) resulted in growth impairment even under normal growth conditions (Wang et al. 2013; Okegawa and Motohashi 2015), which is apparently distinct from phenotypes of other Trx-deficient mutants. Such growth phenotype was observed in Trx-*m* knockdown mutants in rice (Chi et al. 2008). Therefore, Trx-*m* is thought to have some specific roles in plant development, but its detailed functions are still undetermined. The *Arabidopsis trxm1/trxm2/trxm4* triple mutants failed to assemble photosystem II complex properly (Wang et al. 2013). Besides, other studies using *trxm* mutants suggest that Trx-*m* is involved in the regulation of cyclic electron transport around photosystem I and non-photochemical quenching (Courteille et al. 2013; Da et al. 2018). Considering these reports, the main function of Trx-*m* may be related to the biogenesis and maintenance of photosynthetic electron transport machineries, rather than the metabolic control in the stroma. This possibility is partly supported by the proteomic study indicating that Trx-*m* is localized adjacent to the thylakoid membrane (Peltier et al. 2002), but further biochemical evidence is needed to conclude this. It should be mentioned that another isoform of Trx-*m* (Trx-*m*3) is expressed in non-green plastids and is essential for meristem maintenance (Benitez-Alfonso et al. 2009).

In *Arabidopsis* and tobacco, reduced expression of Trx-*z* resulted in a severe albino phenotype (Arsova et al. 2010). Based on the yeast two-hybrid assay, Trx-*z* was shown to bind to fructokinase-like protein (FLN). The association of Trx-*z* with FLN may be essential for plastid-encoded RNA polymerase (PEP)-dependent gene expression. This hypothesis is supported by proteomic studies showing that both Trx-*z* and FLN are the component of PEP complex (Schroter et al. 2010; Steiner et al. 2011). However, the importance of redox regulation during this crosstalk is still a matter of debate. Because redox activity of Trx-*z* and FLN may be dispensable for plant growth, it cannot be excluded that these proteins are only structural components of PEP complex (Wimmelbacher and Bornke 2014).

5 NTRC: A Notable Redox-Mediator Protein

NTR is a redox-mediator protein that contains a flavin adenine dinucleotide (FAD) cofactor and a redox-active disulfide bond (Jacquot et al. 2009). In *Arabidopsis*, two isoforms of NTR (NTRA and NTRB) are known to be present in cytosol and mitochondria; however, NTRC was exceptionally identified in chloroplasts as a unique bimodular NTR which harbors Trx domain at a C-terminus (Serrato et al. 2004). As using NADPH as a source of reducing power, NTRC can work independently from

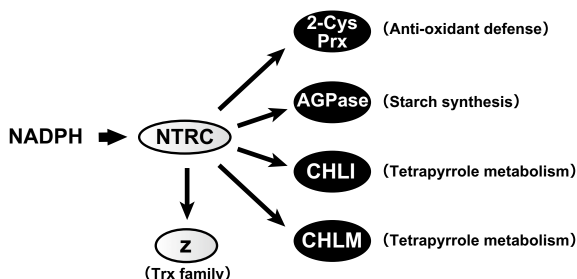

Fig. 3 Target proteins of NADPH-thioredoxin reductase C (NTRC) in chloroplasts. Pathways for NTRC-mediated reducing power transfer are described. Abbreviations: AGPase, ADP-glucose pyrophosphorylase; CHLI, Mg-chelatase I subunit; CHLM, Mg protoporphyrin IX methyltransferase; 2-Cys Prx, 2-Cys peroxiredoxin

light-driven FTR/Trx redox cascade. NTRC-dependent redox regulation is thought to have some indispensable roles for plants, because an *Arabidopsis* NTRC-deficient mutant (*ntrc* mutant) shows an impaired growth phenotype with pale-green leaves. To date, a number of studies have addressed biochemical and physiological aspects of this novel protein (Cejudo et al. 2012).

NTRC-targeted chloroplast proteins, which have been suggested based on the biochemical studies, are described in Fig. 3. Impacts of the absence of NTRC on *Arabidopsis* plant phenotype are summarized in Table 2. The first-reported and the most well-known target of NTRC is 2-Cys Prx (Moon et al. 2006; Perez-Ruiz et al. 2006). The biochemical reaction mode of NTRC with 2-Cys Prx has been clarified in detail (Perez-Ruiz and Cejudo 2009; Bernal-Bayard et al. 2012). Prior to these findings, 2-Cys Prx was shown to receive reducing power from Trxs (Konig et al. 2002; Collin et al. 2003) and CDSP32, a protein containing two Trx modules (Broin et al. 2002). However, NTRC emerges higher efficiency in reducing 2-Cys Prx than these redox-mediator proteins (Moon et al. 2006; Perez-Ruiz et al. 2006; Bernal-Bayard et al. 2014; Yoshida and Hisabori 2016). Given the hypersensitivity to several abiotic stresses in *ntrc* mutants (Table 2), NTRC/2-Cys Prx redox pathway is likely to constitute the major antioxidant system in chloroplasts. As present in some cyanobacteria (Sueoka et al. 2009; Pascual et al. 2011; Sanchez-Riego et al. 2016; Mihara et al. 2017), this is considered to be an evolutionally conserved system.

The phenotype of *ntrc* mutant is more severe than that of 2-Cys Prx-deficient plant, indicating additional functions of NTRC (Pulido et al. 2010). It has been demonstrated by in vitro studies that AGPase, CHLI, and Mg-protoporphyrin IX methyltransferase (CHLM, involved in the tetrapyrrole metabolism) are subjected to NTRC-dependent redox regulation (Fig. 3) (Michalska et al. 2009; Richter et al. 2013; Perez-Ruiz et al. 2014; Yoshida and Hisabori 2016). AGPase and CHLI are also redox-regulated by Trx (Ballicora et al. 2000; Ikegami et al. 2007; Luo et al. 2012; Thormahlen et al. 2013), but at least for CHLI reduction, NTRC seems to have higher efficiency than Trx (Perez-Ruiz et al. 2014; Yoshida and Hisabori 2016). In accordance with these biochemical results, *ntrc* mutants show the phenotypes of

Table 2 Phenotypes of *Arabidopsis* NTRC-deficient mutants and multiple mutants deficient in NTRC and FTR/Trx pathways

Arabidopsis mutant	Notable phenotype	References[a]
ntrc	Growth impairment with pale-green leaves	Serrato et al. (2004), Perez-Ruiz et al. (2006)
	Hypersensitivity to abiotic stresses	Serrato et al. (2004), Perez-Ruiz et al. (2006)
	Photoperiod-dependent growth inhibition	Perez-Ruiz et al. (2006), Lepisto et al. (2009)
	Accumulation of oxidized 2-Cys Prx	Perez-Ruiz et al. (2006), Kirchsteiger et al. (2009)
	Impaired redox regulation of AGPase and lowered starch content	Michalska et al. (2009)
	Impaired chlorophyll synthesis	Stenbaek et al. (2008), Richter et al. (2013)
	Elevated NPQ	Carrillo et al. (2016), Naranjo et al. (2016b)
ntrc/trxf1	Severe growth impairment	Thormahlen et al. (2015)
	Changes in metabolite profiles	
ftrb/ntrc	Lethal phenotype under autotrophic conditions	Yoshida and Hisabori (2016)
	Disruption of photosynthetic system	
ntrc/trxf1/trxf2	Severe growth impairment	Ojeda et al. (2017)
	Abnormal structure of chloroplasts	
ntrc/trxx	Severe growth impairment	Ojeda et al. (2017)
	Abnormal structure of chloroplasts	
ntrc/trxm1/trxm2/trxm4	Severe growth impairment	Da et al. (2017)
	Impairment of tetrapyrrole metabolism	

[a]Only representative references are described
Abbreviations *AGPase* ADP-glucose pyrophosphorylase; *2-Cys Prx* 2-Cys peroxiredoxin; *NPQ* non-photochemical quenching

lowered starch content and impaired chlorophyll synthesis (Table 2). Furthermore, other proteins including glutamyl-transfer RNA reductase (GluTR, involved in the tetrapyrrole metabolism), FBPase, PRK, and ATP synthase CF_1-γ subunit have been also suggested to interact with NTRC, based on the bimolecular fluorescence complementation or co-immunoprecipitation experiments (Richter et al. 2013; Nikkanen et al. 2016). However, it is still unconcluded whether they are actually capable of receiving reducing power from NTRC. It should be mentioned that some studies

showed the inability of NTRC to reduce FBPase (Yoshida and Hisabori 2016; Ojeda et al. 2017).

In recent years, the exchange of reducing power between NTRC and Trxs has been suggested (Toivola et al. 2013; Nikkanen et al. 2016). Using a chromatography-based method, we revealed that NTRC associates with Trx-*z* with high affinity. The following biochemical assay indicated that NTRC can reduce Trx-*z* but not other Trx subtypes (Yoshida and Hisabori 2016). We therefore concluded that Trx-*z* is a unique Trx targeted by NTRC (Fig. 3); however, it should be investigated in more detail how this interplay works *in planta*.

6 Significance of Cooperative Redox Regulation by FTR/Trx and NTRC Pathways

As documented above, accumulating evidence suggests that NTRC has redox-regulatory functions distinct from those of FTR/Trx system. Recent studies have addressed the functional coordination of FTR/Trx and NTRC pathways, by constructing multiple mutants in *Arabidopsis* (Table 2).

Combined deficiency of NTRC and one Trx subtype (Trx-*f*, Trx-*m*, or Trx-*x*) results in more remarkable growth phenotype than that of *ntrc* single mutants. This severe growth impairment is accompanied by pleiotropic phenotypes, including changes in metabolite profiles and abnormal ultrastructure of chloroplasts (Thormahlen et al. 2015; Da et al. 2017; Ojeda et al. 2017). Most strikingly, double mutants impaired in FTR and NTRC expression display lethal phenotype under autotrophic growth conditions (Yoshida and Hisabori 2016). These mutants can survive in the sucrose-containing medium, but suffer from a drastic loss of photosynthetic performance and the resulting growth retardation. These all data indicate that FTR/Trx and NTRC pathways cooperatively control a range of chloroplast functions, which is critical for plant development and viability.

Apart from FTR/Trx redox cascade, NTRC can function even under non-photosynthetic conditions by using NADPH produced by the oxidative pentose phosphate pathway. This property is possibly beneficial at the early stage of plastid differentiation when the electron transport system is still immature. It therefore seems to be attractive to study how the engagement of each FTR/Trx or NTRC pathway changes depending on the developmental phase. The functional dynamics of two redox systems should be comprehensively dissected by future researches.

7 Concluding Remarks

It has been increasingly uncovered that chloroplast redox network is highly organized, dynamically responds to environments, and plays a critical role in plant biomass

production. This regulatory system confers the elegant strategy on plants for surviving under ever-fluctuating natural conditions. However, the whole picture of chloroplast redox network is not yet understood. For example, the biochemical characterization of putative redox-mediator proteins (Trx-like proteins) remains to be fully attained, although some studies have addressed this issue (Dangoor et al. 2009, 2012; Chibani et al. 2012; Eliyahu et al. 2015). Furthermore, it is of an intriguing issue to study how a complex set of these proteins responds to environmental stresses and serves to control a wide variety of chloroplast functions. More comprehensive and in-depth studies will reveal an expanded map of chloroplast redox network and its biological functions.

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