Review

Daniel Wittmann, Neha Sinha and Bernhard Grimm*

Thioredoxin-dependent control balances the metabolic activities of tetrapyrrole biosynthesis

<https://doi.org/10.1515/hsz-2020-0308>Received September 12, 2020; accepted October 13, 2020;
published online October 28, 2020

Abstract: Plastids are specialized organelles found in plants, which are endowed with their own genomes, and differ in many respects from the intracellular compartments of organisms belonging to other kingdoms of life. They differentiate into diverse, plant organ-specific variants, and are perhaps the most versatile organelles known. Chloroplasts are the green plastids in the leaves and stems of plants, whose primary function is photosynthesis. In response to environmental changes, chloroplasts use several mechanisms to coordinate their photosynthetic activities with nuclear gene expression and other metabolic pathways. Here, we focus on a redox-based regulatory network composed of thioredoxins (TRX) and TRX-like proteins. Among multiple redox-controlled metabolic activities in chloroplasts, tetrapyrrole biosynthesis is particularly rich in TRX-dependent enzymes. This review summarizes the effects of plastid-localized reductants on several enzymes of this pathway, which have been shown to undergo dithiol-disulfide transitions. We describe the impact of TRX-dependent control on the activity, stability and interactions of these enzymes, and assess its contribution to the provision of adequate supplies of metabolic intermediates in the face of diurnal and more rapid and transient changes in light levels and other environmental factors.

Keywords: chlorophyll synthesis; chloroplast biogenesis; NTRC; post-translational control; thiol switch; thioredoxin.

Introduction

Apart from exploiting the benefits of oxygen, life in an aerobic environment must cope with its attendant drawbacks – the formation of reactive oxygen species (ROS), including singlet oxygen, the superoxide anion and hydrogen peroxide. Although accumulation of ROS is mainly associated with cell damage caused by the irreversible oxidation of proteins, lipids and nucleic acids, ROS also play a major role in triggering appropriate responses to environmental stresses. Due to the oxygenic nature of plant photosynthesis, light absorption by the photosynthetic pigments, as well as energy transfer and energy conversion throughout the photosynthetic electron transport chain present severe challenges for organisms that are vulnerable and sessile. The drastic effects of the abiotic stressors oxygen and light make reliable protective mechanisms in spatial proximity to the photosynthetic machinery essential.

The efficacy of light-dependent and oxygen-producing photosynthesis also depends on the availability of adequate amounts of the photosynthetic pigments chlorophyll (Chl), carotenoids and phycobilins. Chl and bilins are end-products of tetrapyrrole biosynthesis (TBS). In higher plants, this pathway is precisely regulated to avoid the excess accumulation of photoreactive intermediates, which would cause subcellular photo-oxidative damage. The TBS pathway is not unique to plants, but is indispensable for nearly all known organisms. In plants, the chloroplast-localized TBS pathway produces four main tetrapyrrole end products. All have a large macrocyclic structure consisting of four pyrrole rings, linked in either a linear or cyclic fashion via diverse combinations of methine bridges. Cyclic tetrapyrroles comprise Chl, heme and siroheme. Chl *a* and *b* are essential for light absorption and excitation energy transfer in antenna proteins of photosystems I and II, as well as for charge separation in the core complexes of the photosystems. Heme acts as the prosthetic group of cytochromes and is involved in photosynthetic and mitochondrial electron transport, among other functions. As the prosthetic cofactor in nitrite and sulfite reductases,

*Corresponding author: **Bernhard Grimm**, Humboldt-Universität zu Berlin, Faculty of Life Science, Institute of Biology/Plant Physiology, Philippstraße 13 (Building 12), 10115 Berlin, Germany, E-mail: bernhard.grimm@rz-hu-berlin.de. <https://orcid.org/0000-0002-9730-1074>

Daniel Wittmann and Neha Sinha, Humboldt-Universität zu Berlin, Faculty of Life Science, Institute of Biology/Plant Physiology, Philippstraße 13 (Building 12), 10115 Berlin, Germany. <https://orcid.org/0000-0003-2965-1440> (D. Wittmann), <https://orcid.org/0000-0002-5589-0309> (N. Sinha)

siroheme plays a role in nitrogen and sulfur assimilation. In addition, the linear tetrapyrrole phytychromobilin acts as the chromophore of phytychromes and is essential for red-light perception and signal transmission (Grimm, 2019a/b; Tanaka et al. 2011). Other phycobilins assembled in phycobilisomes of cyanobacteria or red algae participate in light-harvesting.

The TBS of photosynthetic organisms shares common steps, starting with the tightly regulated formation of 5-aminolevulinic acid (ALA). But many more regulatory mechanisms have been identified in later steps of TBS. The regulation of tetrapyrrole metabolism takes place both at the transcriptional level (Kobayashi and Masuda 2016) and via post-translational mechanisms that act on TBS enzymes, including redox control (Brzezowski et al. 2015). Redox regulation presumably enables fast and reliable switching between activated and inactivated states of the TBS pathway during the diurnal transition from dark to light. Thus, it is very likely that redox-dependent thiol switches control protein stability, enzyme activity and intra- and intermolecular changes in protein configuration and protein complex formation in the context of the TBS.

This review will discuss (i) TBS enzymes that are subject to either of two thioredoxin (TRX) systems – the classical TRX variants (which are reduced during photosynthetic electron transport via ferredoxin (Fdx) and the Fdx-Trx reductase) and the NADPH-dependent TRX reductase C (NTRC)-mediated pathway (which uses NADPH as the electron donor); (ii) review the biochemical and physiological effects of the loss of these catalytic reductants on TBS enzymes in *Arabidopsis thaliana*, and (iii) describe the essential thiol groups of the redox-sensitive TBS enzymes so far identified; (iv) the catalytic and structural consequences of redox-dependent transitions between thiol groups and disulfide bonds on the performance of TBS enzymes will then be compared to those observed for other plastid-localized and redox-controlled proteins. Finally, we speculate on possible interdependencies between redox-controlled proteins involved in the initial and rate-limiting steps of TBS during plant growth.

Overview of tetrapyrrole biosynthesis

Tetrapyrroles play important roles in biological processes that are vital to life on Earth (Brzezowski et al. 2015; Tanaka et al. 2011). Due to their spectral and biochemical properties, they have been defined as the “pigments of life” (see

Battersby 1988). ALA is the universal precursor of TBS. Animals, fungi, yeast, and some bacteria (including purple non-sulfur bacteria), synthesize ALA by a distinct route involving the condensation of glycine and succinyl-coenzyme A by ALA synthase. Plants, algae, many bacteria and archaea synthesize ALA from activated glutamate. Activation is carried out by glutamyl-tRNA synthetase (GluRS), which couples the amino acid to tRNA^{Glu}. The product is then used for both protein synthesis and TBS. Glutamyl-tRNA reductase (GluTR) is the first committed enzyme of TBS and reduces the activated carboxyl group of glutamyl-tRNA to a formyl group, yielding glutamate-1-semialdehyde (GSA, Figure 1). Subsequently, a pyridoxamine phosphate (PMP)-mediated intermolecular exchange of an amino group converts GSA into ALA, in a reaction catalyzed by GSA aminotransferase (GSAAT). ALA synthesis determines the flux rate of metabolites through the pathway (Grimm 2019a/b; Mochizuki et al. 2010; Tanaka et al. 2011).

ALA dehydratase (ALAD), also known as porphobilinogen synthase, then catalyzes the asymmetric condensation of two ALA molecules to form the first non-linear intermediate of TBS, the monopyrrole porphobilinogen. Four molecules of porphobilinogen are subsequently coupled to the cofactor dipyrromethane of hydroxymethylbilane (HMB) synthase (HMBS) to produce the linear tetrapyrrole HMB. In the following step, HMB is converted by uroporphyrinogen III (UroIII) synthase into UroIII, the first closed macrocycle in the pathway. It is important to note here that UroIII and products of the later metabolic steps in the pathway act as photosensitizers. Their accumulation in plant cells causes photodynamic damage by generating singlet oxygen.

Siroheme is formed from UroIII in a three-step-reaction catalyzed in plants by the sequential action of an S-adenosyl-L-methionine-dependent methyltransferase, an unknown dehydrogenase and sirohydrochlorin ferrochelatase (Tripathy et al. 2010). In the main branch of TBS, UroIII decarboxylase (UROD) and coproporphyrinogen III oxidase (CPOX) catalyze in consecutive reactions the formation of protoporphyrinogen IX (Protopogen). Next, Protopogen is converted to protoporphyrin IX (Proto) by the Protopogen oxidase (PPOX). At this point, tetrapyrrole metabolism splits into two distinct pathways, the Fe branch and the Mg branch. Whereas the Fe branch leads to the formation of protoheme and its derivatives, the catalytic reactions of the Mg branch result in Chl formation. In the Fe branch, ferrochelatase (FeCh) inserts Fe²⁺ into Proto to produce protoheme. The following steps yield biliverdin and phytychromobilin via an oxidation reaction catalyzed by heme oxygenase and an

Figure 1: Scheme of the plastid-localized tetrapyrrole biosynthetic pathway.

The metabolites and the abbreviations used for all enzymes of the pathway are specified. The blue, yellow, pink, red and cyan colored ovals highlight the redox regulators in either *Arabidopsis thaliana* (At), *Chlamydomonas reinhardtii* (Cr), *Pisum sativum* (Ps), *Spinacia oleracea* (So), *Synechocystis* sp. PCC 6803 (Syn), respectively. The redox-targeted enzymes of the pathway are indicated in bold and listed in Table 2, together with the interacting reductants. Some of the additional regulatory factors are specified in green boxes combined with a reference in green (stimulatory) or red (inhibitory) to the interacting enzyme. Subunits of the Mg chelatase (MgCh) and the light-dependent (LPOR) and light-independent (DPOR) protochlorophyllide oxidoreductase (POR) are indicated by grey ovals. For further details, see the list of abbreviations in the text.

isomerization mediated by phytylchelatase synthase. Within the Mg branch, magnesium chelatase (MgCh) inserts a Mg^{2+} ion into the Proto macrocycle, a step which requires ATP. Then the transfer of a methyl group from S-adenosyl-L-methionine (SAM) to Mg protoporphyrin IX (MgP) by MgP methyltransferase (CHLM) produces MgP monomethylester (MgPME). MgPME cyclase (the catalytic subunit of which is designated as 'CHL27' in Arabidopsis) integrates an atomic oxygen into MgPME to form 3,8-divinyl protochlorophyllide (Pchlde). In angiosperms, the strictly light-dependent protochlorophyllide oxidoreductase (POR) reduces 3,8-divinyl-Pchlde to 3,8-divinyl-chlorophyllide (Chlide). Besides angiosperms, other plants, algae and cyanobacteria also contain a light-independent POR. The 8-vinyl group of 3,8-divinyl-Chlide is reduced by a divinyl-reductase to form 3-vinyl Chlide. In the final steps of the Chl branch, Chlide is esterified at the C17-propionate group of ring D with phytyl pyrophosphate by Chl synthase to form Chl *a* and Chl(*ide*) *a* oxygenase catalyzes Chl *b* formation (Brzezowski et al. 2015; Tanaka and Tanaka 2007).

The thioredoxin network in chloroplasts

Regulation of the redox status of proteins is one of the essential post-translational mechanisms that contributes to the biogenesis and maintenance of chloroplasts by modulating the activity, folding and stability of plastid-localized proteins. The thiol groups of redox-active cysteine (Cys) residues are the targets of redox regulation and can be reversibly oxidized to intra- or intermolecular disulfide bonds, or mixed disulfide bonds with glutathione or sulfides (Dietz and Hell, 2015; Nikkanen et al. 2016; Richter and Grimm 2013). The thiol switch between oxidized and reduced states of Cys residues is mainly controlled by two redox regulatory systems in chloroplasts, TRXs and NTRC (Dietz 2016; Geigenberger et al. 2017). TRXs are universal oxidoreductases of 12–14 kDa with a canonical TRX fold that includes an active-site motif with two conserved Cys (WCGPC) that mediate oxidoreductase activity (Holmgren 1995; Martin 1995). The presence of redox-active dithiols in their active sites enables these proteins to catalyze the reduction of disulfide bonds in many target proteins, thus modifying the activity and stability of the latter. The typical pathway of redox regulation in chloroplasts includes Fdx, which is reduced in the light by the photosynthetic electron transport chain. These reducing equivalents are then made available to the large set of

TRXs by the activity of a Fdx-dependent TRX reductase (Buchanan and Balmer 2005; Brzezowski et al. 2015; Cejudo et al., 2019; Yoshida and Hisabori 2017). Nucleus-encoded and plastid-localized TRXs form a large protein family, comprising the isoforms TRX-f1 and f2, TRX-m1, m2, m3 and m4, TRX-x, TRX-y1, and y2 and TRX-z (Balsera et al. 2014; Michalska et al. 2009; Michelet et al. 2013; Serrato et al. 2013).

In addition, chloroplasts use NTRC, which consists of an N-terminal NTR domain and a C-terminal TRX-like domain. NTRC comprises a complete redox-based regulatory system that can act independently of the photosynthetic machinery (Serrato et al. 2004). Thus, NTRC accepts electrons not only from photosynthetically generated NADPH, but also that provided by the oxidative pentose phosphate pathway (OPPP), which is active in the dark. Therefore, NTRC can act as an electron donor for starch metabolism and antioxidant defense mechanisms both during the day and at night (Perez-Ruiz et al. 2006).

The plastid redox activities in higher plants are expanded by several atypical TRX-like proteins, which differ from the canonical TRXs in their active-site motifs (Meyer et al. 2008; Meyer et al. 2002). Although the participation of some TRX-like proteins in the plastid redox network has been verified (see Table 1) note that regulation of TBS by TRX-like proteins has hardly been reported. The broad diversity of TRXs and TRX-like proteins in chloroplasts introduces some functional redundancy, as revealed by the fact that single knock-out mutants for TRXs do not always lead to obvious phenotypes. However, in addition to this redundancy, it is to be expected that differences in their midpoint redox potentials (*Em*) confer specific properties and capacities to reduce thiol groups on the various TRXs.

Although the TRX-dependent activation of target enzymes by the reduction of redox-sensitive disulfide bonds has been studied for a long time (Buchanan 2016), relatively little information exists regarding the potential impact of redox-dependent inactivation mechanisms on the primary metabolism of chloroplasts, which could be induced by adverse environmental conditions or after the transition from light to dark. Three recent studies, which were published almost simultaneously, indicated that electron flow between NTRC/TRX/TRX-like proteins and oxidized 2-cysteine peroxiredoxins (2CPs) is crucial for the short-term inactivation of redox targets (Ojeda et al. 2018a; Vaseghi et al. 2018; Yoshida et al. 2018). When the supply of NADPH is inadequate (e.g. in darkness, under environmental stress), a pool of oxidized NTRC can rapidly accumulate, which is not available for the reduction of 2CPs. The oxidized 2CPs then form an

electron sink for the reduced TRXs (Ojeda et al. 2018b; Perez-Ruiz et al. 2017). The resulting oxidized TRXs serve as electron acceptors for redox-controlled enzymes of the Calvin-Benson cycle (CBC), which are inactivated in the process (Vaseghi et al. 2018).

Conversely, the proposed drainage of electrons from TRXs to oxidized 2CPs in pale green *ntrc* knockout mutants should result in increased amounts of oxidized targets for TRXs (Perez-Ruiz et al. 2017). Furthermore, in the triple *ntrc/Δ2cp* mutant (which lacks *NTRC* and *2CPB* and carries a severe knock-down mutation in *2CPA*), the *ntrc* phenotype is fully complemented and the seedlings resemble WT (Perez-Ruiz et al. 2017). This was attributed to the recovery of the reducing power of the TRX system owing to the virtual absence of electron leakage to 2CPs in the triple mutant (Perez-Ruiz et al. 2017). Simultaneously, levels of the TBS enzymes CHLM and GluTR were restored to WT-like levels in the *ntrc/Δ2cp* mutant, which effectively corresponds to the rescue of the *ntrc* phenotype in the triple mutant (Richter et al. 2018). Further studies should explore whether the instability of other TBS enzymes in the absence of NTRC is rescued in *ntrc/Δ2cp*. However, initial hints of cooperative action of TRXs and NTRC in regulating TBS metabolism suggest that interdependency of redox regulators is essential for the proper functioning of the TBS pathway, as discussed below.

Redox control of TBS enzymes

Initial studies using thiol-dependent affinity chromatography identified TBS enzymes amongst others as potential targets of TRXs (see Table 2 and Figure 1, Balmer et al. 2003; Gonzalez et al. 2019; Marchand et al. 2006; Perez-Perez et al. 2017). An increasing number of proteomic analyses have provided an inventory of potentially redox-dependent TBS proteins, whose stability and activity can be assessed in the search for acclimation strategies that protect plants from photo-oxidative damage (with the ultimate goal of improving plant water usage efficiency, stress tolerance and yield). For example, the use of oxidative isobaric tags for relative and absolute quantitation (OxiTRAQ) in proteomic studies has identified GSAAT, GluRS, GluTR, PPOX, HMBS, UROD, CPOX, CHLH, CHLI and POR as enzymes whose Cys residues undergo oxidative modification and/or carbamidomethylation in *Arabidopsis* cells in response to salicylate or the flagellin peptide flg22 – components of two defense pathways which are known to disturb cellular redox homeostasis (Liu et al. 2015).

Table 1: Survey of all plastid-localized thioredoxins (TRX) and thioredoxin-like (TRXL) proteins in *Arabidopsis thaliana*.

TRX/TRXL	Locus	Localization	Function(s)
TRX-f1	AT3G02730	Stroma (Cain et al. 2009)	Activation of CBC (e.g. FBPase, SBPase; Naranjo et al. 2016a; Okegawa and Motohashi 2015), starch synthesis (Sparla et al. 2006; Seung et al. 2013; Skryhan et al. 2015; Thormahlen et al. 2013), TBS (Luo et al. 2012; Nikkanen et al. 2016), activation of NADP-MDH (malate valve; Yoshida et al. 2015), reductive inactivation of G6PDH (OPPP; Nee et al. 2009)
TRX-f2	AT5G16400	Stroma (Cain et al. 2009)	Activation of CBC (e.g. FBPase, SBPase; Naranjo et al. 2016a), activation of NADP-MDH (malate valve; Yoshida et al. 2015), reductive inactivation of G6PDH (OPPP; Nee et al., 2009)
TRX-m1	AT1G03680	Stroma (Cain et al. 2009)	Activation of TBS (Da et al. 2017), CBC (e.g. FBPase, SBPase; Okegawa and Motohashi 2015), biogenesis of PSII and regulation of light harvesting (NPQ; Wang et al. 2013), regeneration of MSRB (oxidative stress response; Vieira Dos Santos et al. 2007)
TRX-m2	AT4G03520	Stroma (Cain et al. 2009)	Activation of TBS (Da et al. 2017), CBC (e.g. FBPase, SBPase; Okegawa and Motohashi 2015), biogenesis of PSII and regulation of light harvesting (NPQ; Wang et al. 2013)
TRX-m3	AT2G15570	Stroma (Cain et al. 2009)	Meristem development, phloem unloading to meristem (Benitez-Alfonso et al. 2009)
TRX-m4	AT3G15360	Stroma (Cain et al. 2009)	Activation of TBS (Da et al. 2017), CBC (e.g. FBPase, SBPase; Okegawa and Motohashi 2015), biogenesis of PSII and regulation of light harvesting (NPQ; Wang et al. 2013)
TRX-x	AT1G50320	Stroma (Cain et al. 2009)	Antioxidant function: Electron transfer to 2-CPs (Bernal-Bayard et al. 2014; Collin et al. 2003)
TRX-y1	AT1G76760	Stroma (Cain et al. 2009)	Oxidative stress responses: Electron transfer to PRXQ (Collin et al. 2004) maintenance of reduced ascorbate pool during drought stress by reduction of MDHARs (Vanacker et al. 2018)
TRX-y2	AT1G43560	Stroma (Cain et al. 2009)	Oxidative stress responses: Electron transfer to PRXQ (Collin et al. 2004), regeneration of MSRB (Vieira Dos Santos et al. 2007), maintenance of reduced ascorbate pool during drought stress by reduction of MDHARs (Vanacker et al. 2018)
TRX-z	AT3G06730	Colocalizes with plastid nucleoids in the stroma (Arsova et al. 2010)	Involved in plastid transcription: Structural subunit of PEP (Wimmelbacher and Bornke 2014), electron transfer to the PEP subunits FLN1, FLN2 (Arsova et al. 2010) and PRIN2 (Diaz et al. 2018); activation of different antioxidative enzymes (Bohrer et al. 2012; Chibani et al. 2011; Yoshida et al. 2015)
NTRC	AT2G41680	Most abundant in stroma, partially in thylakoid fraction (Serrato et al. 2004)	Main reductant of 2-CPs (antioxidant function; Moon et al. 2006, Perez-Ruiz et al. 2006) and maintenance of plastid redox homeostasis (Perez-Ruiz et al. 2017), regulation of starch synthesis (Michalska et al. 2009; Skryhan et al. 2015), TBS (CHLI, CHLM; Da et al. 2017; Richter et al. 2013; Perez-Ruiz et al. 2014; Richter et al. 2016), CF1-ATPase activity (Carrillo et al. 2016), NPQ (Naranjo et al. 2016); regulation of the cyclic electron flow (Nikkanen et al. 2018)
ACHT1	AT4G26160	Associated with thylakoid membrane, partially stromal (Dangoor et al. 2009)	Light sensing, electron transfer to 2-CPs (antioxidant function; Dangoor et al. 2009; Dangoor et al. 2012)
ACHT2	AT4G29670	Chloroplast (Dangoor et al. 2009)	Electron transfer to 2-CPs (antioxidant function; Cerveau et al. 2016; Dangoor et al. 2009)
ACHT3	AT2G33270	Chloroplast (Dangoor et al. 2009)	Unknown
ACHT4	AT1G08570	Associated with thylakoid membrane, partially stromal (Dangoor et al. 2009)	Negative regulation of starch synthesis (oxidation of the APS1 subunit of the AGPase; Eliyahu et al. 2015), electron transfer to 2-CPs (antioxidant function; Dangoor et al. 2009)
ACHT5	AT5G61440	Chloroplast (Dangoor et al. 2009)	Unknown
TRXL2.1/ WCRKC1	AT5G06690	Stroma (Cain et al. 2009)	Oxidative inactivation of redox-regulated enzymes of the CBC, electron transfer to 2-CPs (antioxidant function; Yoshida et al. 2018), oxidative activation of G6PDH (Yoshida et al. 2019), involved in stress responses by activation of TPX and MSR (Chibani et al. 2012)
TRXL2.2/ WCRKC2	AT5G04260	Stroma (Cain et al. 2009)	Inactivation of redox-regulated enzymes, electron transfer to 2-CPs (antioxidant function; Yoshida et al. 2018), oxidative activation of G6PDH (Yoshida et al. 2019)

Table 1: (continued)

TRX/TRXL	Locus	Localization	Function(s)
CDSP32	AT1G76080	Stroma (Rey et al. 1998)	Regeneration of MSRB (Tarrago et al. 2010), electron transfer to 2-CPs (antioxidant function; Broin et al. 2002)
APR1	AT4G04610	Stroma (Cain et al. 2009)	Functions as glutathione-dependent sulfate reductase (sulfate assimilation; Chen et al. 2019; Lee et al. 2011)
APR2	AT1G62180	Stroma (Cain et al. 2009)	Functions as glutathione-dependent sulfate reductase (sulfate assimilation; Lee et al. 2011), increases tolerance to selenate toxicity (Grant et al. 2011)
APR3	AT4G21990	Stroma (Cain et al. 2009)	Functions as glutathione-dependent sulfate reductase (sulfate assimilation; Lee et al. 2011)
CcdA	AT5G54290	Thylakoid membrane (Motohashi and Hisabori 2010)	Transfer of electrons from stroma to luminal site of the thylakoid membrane (Motohashi and Hisabori 2010), biogenesis of the cytochrome <i>b₆f</i> complex (Page et al. 2004)
HCF164	AT4G37200	Thylakoid membrane (active site luminal; Lennartz et al. 2001)	Biogenesis of the cytochrome <i>b₆f</i> complex (Lennartz et al. 2001; Motohashi and Hisabori 2006; Page et al. 2004)
SOQ1	AT1G56500	Thylakoid membrane (active site luminal; Brooks et al. 2013)	Involved in regulation of lightharvesting by inhibition of NPQ (Brooks et al. 2013).
LTO1	AT4G35760	Thylakoid membrane (active site luminal; Karamoko et al. 2011)	Involved in assembly of PSII (Karamoko et al. 2011)

Abbreviations: 2-CP: 2-cysteine peroxiredoxin; ACHT: ATYPICAL CYS HIS RICH THIOREDOXIN; AGPase: ADP-glucose pyrophosphorylase; APR: adenosine 5'-phosphosulfate reductase; CBC: Calvin-Benson Cycle; CcdA: Cytochrome c-type biogenesis *ccda*-like chloroplastic protein; CDSP32: CHLOROPLASTIC DROUGHT-INDUCED STRESS PROTEIN OF 32 KD; CF1-ATPase: F1 subunit of thylakoid ATP synthase; CHLI: ATPase subunit of the magnesium chelatase; CHLM: magnesium protoporphyrin IX methyltransferase; FBPase: fructose-1,6-bisphosphatase; FLN: fructokinase-like protein; G6PDH: Glucose-6-phosphate 1-dehydrogenase; HCF164: HIGH CHLOROPHYLL FLUORESCENCE 164; LTO1: LUMEN THIOL OXIDOREDUCTASE 1; MDHAR: monodehydroascorbate reductase; MSR: methionine sulfoxide reductase; NADP-MDH: NADP-dependent malate dehydrogenase; NPQ: non-photochemical quenching; NTRC: NADPH-dependent thioredoxin reductase C; OPPP: oxidative pentose phosphate pathway; PEP: plastid encoded RNA polymerase; PRIN2: Protein PLASTID REDOX INSENSITIVE 2; PRX: peroxiredoxin; PSII: photosystem II; SBPase: sedoheptulose-1,7-bisphosphatase; SOQ1: SUPPRESSOR OF QUENCHING 1; TBS: tetrapyrrole biosynthesis; TPX: thiol peroxidase; TRX: Thioredoxin; TRXL: Thioredoxin-like.

In addition, the Arabidopsis *ntrc* knock-out line and mutants deficient in the expression of one or more *TRX* genes display pale green leaf pigmentation and retarded growth (Da et al. 2017; Richter et al. 2013; Richter et al. 2016; Serrato et al., 2004). Indeed, simultaneous knockout of *NTRC* and *TRXf1*, or the silencing of the *TRXm1*, *m2* and *m4* genes in the *ntrc* background leads to even more drastic phenotypes in terms of growth rates and Chl amounts (Thormahlen et al. 2015). Thus, these lines phenotypically resemble mutants with lower accumulation or deficient synthesis of Chl. Although the expression of genes involved in TBS is not significantly altered in the *NTRC*/*TRX*-deficient seedlings, the reduced Chl content is accompanied by a decrease in ALA-synthesizing capacity relative to WT. Immunoblots of protein extracts from these mutant seedlings revealed that levels of several proteins involved in Chl synthesis, such as GluTR, GSAAT, ALAD, CHLI, CHLM and POR, were reduced to varying extents owing to *TRX* and/or *NTRC* deficiency – which suggests that a fairly large set of TBS enzymes is under redox control (Da et al. 2017; Richter et al. 2013; Wittmann et al. 2018). In the following, we describe the current state of research on

the redox-dependent TBS enzymes and highlight their dependencies on plastid reductants for function.

Glutamyl-tRNA reductase

The Arabidopsis genome contains three different genes for GluTR: *HEMA1* (At1g58290), *HEMA2* (At1g09940) and the putative pseudogene *HEMA3* (At2g31250) (Matsumoto et al. 2004). GluTR1 is the most abundant isoform in *A. thaliana* and its loss results in a pale, non-viable *hema1* mutant under photoautotrophic growth conditions (Apitz et al. 2014). As GSA, the catalytic product of GluTR, marks the rate-limiting step in plant TBS, multiple mechanisms of post-translational regulation have emerged for GluTR. The interaction of GluTR with either the GluTR-binding protein (GBP; Zhao et al. 2014; Zhao and Han 2018) or FLUORESCENCE IN BLUE (FLU; Hou et al. 2019; Meskauskiene et al. 2001) ensures optimal synthesis of GSA during day and night. Aggregation of GluTR is prevented by the chaperone cpSRP43 (Wang et al. 2018). GluTR is a target of the caseinolytic protease (Clp, Apitz et al. 2016), and GluTR

Table 2: Potentially redox-controlled enzymes and other factors involved in TBS, which interact with TRXs.

Target protein of TBS	Interacting TRX
GluTR	NTRC (Arabidopsis; Richter et al. 2013), TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
GBP	TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
GSAAT	TRX-f, TRX m (spinach; Balmer et al. 2003), TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
ALAD	TrxA (Synechocystis; Lindahl and Florencio 2003), TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017), TRX-f1, TRX m4 (Arabidopsis; Wittmann et al. 2018), NTRC (Arabidopsis; Wittmann et al. 2018; Gonzalez et al. 2019)
PBGD	TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
UROS	TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
UROD	TRX-f, TRX m (spinach; Balmer et al. 2003), TRX-h3 (Arabidopsis; Marchand et al. 2006), TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
CPOX	TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
MgCh	CHLH TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017) CHLI TRX-f, TRX-m (Spinach; Balmer et al. 2003), TRX-f (pea; Luo et al. 2012), NTRC (Arabidopsis; Gonzalez et al. 2019; Perez-Ruiz et al. 2014), TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
GUN4	CHLD TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
CHLM	TRX-h1 (Chlamydomonas; Perez-Perez et al. 2017)
POR	NTRC (Arabidopsis; Richter et al. 2013), TRX-m1, m2, m4 (Arabidopsis; Da et al. 2017) NTRC (PORB, Arabidopsis; Gonzalez et al. 2019), TRX-h1 (light-dependent POR (LPOR) and the light-independent POR (DPOR) subunits chlN and chlL (Chlamydomonas; Perez-Perez et al. 2017)

The listed interactions were identified by different interaction assays. The redox-active cysteines of the bold enzymes were identified by site directed mutagenesis. Target proteins are displayed in the order of their occurrence in the TBS pathway (see also Figure 1). Alphabetical order of abbreviations: ALAD: aminolevulinic acid dehydratase; CHLM: magnesium protoporphyrin IX methyltransferase; CHLP: geranylgeranyl reductase; CPOX: coproporphyrinogen-Oxidase; GBP: GluTR-binding protein; GGPS: Geranylgeranyl diphosphate synthase; GluTR: glutamyl-tRNA reductase; GUN4: GENOMES UNCOUPLED 4; PBGD:porphobilinogendeaminase; MgCh: magnesium chelatase (subunits CHLD, CHLI, CHLH); POR: NADPH:protochlorophyllide oxidoreductase; UROD: uroporphyrinogen III decarboxylase; UROS: uroporphyrinogen III synthase.

proteolysis is controlled by a heme-dependent feedback loop. Binding of heme to GBP attenuates the GluTR-GBP interaction, and makes the enzyme more accessible to the selector and chaperone subunits of the Clp protease (Richter et al. 2019, see also Section 5.2 below).

Finally, GluTR is subject to redox control. GluTR interacts with NTRC and TRX isoforms, and its concentration

decreases in the *ntrc* mutant (Richter et al. 2013). Moreover, the rate of ALA synthesis is also significantly decreased in *ntrc*, which is likely to be the main reason for the mutant's pale green leaf phenotype. As levels of *HEMA1* and *HEMA2* transcripts are essentially unaffected, the decrease in GluTR content can be largely assigned to post-translational impairment of GluTR stability caused by NTRC deficiency. However, an indirect impact of NTRC on GluTR activity cannot be excluded (Richter et al. 2013, Section 3), although GluTR content is even lower in mutants lacking both *NTRC* and *TRXf1* (Wittmann et al. 2018). Strikingly, simultaneous depletion of three m-type TRXs led to an increase in GluTR accumulation, but also to decreased ALA synthesis, as determined by *in vivo* assays of the sequential GluTR and GSAAT reactions (Luo et al. 2012; Da et al. 2017).

Mature GluTR1 in *A. thaliana* contains nine Cys residues, four of which (Cys144, Cys302, Cys389 and Cys501) are conserved in higher plants (Richter and Grimm 2013). Cys144 is found in algal and bacterial GluTR homologs, such as Chlamydomonas and Chlorobium, and is directly involved in the catalytic mechanism (Moser et al. 1999; Schauer et al. 2002). Cys302 and Cys389 are localized in the NADPH-binding domain, and Cys501 is positioned in the GluTR1 dimerization domain. Cys501 could play a role in redox-dependent homo-dimerization of GluTR or the interaction of its C-terminus with the negative regulator protein FLU. This idea is supported by an analysis of the reductome of Chlamydomonas, which identified Cys486 in the CrGluTR (homologous to Cys501 in AtGluTR) as a TRX target (Perez-Perez et al. 2017).

Glutamate 1-semialdehyde aminotransferase (GSAAT)

In Arabidopsis, two homologous genes for GSAAT, *AtGSA1* (At5g63570) and *AtGSA2* (At3g48730), share 90% sequence identity. The X-ray structure of *AtGSAAT1* has been reported at 1.25 Å resolution. Like a previous crystal structure of a cyanobacterial homolog (Hennig et al. 1997), *AtGSAAT1* forms an asymmetric dimer and displays asymmetry in cofactor binding. While one monomer binds PMP with the gating loop fixed in the open state, the other monomer binds either pyridoxamine phosphate (PMP) or pyridoxal phosphate (PLP) and the gating loop is poised to close (Song et al. 2016).

GSAAT is one of the TBS enzymes identified as a potential target of TRX-f and TRX-m by thiol-dependent affinity chromatography (Balmer et al. 2003; Marchand et al. 2006). Arabidopsis seedlings simultaneously lacking TRX-m1, TRX-m2 and TRX-m4 show a decreased GSAAT

content (Da et al. 2017). In addition, *ntrc/trxf1* mutant lines exhibit a slightly decreased GSAAT content compared to WT (Wittmann et al. 2018). Recent studies using a mono-cysteinic TRX-column have identified TRX-targeted Cys82, Cys157, Cys179, Cys287 and Cys404 of GSAAT in the reductome of *Chlamydomonas* (Perez-Perez et al. 2017).

AtGSAAT1 has 4 Cys residues, Cys148, Cys168, Cys189 (all in the catalytic site and the PLP/PMP-binding domain) and Cys396. Sequence alignment showed that Cys148 and Cys168 are conserved in all GSAAT isoforms of higher plants and in the single GSAAT present in *Chlamydomonas reinhardtii* (Cr), whereas Cys189 is found in *AtGSAAT* and its homolog in *Chlorobium*, a genus of green sulphur bacteria. The PyMOL-modelled *Arabidopsis* and *Synechocystis* GSAAT structures show a potential intramolecular disulfide bond between Cys168 and Cys189, which are ~10 Å apart.

Based on modelling of the crystal structure of GSAAT from *Synechococcus* onto that of GluTR from *Methanopyrus kandleri*, it was proposed that the unusual V-shaped void in homodimeric GluTR is occupied by the GSAAT dimer, allowing efficient ALA synthesis from activated glutamate. It can be speculated that stabilization of a heterotetrameric GluTR₂-GSAAT₂ complex and/or elevated ALA-synthesizing activity require established disulfide bridges between the GluTR and GSAAT components. Then, TRX or NTRC could reduce these disulfide bonds (Hennig et al. 1997; Schubert et al. 2002; reviewed by Richter and Grimm 2013). However, the interaction of GluTR with GSAAT has yet to be demonstrated *in vitro* and *in planta*.

Further studies have confirmed GSAAT as a valid thiol enzyme. A redox proteomic analysis identified 85 leaf proteins that displayed marked quantitative variations in *Citrus aurantium* L. exposed to salt stress. Among them, GSAAT was significantly upregulated in response to excess NaCl. Interestingly, GSAAT accumulation was prevented when seedlings were pretreated with either H₂O₂ or sodium nitroprusside (an NO-releasing chemical). This indicates the involvement of two distinct redox signaling pathways that can induce an intracellular response against subsequent stress-induced oxidative or nitrosylation damage (Jasid et al. 2006; Tanou et al. 2009). In addition, affinity chromatography using biotinylated glutathione and streptavidin as ligands has also identified GSAAT, UROD and CHLI as glutathionylated proteins in an extract from *Chlamydomonas* cells (Zaffagnini et al. 2012). Moreover, studies using complementary proteomics approaches – saturation labeling and differential in-gel electrophoresis,

and isotope-coded affinity tagging – identified GSAAT among 118 potential redox-responsive proteins in the context of jasmonate signaling in guard cells of *Brassica napus* (Zhu et al. 2014). *In vivo* trapping methods for Cys-bound sulfenic acid using a dimedone-based DYn-2 probe in *Arabidopsis* cells exposed to hydrogen peroxide identified 226 sulfenylated proteins, including the TBS enzymes GSAAT and ALAD (Akter et al. 2015).

5-Aminolevulinic acid dehydratase

In *Arabidopsis thaliana*, two isoforms of ALAD are encoded by the *HEMB1* and *HEMB2* genes. Knockout of *HEMB1*, which encodes the predominant isoform, leads to an embryo-lethal phenotype (Tang et al., 2012). The mature ALAD1 of *A. thaliana* has six Cys residues, four of which (Cys152, Cys251, Cys404 and Cys426) are highly conserved in higher plants (Wittmann et al. 2018). Initial indications of redox control of ALAD were obtained when addition of TRX to *in vitro* assays enhanced ALAD activity (Balange and Lambert 1983). Subsequent *in vitro* experiments showed that recombinant ALAD was inactivated upon oxidation and activated by DTT treatment or the addition of recombinant TRXs (Wittmann et al. 2018). In plants lacking the three m1, m2 and m4-type TRXs, the ALAD content was significantly depleted (Da et al. 2017), but also slightly decreased in *ntrc/trxf1* double mutants (Wittmann et al. 2018). The decreased ALAD content was correlated with lower enzyme activity, confirming that redox control alters the stability of ALAD rather than its catalytic activity. It was therefore proposed that the level of ALAD activity is modulated according to need by redox-dependent control of its stability. Indeed, ALAD interacts in both *in vivo* and *in vitro* assays with NTRC, TRX-f1/2 and TRX-m4 (Wittmann et al. 2018).

ALAD was also identified as a putative target of TRXs in proteomic screens of *Synechocystis* (Lindahl and Florencio 2003) and *Chlamydomonas* (Perez-Perez et al. 2017). Interestingly, an *in vivo* approach identified NTRC-containing complexes with CHLI, PORB and ALAD among potentially redox-controlled TBS enzymes in *Arabidopsis* (Gonzalez et al. 2019). In a large-scale approach using co-fractionation mass spectrometry on protein extracts of 13 different plant species ranging from *Chlamydomonas* to eudicots and including *Arabidopsis*, ALAD1 was listed as a potential interaction partner for NTRC and 2CPs (McWhite et al. 2020).

Magnesium chelatase - the MgCh subunit CHLI

MgCh is a heteromeric enzyme composed of the catalytic CHLH subunit and two heptameric rings, made up of CHLI and CHLD subunits, respectively. The latter two proteins belong to the AAA+ (ATPases associated with various cellular activities) protein family (Tanaka et al. 2011). Even though both CHLI and CHLD possess these ATPase domains, ATPase activity has only been reported for CHLI (Jensen et al. 1999), while CHLD functions in a cooperative interaction between the AAA+ motor of CHLI for ATP hydrolysis and the active site on CHLH (Adams and Reid 2013; Farmer et al. 2019). CHLI and CHLD form the double-ring structure in the presence of Mg^{2+} and ATP prior to their association with the Proto-binding CHLH subunit (Fodje et al. 2001). The Arabidopsis genome encodes two CHLI isoforms, with CHLI1 being the dominant isoform in photosynthetic tissue. Nevertheless, CHLI2 is fully functional and able to complement the pale green *chli1* knockout mutant upon expression driven by the *CHLI1* promoter (Huang and Li 2009).

One of the first biochemical indications of redox regulation of TBS enzymes was provided for the recombinant MgCh subunit CHLI, which showed reduced ATPase activity upon treatment with N-ethylmaleimide, a thiol-reactive reagent (Jensen et al. 2000). The physical interaction of CHLI with TRX-f was later confirmed, suggesting formation of a temporary intermolecular disulfide bond between the reductant and the CHLI (Stenbaek and Jensen 2010). It was shown that TRX-f breaks an intramolecular disulfide bond between the redox-sensitive thiol residues of Cys354 and Cys396 at the C-terminal end of CHLI. This thiol switch contributes substantially to the redox-induced activation of the ATPase activity of CHLI1 (Ikegami et al. 2007). Moreover, under oxidative conditions, *AtCHLI1* forms a second, redox-sensitive disulfide bond between Cys102 and Cys193, which apparently does not affect the ATPase activity. Upon illumination of plants, both disulfide bonds are reduced, indicating the need for the reduced state of CHLI in daytime (Ikegami et al. 2007). It was subsequently confirmed that the ATPase activity of both *AtCHLI1* (Ikegami et al. 2007) and *AtCHLI2* (Kobayashi et al. 2008) can be directly stimulated by TRX-f from spinach.

VIGS-*TRXm/TRXf*-inactivated lines show reduced CHLI accumulation and activity in pea plants. These changes coincide with a stronger decrease in the total Chl content (Luo et al. 2012). Additionally, NTRC was observed to stimulate the CHLI activity *in vitro* (Stenbaek and Jensen 2010). Four years later, the interaction between these two

proteins was confirmed by *in vivo* and *in vitro* approaches (Gonzalez et al. 2019; Perez-Ruiz et al. 2014). However, the Cys354-Cys396 disulfide bond of CHLI can also be efficiently reduced by TRX-y1 and TRX-m2 *in vitro* (Yoshida and Hisabori 2016). Gel-shift assays revealed a decrease in the level of reduced CHLI in Arabidopsis *ntrc* and *trxfl* mutants, and VIGS-*TRXm/TRXf* pea plants, compared to WT (Luo et al. 2012; Perez-Ruiz et al. 2014). Moreover, CHLI was less abundant in total leaf extracts from both Arabidopsis mutants (Perez-Ruiz et al. 2014). Hence, in addition to the direct modulation of enzyme activity, redox-dependent regulation also affects CHLI stability.

Magnesium chelatase - the MgCh subunit CHLH

Interestingly, apart from the obviously NTRC- and TRX-enhanced ATPase activity of CHLI, no effect of either reductant on the CHLH-dependent catalysis of Mg chelation could be demonstrated (Jensen et al. 2000; Ikegami et al. 2007; Stenbaek and Jensen 2010). To date, no interaction has been established between CHLH and either NTRC or TRX in higher plants (Balmer et al. 2003; Gonzalez et al. 2019). However, the addition of DTT to the enzyme assays increases the chelation activity of the MgCh complex (Jensen et al. 2000). At the same time, these authors noticed that CHLH activity was sensitive to treatment with the sulfhydryl-reacting agent N-ethylmaleimide. Notably, in the Arabidopsis *ntrc* mutant CHLH content is decreased (Richter et al. 2013), while VIGS-*TRXf/TRXm* pea plants contained lower CHLI content and significantly increased CHLH content (Luo et al. 2012).

In contrast, a proteomic screen of *Chlamydomonas reinhardtii* proteins not only identified CHLH as a TRX interaction partner, but also pinpointed the TRX-sensitive residues as Cys497 and Cys774 (Perez-Perez et al. 2017). The first Cys is conserved in the Arabidopsis CHLH sequence (Cys482), while CrCys774 is replaced by a valine residue. However, both sequences contain a further conserved Cys residue (CrCys784, AtCys769) in close proximity to the crucial CrCys774 position. At present, it cannot be excluded that disulfide bonds can be formed with different redox-sensitive thiol groups in the algal and plant CHLH isoforms.

Despite the general rule that more complex organisms tend to have more Cys residues (Miseta and Csutora 2000), CrCHLH has 13 in all, and AtCHLH only 8. It is conceivable that not all of these Cys residues are redox sensitive. Moreover, it can also be assumed that plant CHLH is additionally controlled by other post-translational mechanisms

and it is not excluded that higher plants lost their redox regulation of the CHLH subunit as result of the potential reliability of the redox-dependent regulation of CHLI. Verifying CHLH's requirement for reductant-mediated control of its activity or stability is a task for the near future.

Mg-protoporphyrin IX methyltransferase

Analysis of the accumulation profile of TBS enzymes in the *ntrc* mutant, in VIGS-*TRXm1/m2/m4* RNAi mutants in the WT (triple mutant) and in the *ntrc* (quadruple mutant) background revealed a progressive decrease in CHLM stability. This drop in CHLM content was not due to reduced transcription of the *CHLM* gene, but resulted in diminished activity. NTRC and TRX-m isoforms interacted with CHLM in *in vitro* assays and exerted a stimulatory effect on CHLM activity (Da et al. 2017; Richter et al. 2013). Hence, alterations in the redox state of CHLM were proposed to account for the gradual decrease in protein stability. It was concluded that, in the context of redox control, stimulation of CHLM activity is overridden by the decrease in the enzyme's stability (Da et al. 2017). Labeling of CHLM with 4'-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid indicated that WT CHLM was entirely reduced during light exposure, while 35%, 28% and 45% of the CHLM content was oxidized in *ntrc*, the VIGS-*TRXm2/m4/m1* plants and the quadruple mutant, respectively (Da et al. 2017). On the other hand, even the quadruple mutant line retained a substantial fraction of reduced CHLM. This observation indicate either that the residual amounts of NTRC and TRX-m were sufficient to reduce CHLM to a certain extent, or that additional TRX isoforms can partially compensate for their loss. In samples from light-exposed plants, supplementation with DTT significantly increased CHLM activity only in the quadruple mutant, but not in single reductant mutants. This finding can be explained if the four-fold mutants contain a significantly larger amount of oxidized CHLM than those lacking one, two or three reductants. But why the stimulatory impact of DTT on CHLM activity was not observed in extracts from *ntrc* and the *trxm1/m2/m4* triple mutant remains unclear.

Arabidopsis CHLM contains three conserved Cys residues at positions 111, 115 and 177. Replacement of Cys177 by serine (Ser) led to a redox-insensitive CHLM *in vitro*. Substitution of Ser for either Cys111 or Cys115 diminished CHLM activity, but the enzyme retained its redox-sensitive property in each case (Richter et al. 2016). The crystal structure of Synechocystis CHLM revealed that the homologous Cys115 residues form part of the conserved DXGCGXG motif localized in the β 1- α D loop of the SAM-binding pocket,

while Cys111 is placed close to the pocket (Chen et al. 2014). It was therefore proposed that oxidizing conditions facilitate formation of a disulfide bond between Cys177 and either of the two Cys residues (Cys111 and Cys115), which leads to conformational changes that hinder SAM binding and thus decrease CHLM activity. An intermolecular disulfide bond was excluded, because CHLM dimerisation was not observed in plant extracts of these mutant lines, when proteins were separated by non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (Richter et al. 2016). Ultimately, attempts to confirm the proposed redox-sensitive disulfide bond between Cys177 and either Cys111 or Cys115 failed, because the simultaneous replacement of Cys111 and Cys115 by Ser residues led to a recombinant mutant CHLM protein with barely detectable activity. The double mutant CHLM with Ser substitution of the residues Cys115 and Cys177 also showed a general loss of activity, while the CHLM(Cys111Ser/Cys177Ser) protein could not be stably expressed in *E. coli* and, therefore, could not be analyzed (Richter et al. 2016). The impact of these mutations on the activity and stability of CHLM made it impossible to conclude if and when either Cys111 or Cys115 – or both residues – serve as the preferred interaction partner for Cys177. From the structural point of view, both combinations would facilitate disulfide bonding as both Cys111 and Cys115 are positioned at a reasonable distance from Cys177 (Chen et al. 2014; Richter et al. 2016).

Protochlorophyllide oxidoreductase

In higher plants, POR is quite an unusual enzyme as it uses the photo-excited substrate Pchl_{ide} as its own chromophore for enzymatic activation. The three Arabidopsis isoforms PORA (AT5G54190), PORB (AT4G27440), and PORC (AT1G03630) differ in their expression patterns, mRNA stability and plastid import pathways (reviewed in Armstrong et al. 1995; Benli et al. 1991; Reinbothe et al. 1995a, b; 1996a, b; Schulz et al. 1989). All POR homologs characterized so far contain four evolutionarily conserved Cys residues, which are implicated in Pchl_{ide} binding and catalysis. Treatment of Synechocystis POR with N-phenylmaleimide chemically modifies Cys195 and Cys222, and decreases its activity (Oliver and Griffiths 1981; Townley et al. 2001). Fluorescence emission spectroscopy has identified the other conserved residues (Cys276 and Cys303) of PORB and their homologs in PORA as distinct Pchl_{ide}-binding sites. Moreover, the expression of barley PORB(Cys303Ala) in a *PORB*-deficient Arabidopsis mutant did not give rise to high-molecular-weight POR complexes, whereas expression of

PORB(Cys276Ala) in the *porb* mutant yielded seedlings that were hypersensitive to high light levels (Buhr et al. 2008, 2017; Reinbothe et al. 2006).

Experiments based on thiol-dependent affinity chromatography suggested that POR interacted with TRX-f and TRX-m (Balmer et al. 2003; Marchand et al. 2006). Later, several studies showed that *NTRC*-, *TRXf*- or *TRXm*-deficient *Arabidopsis* mutants accumulated significantly diminished amounts of POR, whereas *POR* transcript levels were not significantly altered compared to WT. Therefore, the altered stability of POR in mutants with inadequate reducing power argues for a redox-based post-translational control of its half-life in the WT (Richter et al. 2013; Da et al. 2017; Wittmann et al. 2018).

Analysis of the redox-sensitive residues of PORB in OxiTRAQ experiments in *Arabidopsis* revealed that Cys114, Cys166 and Cys276 are carbamidomethylated upon exposure to salicylic acid-induced stress (Liu et al. 2015). Recently, in *Chlamydomonas*, proteome and reductome approaches identified light-dependent POR as a TRX-interacting partner and also showed Cys169, Cys278 and Cys304 to be TRX sensitive. On the other hand, two of the three subunits of the light-independent POR found in cyanobacteria, algae and gymnosperms – ChlL (with Cys18, Cys36 and Cys195) and ChlN (Cys326 and Cys455) – are targets of TRXs (Perez-Perez et al. 2017). Recent studies using an NTRC–tandem affinity purification-Tag approach identified PORB as an interacting protein. Here, 2CP was also co-purified by NTRC, indicating the possible relevance of the NTRC–2CP system to the redox regulation of POR enzymes (González et al. 2019).

Physiological significance of redox control in chloroplasts

Because of rapidly changing environmental conditions and the need for balance between photosynthesis and other essential cellular processes, mechanisms have evolved to ensure tight control of all biochemical pathways in chloroplasts. Among a large number of diverse post-translational modifications of proteins, thiol-based redox control affects multiple plastid-localized metabolic activities in different subcompartments (Dietz and Hell 2015). This control mechanism mainly depends on sensing of the redox states of the chloroplast, which vary with changing light intensities and the day-night cycle.

Comparison of redox control of TBS enzymes with redox-dependent regulation of enzymes of the Calvin-Benson cycle (CBC) and starch synthesis

Intensive studies on redox regulation of the CBC enzymes have shown that the activities of glycerol-3-phosphate dehydrogenase (GAPDH), fructose 1,5-bisphosphatase (FBPase), sedoheptulose-1,7-bisphosphatase (SBPase) and phosphoribulose kinase (PRK) are modulated by TRX-induced thiol switches. In addition, the activity of regulators of some CBC enzymes, e.g. ribulose-1,5-bisphosphate carboxylase/oxygenase-activase and CP12, an antagonistically acting dark inhibitor found in a protein complex that includes PRK and GAPDH, is modulated in a redox-dependent manner (Buchanan 2016; McFarlane et al. 2019; Michelet et al. 2013).

As an example for light-dependent redox regulation of CBC enzymes, the *trxf1/ntrc* double mutant displayed a 70% decrease in the ratio of reduced/oxidized FBPase upon illumination, while the ratios in the single *trxf1* and *ntrc* mutants were decreased by 40 and 20%, respectively, relative to WT (Thormahlen et al. 2015). It is noteworthy that *NTRC*- and *TRXf1*-deficient single and double mutants show little change in FBPase levels compared to WT (Thormahlen et al. 2015). Similarly, plants expressing a nonfunctional NTRC with either an inactivated NTR or TRX domain in the *ntrc* background contained only the oxidized forms of FBPase and PRK under low light conditions but, relative to WT or *ntrc*, amounts of these enzymes did not decline (Nikkanen et al. 2016). It was suggested that conversion of the reduced to the oxidized form inactivates FBPase activity without affecting its stability. Moreover, in the redox-sensitive FBPase variant, the Mg^{2+} cofactor-binding site is displaced from the active site after the oxidation of redox-active thiol groups (Chiadmi et al. 1999; Dai et al. 2004).

In contrast to the redox-mediated inactivation of CBC enzymes, the redox sensitivity of TBS enzymes generally alters their stability *in planta* rather than their catalytic activity (Figure 2). As reviewed Sections 4.14 above, amounts of redox-sensitive TBS proteins in TRX- and NTRC-deficient seedlings were often diminished, most probably due to enhanced proteolysis of the oxidized thiol proteins. However, it was also observed that the addition of DTT to *in vitro* enzyme assays with recombinant TBS proteins or plant extracts boosted the activity of the enzyme concerned. But in principle, the impact of the redox states of TBS enzymes on their own stability was always more

Figure 2: Model of the proposed degradation of thiol TBS enzymes in response to the formation of intra- or intermolecular disulfide bonds.

Intra- and intermolecular disulfide bonds can potentially lead to structural alterations that facilitate recognition and degradation by plastid-localized proteases. Intermolecular disulfide bonds in homodimers of an enzyme or heterodimers between enzymatic and non-enzymatic proteins, such as the reductants, could also serve as signals for proteolytic degradation.

pronounced than any change in their intrinsic activity (Da et al. 2017).

Interestingly, GAPDH is inactivated by oxidized CP12 in the dark, in a process that leads to the formation of an inactive ternary complex with PRK (Trost et al. 2006; Fermani et al. 2012). In addition, a GAPDH B-subunit with redox-active Cys residues has evolved in higher plants, and the A2B2 GAPDH heterotetramer is self-regulating, i.e. independent of CP12 (Fermani et al. 2007; Lopez-Calcano et al. 2014). In contrast to these findings, no bona fide redox-dependent interaction between a TBS enzyme and its regulator has yet been identified.

Surprisingly, no clear-cut conditional day-night switch between the oxidized and reduced forms of TBS enzymes has yet been detected in higher plants. As mentioned above, CHLI does not strictly change its redox state during the day-to-night transition. Apart from the reduced form, CHLI was found to be partially oxidized (ox1) in WT pea seedlings during illumination, while in *VIGS-TRXm/TRXf* mutants a second oxidized form (ox2) (Luo et al. 2012) – and in *Arabidopsis* even a third (Perez-Ruiz et al. 2014) – accumulated upon light exposure. In *VIGS-TRXf/TRXm* pea seedlings, up to 22% of the CHLI content is found in the oxidized form (Luo et al. 2012), while *Arabidopsis* WT and *trxx* mutants accumulated up to 17 and 18% oxidized CHLI during the day, compared to 22 and 32% in the *trxf1* and *ntrc* mutants, respectively. These studies indicate that a portion of CHLI is present in the oxidized form even in light-exposed WT leaf samples, while *TRX*-deficient mutants accumulate almost twice as much. As even quadruple mutants for *TRX* fail to achieve complete oxidation of redox-controlled enzymes of TBS, it is assumed that either generally a wider range of *TRX* isoforms serve in the reduction of these TBS enzymes, or other *TRX* isoforms or *NTRC* can partially compensate for the absence of *TRX* isoforms that normally participate in controlling the redox state of CHLI (Luo et al. 2012; Perez-Ruiz et al. 2014).

The turnover of the ADP-glucose pyrophosphorylase (AGPase) subunit APS1 is known to be affected by its redox state. The APS1(Cys81Ser) mutant mimics the reduced conformation, i.e. the normally less abundant monomeric APS1 variant seen in the WT. It was suggested that the monomeric APS1 might be more susceptible to proteolytic degradation (Hadrach et al. 2012), and APS1 was later identified as a potential substrate for the selector protein ClpS of the Clp system (Nishimura et al. 2013). The same approach also pointed to GluTR and ALAD as targets for Clp-dependent degradation. Levels of both enzymes were modified in *TRX-m* or/and *NTRC*-deficient mutants Sections 4.14.3. As mentioned above, these proteins and other TBS enzymes are apparently more sensitive to proteolysis in their oxidized form. Thus, the redox-dependent stability of target proteins and the potentially redox-dependent mode of action of the Clp system should be addressed in future investigations.

In summary, redox-sensitive TBS enzymes are found mainly in the reduced form during photoperiodic growth, and the oxidation state of TBS enzymes is not limited to the dark phase. Based on the observation of diminished TBS protein levels in *NTRC*- and *TRX*-deficient mutants, it is proposed that oxidized TBS enzymes are not specifically inactivated, but are preferentially released for protein degradation. Nevertheless, why redox-dependent TBS enzymes exhibit patterns of thiol switching distinct from those exhibited by redox-controlled CBC enzymes remains an intriguing question.

Light-dark control of Chl synthesis

Over the course of angiosperm evolution, light-dependent Chl synthesis became the norm, enabling plants to efficiently perform all photosynthetic processes. However, the daily change from light to darkness and back requires precise switching between activated and repressed Chl synthesis. Owing to the variability of environmental

conditions, and the cytotoxic impact of accumulating photoreactive Chl precursors, control circuits have evolved which adjust the Chl demand and tune the metabolic flux through each enzymatic step. In the dark, suppression of ALA synthesis in photosynthetically active green tissue and shutdown of the Mg branch for Chl synthesis are obligatory to avoid the accumulation of photoreactive tetrapyrrole intermediates (i.e., Proto or Pchlide). However, synthesis of ALA in at least small quantities for heme, phycobilin and siroheme production is thought to be maintained during the night.

The light-dependent catalytic reaction mediated by POR is responsible for the strictly light-driven mode of Chl biosynthesis in higher plants. A number of regulatory strategies have been identified which prevent the accumulation of phototoxic intermediates during the night and modulate the need for Chl under varying light intensities by reducing the flow of substrate into the Chl-synthesizing branch of TBS. (i) The Pchlide-dependent inactivation of GluTR by the negative regulator FLU, which leads to a decline in ALA synthesis (Meskauskiene et al. 2001; Zhang et al. 2015). (ii) The slow-down of MgCh activity by GENOMES UNCOUPLED 4 phosphorylation (Richter et al. 2018) and, very probably, the redox-dependent inactivation of CHLI-catalyzed ATP hydrolysis (Ikegami et al. 2007). (iii) It has also been proposed that porphyrin intermediates can be consumed through drainage into the heme synthesis pathway when Chl synthesis is blocked in darkness. This additional dark accumulation of heme could then trigger a heme-dependent inactivation of ALA synthesis. It took some effort to identify this heme-dependent post-translational feedback control on ALA synthesis. Indeed, the suggested redirection of metabolite flow into the heme branch triggers a feedback control of ALA synthesis by accumulating heme. Heme was shown to bind to GBP and loosen its interaction to GluTR, so that free GluTR becomes more accessible to Clp-mediated protein degradation (Richter et al. 2019).

In darkness, a membrane-bound inactivation complex consisting of FLU, GluTR and other enzymes of the Chl branch, such as POR, MgPME cyclase subunit CHL27 and divinyl reductase, is formed when Pchlide, the substrate of the light-dependent POR, accumulates (Kauss et al. 2012). Upon exposure to light, GluTR is released from this inactivation complex and ALA formation is stimulated. However, it is not clear how GluTR is recruited to the membrane-bound FLU and what triggers the GluTR–FLU interaction. Given the various redox states of chloroplasts

required for balanced metabolic flow during light-dark transitions, GluTR, FLU, POR and other proteins of the inactivation complex may well be targets for redox-mediated control in TBS.

Structural and biochemical analyses have revealed the homodimerization of GluTR (Czarnecki et al. 2011; Zhao et al. 2014). During fractionation in non-reducing SDS-polyacrylamide gels, a substantial portion of GluTR remains in the dimer form (our unpublished data). This structure was suggested to facilitate substrate channeling to the dimeric GSAAT (Moser et al. 2001) and a redox-mediated interaction may be involved here. In the light, GluTR is active and is predominantly found in the soluble fraction, i.e. the stroma of chloroplasts (Schmied et al. 2018). During the night, the proportion of GluTR in the stroma is diminished and GluTR interacts with FLU at the membrane, resulting in inactivation of ALA synthesis. But how GluTR is inactivated by FLU and released from membrane-associated FLU to stimulate ALA synthesis remains unknown. We set out to examine the potential redox-mediated GluTR inactivation by FLU employing several approaches:

FLU has two conserved Cys residues at positions 119 and 292, outside and within its protein-interacting tetratricopeptide-repeat domain, respectively. When expressed *in planta*, FLU(Cys119Ser) fails to form dimers under oxidizing conditions (unpublished data), indicating that Cys119 of FLU is required for the formation of intermolecular disulfide bonds. Following the idea that oxidizing conditions support the interaction between dimers of FLU and GluTR suggested by the 3D structure analysis of GluTR (Zhang et al. 2015), it is speculated that the dimerization of FLU could strengthen a stable interaction with GluTR, whereas FLU(Cys119Ser) is potentially defective in GluTR inactivation.

Since both proteins are readily accessible to thiol-based redox control for the suppression of ALA synthesis, it is possible that (i) homodimerization of GluTR and FLU, (ii) assembly of the FLU–GluTR complex, or (iii) the transfer and attachment of GluTR to the thylakoid membrane depend on disulfide bond formation, and therefore redox-mediated control. These processes could be prerequisites for rapid and dynamic inactivation of GluTR by FLU, although it cannot be excluded that the redox control of POR (or any other protein that contributes to the inactivation complex of GluTR) participates in the modulation of metabolic activities during light exposure and darkness. At all events, these assumptions need to be tested in further biochemical studies.

Final remarks

Rates of flux through several metabolic pathways, including CBC, starch and Chl synthesis, are coupled to photosynthetic activity. Because plants cannot predict changing light conditions during the day, they need to sense light changes and use that information to modulate rapidly enzyme activities (Buchanan 2016). This review provides an interim report on efforts to elucidate redox control in TBS. It is remarkable that more than half of the enzymes in TBS interact with TRX/NTRC and are (potentially) under redox control. Whether each TBS enzyme is preferentially controlled by a specific reductant remains an open question. However, although we are far from any comprehensive view, TRX-f seems to be relatively more important for the regulation of certain stroma-localized plastid metabolic pathways, such as CBC and starch synthesis (Buchanan 2016), even though there are some redundancies with the m-type TRXs. Conversely, m-type TRXs appear to be more closely involved in the biogenesis of the photosystems (Wang et al. 2013) and most probably in TBS (Da et al. 2017).

Acknowledgment: Our research was funded by the Deutsche Forschungsgemeinschaft (DFG) project entitled “Thiol-Based Redox Control of Tetrapyrrole Metabolism: Posttranslational Control of Tetrapyrrole Biosynthesis Enzymes by NADPH-Dependent Thioredoxin Reductase C and Thioredoxins” (Gr 936/17-2) to B.G., which is part of the Priority Programme SPP1710 “Dynamics of Thiol-Based Redox Switches in Cellular Physiology”.

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

- Adams, N.B.P. and Reid, J.D. (2013). The allosteric role of the AAA+Domain of ChlD protein from the magnesium chelatase of *Synechocystis* Species PCC 6803. *J. Biol. Chem.* 288: 28727–28732.
- Akter, S., Huang, J., Bodra, N., De Smet, B., Wahni, K., Rombaut, D., Pauwels, J., Gevaert, K., Carroll, K., Van Breusegem, F., et al (2015). DYN-2 based identification of *Arabidopsis* Sulfenomes. *Mol. Cell. Proteomics* 14: 1183–1200.
- Apitz, J., Nishimura, K., Schmied, J., Wolf, A., Hedtke, B., van Wijk, K.J., and Grimm, B. (2016). Posttranslational control of ALA synthesis includes GluTR degradation by Clp protease and stabilization by GluTR-binding protein. *Plant Physiol.* 170: 2040–2051.
- Apitz, J., Schmied, J., Lehmann, M.J., Hedtke, B., and Grimm, B. (2014). GluTR2 complements a hema1 mutant lacking glutamyl-tRNA reductase 1, but is differently regulated at the post-translational level. *Plant Cell Physiol.* 55: 645–657.
- Armstrong, G.A., Runge, S., Frick, G., Sperling, U., and Apel, K. (1995). Identification of NADPH:protochlorophyllide oxidoreductases A and B: a branched pathway for light-dependent chlorophyll biosynthesis in *Arabidopsis thaliana*. *Plant Physiol.* 108: 1505–1517.
- Arsova, B., Hoja, U., Wimmelbacher, M., Greiner, E., Üstün, Ş., Melzer, M., Petersen, K., Lein, W., and Börnke, F. (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.
- Balange, A.P. and Lambert, C. (1983). In vitro activation of δ -aminolevulinic acid dehydratase from far-red irradiated radish (*Raphanus sativus* L.) seedlings by thioredoxin f. *Plant Sci. Lett.* 32: 253–259.
- Balmer, Y., Koller, A., del Val, G., Manieri, W., Schürmann, P., and Buchanan, B.B. (2003). Proteomics gives insight into the regulatory function of chloroplast thioredoxins. *Proc. Natl. Acad. Sci. Unit. States Am.* 100: 370–375.
- Balsera, M., Uberegui, E., Schürmann, P., and Buchanan, B. B. (2014). Evolutionary development of redox regulation in chloroplasts. *Antioxidants Redox Signal.* 21: 1327–1355.
- Battersby, A.R. (1988). Biosynthesis of the pigments of life. *J. Nat. Prod.* 51: 629–642.
- Benitez-Alfonso, Y., Cilia, M., Roman, A.S., Thomas, C., Maule, A., Hearn, S., and Jackson, D. (2009). Control of *Arabidopsis* meristem development by thioredoxin-dependent regulation of intercellular transport. *Proc. Natl. Acad. Sci. Unit. States Am.* 106: 3615–3620.
- Benli, M., Schulz, R.d., and Apel, K. (1991). Effect of light on the NADPH-protochlorophyllide oxidoreductase of *Arabidopsis thaliana*. *Plant Mol. Biol.* 16: 615–625.
- Bernal-Bayard, P., Ojeda, V., Hervás, M., Cejudo, F.J., Navarro, J.A., Velázquez-Campoy, A., and Pérez-Ruiz, J.M. (2014). Molecular recognition in the interaction of chloroplast 2-Cys peroxiredoxin with NADPH-thioredoxin reductase C (NTRC) and thioredoxin. *FEBS Lett.* 588: 4342–4347.
- Bohrer, A.-S., Massot, V., Innocenti, G., Reichheld, J.-P., Issakidis-Bourguet, E., and Vanacker, H. (2012). New insights into the reduction systems of plastidial thioredoxins point out the unique properties of thioredoxin z from *Arabidopsis*. *J. Exp. Bot.* 63: 6315–6323.
- Broin, M., Cuiné, 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.
- Brooks, M.D., Sylak-Glassman, E.J., Fleming, G.R., and Niyogi, K.K. (2013). A thioredoxin-like/ -propeller protein maintains the efficiency of light harvesting in *Arabidopsis*. *Proc. Natl. Acad. Sci. Unit. States Am.* 110: E2733–E2740.
- Brzezowski, P., Richter, A.S., and Grimm, B. (2015). Regulation and function of tetrapyrrole biosynthesis in plants and algae. *Biochim. Biophys. Acta Bioenerg.* 1847: 968–985.
- Buchanan, B.B. (2016). The path to thioredoxin and redox regulation in chloroplasts. *Annu. Rev. Plant Biol.* 67: 1–24.

- Buchanan, B.B. and Balmer, Y. (2005). Redox regulation: a broadening horizon. *Annu. Rev. Plant Biol.* 56: 187–220.
- Buhr, F., El Bakkouri, M., Valdez, O., Pollmann, S., Lebedev, N., Reinbothe, S., and Reinbothe, C. (2008). Photoprotective role of NADPH:protochlorophyllide oxidoreductase A. *Proc. Natl. Acad. Sci. Unit. States Am.* 105: 12629–12634.
- Buhr, F., Lahroussi, A., Springer, A., Rustgi, S., von Wettstein, D., Reinbothe, C., and Reinbothe, S. (2017). NADPH:protochlorophyllide oxidoreductase B (PORB) action in *Arabidopsis thaliana* revisited through transgenic expression of engineered barley PORB mutant proteins. *Plant Mol. Biol.* 94: 45–59.
- Cain, P., Hall, M., Schröder, W.P., Kieselbach, T., and Robinson, C. (2009). A novel extended family of stromal thioredoxins. *Plant Mol. Biol.* 70: 273–281.
- 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.
- Cejudo, F.J., Ojeda, V., Delgado-Requerey, V., Gonzalez, M., and Perez-Ruiz, J.M. (2019). Chloroplast redox regulatory mechanisms in plant adaptation to light and darkness. *Front. Plant Sci.* 10: 380.
- Cerveau, D., Kraut, A., Stotz, H.U., Mueller, M.J., Couté, Y., and Rey, P. (2016). Characterization of the *Arabidopsis thaliana* 2-Cys peroxiredoxin interactome. *Plant Sci.* 252: 30–41.
- Chen, F.F., Chien, C.Y., Cho, C.C., Chang, Y.Y., and Hsu, C.H. (2019). C-terminal redox domain of Arabidopsis APR1 is a non-canonical thioredoxin domain with glutaredoxin function. *Antioxidants* 8: 461.
- Chen, X., Wang, X., Feng, J., Chen, Y., Fang, Y., Zhao, S., Zhao, A., Zhang, M., and Liu, L. (2014). Structural insights into the catalytic mechanism of Synechocystis Magnesium protoporphyrin IXO-methyltransferase (ChlM). *J. Biol. Chem.* 289: 25690–25698.
- Chiadmi, M., Navaza, A., Miginiac-Maslow, M., Jacquot, J.P., and Cherfils, J. (1999). Redox signalling in the chloroplast: structure of oxidized pea fructose-1,6-bisphosphate phosphatase. *EMBO J.* 18: 6809–6815.
- Chibani, K., Tarrago, L., Gualberto, J.M., Wingsle, G., Rey, P., Jacquot, J.-P., and Rouhier, N. (2012). Atypical thioredoxins in poplar: the glutathione-dependent thioredoxin-like 2.1 supports the activity of target enzymes possessing a single redox active cysteine. *Plant Physiol.* 159: 592–605.
- Chibani, K., Tarrago, L., Schürmann, P., Jacquot, J.-P., and Rouhier, N. (2011). Biochemical properties of poplar thioredoxin z. *FEBS Lett.* 585: 1077–1081.
- Collin, V., Issakidis-Bourguet, E., Marchand, C., Hirasawa, M., Lancelin, J.-M., Knaff, D. B., and Miginiac-Maslow, M. (2003). The Arabidopsis Plastidial thioredoxins. *J. Biol. Chem.* 278: 23747–23752.
- Collin, V., Lamkemeyer, P., Miginiac-Maslow, M., Hirasawa, M., Knaff, D.B., Dietz, K.-J., and Issakidis-Bourguet, E. (2004). Characterization of plastidial thioredoxins from *Arabidopsis* belonging to the new y-type. *Plant Physiol.* 136: 4088–4095.
- Czarnecki, O., Hedtke, B., Melzer, M., Rothbart, M., Richter, A., Schröter, Y., Pfannschmidt, T., and Grimm, B. (2011). An *Arabidopsis* GluTR binding protein mediates spatial separation of 5-aminolevulinic acid synthesis in chloroplasts. *Plant Cell* 23: 4476–4491.
- Da, Q., Wang, P., Wang, M., Sun, T., Jin, H., Liu, B., Wang, J., Grimm, B., and Wang, H.-B. (2017). Thioredoxin and NADPH-dependent thioredoxin reductase C regulation of tetrapyrrole biosynthesis. *Plant Physiol.* 175: 652–666.
- Dai, S., Johansson, K., Miginiac-Maslow, M., Schürmann, P., and Eklund, H. (2004). Structural basis of redox signaling in photosynthesis: structure and function of ferredoxin:thioredoxin reductase and target enzymes. *Photosynth. Res.* 79: 233–248.
- Dangoor, I., Peled-Zehavi, H., Levitan, A., Pasand, O., and Danon, A. (2009). A small family of chloroplast atypical thioredoxins. *Plant Physiol.* 149: 1240–1250.
- 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.
- Diaz, M.G., Hernandez-Verdeja, T., Kremnev, D., Crawford, T., Dubreuil, C., and Strand, A. (2018). Redox regulation of PEP activity during seedling establishment in *Arabidopsis thaliana*. *Nat. Commun.* 9: 50.
- Dietz, K.J. (2016). Thiol-based peroxidases and ascorbate peroxidases: why plants rely on multiple peroxidase systems in the photosynthesizing chloroplast?. *Mol. Cell* 39: 20–5.
- Dietz, K.-J. and Hell, R. (2015). Thiol switches in redox regulation of chloroplasts: balancing redox state, metabolism and oxidative stress. *Biol. Chem.* 396: 483–494.
- 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.
- Farmer, D.A., Brindley, A.A., Hitchcock, A., Jackson, P.J., Johnson, B., Dickman, M.J., Hunter, C.N., Reid, J.D., and Adams, N.B.P. (2019). The ChlD subunit links the motor and porphyrin binding subunits of magnesium chelatase. *Biochem. J.* 476: 1875–1887.
- Fermani, S., Sparla, F., Falini, G., Martelli, P.L., Casadio, R., Pupillo, P., Ripamonti, A., and Trost, P. (2007). Molecular mechanism of thioredoxin regulation in photosynthetic A2B2-glyceraldehyde-3-phosphate dehydrogenase. *Proc. Natl. Acad. Sci. Unit. States Am.* 104: 11109–11114.
- Fermani, S., Trivelli, X., Sparla, F., Thumiger, A., Calvaresi, M., Marri, L., Falini, G., Zerbetto, F., and Trost, P. (2012). Conformational selection and folding-upon-binding of intrinsically disordered protein CP12 regulate photosynthetic enzymes assembly. *J. Biol. Chem.* 287: 21372–21383.
- Fodje, M.N., Hansson, A., Hansson, M., Olsen, J.G., Gough, S., Willows, R.D., and Al-Karadaghi, S. (2001). Interplay between an AAA module and an integrin I domain may regulate the function of magnesium chelatase. *J. Mol. Biol.* 311: 111–122.
- 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.
- González, M., Delgado-Requerey, V., Ferrández, J., Serna, A., and Cejudo, F.J. (2019). Insights into the function of NADPH thioredoxin reductase C (NTRC) based on identification of NTRC-interacting proteins *in vivo*. *J. Exp. Bot.* 70: 5787–5798.
- Grant, K., Carey, N.M., Mendoza, M., Schulze, J., Pilon, M., Pilon-Smits, E.A.H., and van Hoewyk, D. (2011). Adenosine 5'-phosphosulfate reductase (APR2) mutation in *Arabidopsis* implicates glutathione deficiency in selenate toxicity. *Biochem. J.* 438: 325–335.
- Grimm, B. (2019a). Metabolism, structure and function of plant tetrapyrroles: introduction, microbial and eukaryotic chlorophyll synthesis and catabolism. In: *Advances in Botanical Research*

- series, Vol. 90. Academic Press. pp. 328. Hardcover ISBN: 9780081027523 eBook ISBN: 9780081027530.
- Grimm, B. (2019b). Structure and function of plant tetrapyrroles: control mechanisms of chlorophyll biosynthesis and analysis of chlorophyll-binding protein. In: *Advances in Botanical Research series*, Vol. 91. pp. 308. Hardcover ISBN: 9780128199824 eBook ISBN: 9780128206348.
- Hädrich, N., Hendriks, J.H.M., Kötting, O., Arrivault, S., Feil, R., Zeeman, S.C., Gibon, Y., Schulze, W.X., Stitt, M., and Lunn, J.E. (2012). Mutagenesis of cysteine 81 prevents dimerization of the APS1 subunit of ADP-glucose pyrophosphorylase and alters diurnal starch turnover in *Arabidopsis thaliana* leaves. *Plant J.* 70: 231–242.
- Hennig, M., Grimm, B., Contestabile, R., John, R.A., and Jansonius, J.N. (1997). Crystal structure of glutamate-1-semialdehyde aminomutase: an 2-dimeric vitamin B6-dependent enzyme with asymmetry in structure and active site reactivity. *Proc. Natl. Acad. Sci. Unit. States Am.* 94: 4866–4871.
- Holmgren, A. (1995). Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. *Structure* 3: 239–243.
- Hou, Z, Yang, Y, Hedtke, B, and Grimm, B (2019). Fluorescence in blue light (FLU) is involved in inactivation and localization of glutamyl-tRNA reductase during light exposure. *Plant J.* 97: 517–529.
- Huang, Y.-S., and Li, H.-M. (2009). *Arabidopsis* CHL12 can substitute for CHL11. *Plant Physiol.* 150: 636–645.
- Ikegami, A., Yoshimura, N., Motohashi, K., Takahashi, S., Romano, P.G.N., Hisabori, T., Takamiya, K.-I., and Masuda, T. (2007). The CHL1 subunit of *Arabidopsis thaliana* Magnesium chelatase is a target protein of the chloroplast thioredoxin. *J. Biol. Chem.* 282: 19282–19291.
- Jasid, S., Simontacchi, M., Bartoli, C. G., and Puntarulo, S. (2006). Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. *Plant Physiol.* 142: 1246–1255.
- Jensen, P.E., Gibson, L.C.D., and Neil HUNTER, C. (1999). ATPase activity associated with the magnesium-protoporphyrin IX chelatase enzyme of *Synechocystis* PCC6803: evidence for ATP hydrolysis during Mg^{2+} insertion, and the $MgATP$ -dependent interaction of the ChlI and ChlD subunits. *Biochem. J.* 339: 127–134.
- Jensen, P.E., Reid, J.D., and Hunter, C.N. (2000). Modification of cysteine residues in the ChlI and ChlH subunits of magnesium chelatase results in enzyme inactivation. *Biochem. J.* 352: 435–441.
- Karamoko, M., Cline, S., Redding, K., Ruiz, N., and Hamel, P.P. (2011). Lumen Thiol Oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in *Arabidopsis*. *Plant Cell* 23: 4462–4475.
- Kauss, D., Bischof, S., Steiner, S., Apel, K., and Meskauskiene, R. (2012). FLU, a negative feedback regulator of tetrapyrrole biosynthesis, is physically linked to the final steps of the Mg^{++} -branch of this pathway. *FEBS Lett.* 586: 211–216.
- Kobayashi, K. and Masuda, T. (2016). Transcriptional regulation of tetrapyrrole biosynthesis in *Arabidopsis thaliana*. *Front. Plant Sci.* 7: 1811.
- Kobayashi, K., Mochizuki, N., Yoshimura, N., Motohashi, K., Hisabori, T., and Masuda, T. (2008). Functional analysis of *Arabidopsis thaliana* isoforms of the Mg-chelatase CHL1 subunit. *Photochem. Photobiol. Sci.* 7: 1188–1195.
- Lee, B.-R., Koprivova, A., and Kopriva, S. (2011). The key enzyme of sulfate assimilation, adenosine 5'-phosphosulfate reductase, is regulated by HY5 in *Arabidopsis*. *Plant J.* 67: 1042–1054.
- Lennartz, K., Plucken, H., Seidler, A., Westhoff, P., Bechtold, N., and Meierhoff, K. (2001). HCF164 encodes a thioredoxin-like protein involved in the biogenesis of the cytochrome b6f complex in *Arabidopsis*. *Plant Cell* 13: 2539–2551.
- Lindahl, M., and Florencio, F.J. (2003). Thioredoxin-linked processes in cyanobacteria are as numerous as in chloroplasts, but targets are different. *Proc. Natl. Acad. Sci. Unit. States Am.* 100: 16107–16112.
- Liu, P., Zhang, H., Yu, B., Xiong, L., and Xia, Y. (2015). Proteomic identification of early salicylate- and flg22-responsive redox-sensitive proteins in *Arabidopsis*. *Sci. Rep.* 5: 8625.
- Lopez-Calcano, P.E., Howard, T.P., and Raines, C.A. (2014). The CP12 protein family: a thioredoxin-mediated metabolic switch?. *Front. Plant Sci.* 5: 9.
- Luo, T., Fan, T., Liu, Y., Rothbart, M., Yu, J., Zhou, S., Grimm, B., and Luo, M. (2012). Thioredoxin redox regulates ATPase activity of magnesium chelatase CHL1 subunit and modulates redox-mediated signaling in tetrapyrrole biosynthesis and homeostasis of reactive oxygen species in pea plants. *Plant Physiol.* 159: 118–130.
- Marchand, C., Le Maréchal, P., Meyer, Y., and Decottignies, P. (2006). Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. *Proteomics* 6: 6528–6537.
- Martin, K. J. (1995). Workers' compensation. *AAOHN J.* 43: 245–250.
- Matsumoto, F., Obayashi, T., Sasaki-Sekimoto, Y., Ohta, H., Takamiya, K.-I., and Masuda, T. (2004). Gene expression profiling of the tetrapyrrole metabolic pathway in *Arabidopsis* with a mini-array system. *Plant Physiol.* 135: 2379–2391.
- McFarlane, C.R., Shah, N.R., Kabasakal, B.V., Echeverria, B., Cotton, C.A.R., Bubeck, D., and Murray, J.W. (2019). Structural basis of light-induced redox regulation in the Calvin-Benson cycle in cyanobacteria. *Proc. Natl. Acad. Sci. U.S.A.* 116: 20984–20990.
- McWhite, C.D., Papoulas, O., Drew, K., Cox, R.M., June, V., Dong, O.X., Kwon, T., Wan, C., Salmi, M.L., Roux, S.J., et al (2020). A pan-plant protein complex map reveals deep conservation and novel assemblies. *Cell* 181: 460–474.
- Meskauskiene, R., Nater, M., Goslings, D., Kessler, F., op den Camp, R., and Apel, K. (2001). FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. Unit. States Am.* 98: 12826–12831.
- Meyer, Y., Siala, W., Bashandy, T., Riondet, C., Vignols, F., and Reichheld, J.P. (2008). Glutaredoxins and thioredoxins in plants. *Biochim. Biophys. Acta Mol. Cell Res.* 1783: 589–600.
- Meyer, Y., Vignols, F., and Reichheld, J.P. (2002). Classification of plant thioredoxins by sequence similarity and intron position. *Methods Enzymol.* 347: 394–402.
- 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. Unit. States Am.* 106: 9908–9913.
- Michelet, L., Zaffagnini, M., Morisse, S., Sparla, F., Perez-Perez, M.E., Francia, F., Danon, A., Marchand, C.H., Fermani, S., Trost, P., et al (2013). Redox regulation of the Calvin-Benson cycle: something old, something new. *Front. Plant Sci.* 4: 470.

- Miseta, A. and Csutora, P. (2000). Relationship between the occurrence of cysteine in proteins and the complexity of organisms. *Mol. Biol. Evol.* 17: 1232–1239.
- Mochizuki, N., Tanaka, R., Grimm, B., Masuda, T., Moulin, M., Smith, A.G., Tanaka, A., and Terry, M.J. (2010). The cell biology of tetrapyrroles: a life and death struggle. *Trends Plant Sci.* 15: 488–498.
- Moon, J.C., Jang, H.H., Chae, H.B., Lee, J.R., Lee, S.Y., Jung, Y.J., Shin, M.R., Lim, H.S., Chung, W.S., Yun, D.-J., et al. (2006). The C-type *Arabidopsis* thioredoxin reductase ANTR-C acts as an electron donor to 2-Cys peroxiredoxins in chloroplasts. *Biochem. Biophys. Res. Commun.* 348: 478–484.
- Moser, J., Lorenz, S., Hubschwerlen, C., Rompf, A., and Jahn, D. (1999). Methanopyrus kandleri Glutamyl-tRNA reductase. *J. Biol. Chem.* 274: 30679–30685.
- Moser, J., Schubert, W.D., Beier, V., Bringemeier, I., Jahn, D., and Heinz, D.W. (2001). V-shaped structure of glutamyl-tRNA reductase, the first enzyme of tRNA-dependent tetrapyrrole biosynthesis. *EMBO J.* 20: 6583–6590.
- Motohashi, K. and Hisabori, T. (2006). HCF164 receives reducing equivalents from stromal thioredoxin across the thylakoid membrane and mediates reduction of target proteins in the thylakoid lumen. *J. Biol. Chem.* 281: 35039–35047.
- Motohashi, K. and Hisabori, T. (2010). CcdA is a thylakoid membrane protein required for the transfer of reducing equivalents from stroma to thylakoid lumen in the higher plant chloroplast. *Antioxidants Redox Signal.* 13: 1169–1176.
- Naranjo, B., Diaz-Espejo, A., Lindahl, M., and Cejudo, F.J. (2016a). Type-II thioredoxins have a role in the short-term activation of carbon metabolism and their loss affects growth under short-day conditions in *Arabidopsis thaliana*. *Exptl. Bot.* 67: 1951–1964.
- Naranjo, B., Migné, C., Krieger-Liszka, A., Hornero-Méndez, D., Gallardo-Guerrero, L., Cejudo, F.J., and Lindahl, M. (2016b). 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.
- Née, G., Zaffagnini, M., Trost, P., and Issakidis-Bourguet, E. (2009). Redox regulation of chloroplastic glucose-6-phosphate dehydrogenase: a new role for f-type thioredoxin. *FEBS Lett.* 583: 2827–2832.
- Nikkanen, L., Toivola, J., and Rintamäki, E. (2016). Crosstalk between chloroplast thioredoxin systems in regulation of photosynthesis. *Plant Cell Environ.* 39: 1691–1705.
- Nishimura, K., Asakura, Y., Friso, G., Kim, J., Oh, S.-H., Rutschow, H., Ponnala, L., and van Wijk, K.J. (2013). ClpS1 is a conserved substrate selector for the chloroplast Clp protease system in *Arabidopsis*. *Plant Cell* 25: 2276–2301.
- Ojeda, V., Pérez-Ruiz, J.M., and Cejudo, F.J. (2018a). 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.
- Ojeda, V., Pérez-Ruiz, J.M., and Cejudo, F.J. (2018b). 2-Cys peroxiredoxins participate in the oxidation of chloroplast enzymes in the dark. *Mol. Plant* 11: 1377–1388.
- Okegawa, Y. and Motohashi, K. (2015). Chloroplastic thioredoxin functions as a major regulator of Calvin cycle enzymes during photosynthesis in vivo. *Plant J.* 84: 900–913.
- Oliver, R.P. and Griffiths, W.T. (1981). Covalent labelling of the NADPH: protochlorophyllide oxidoreductase from etioplast membranes with [3H]N-phenylmaleimide. *Biochem. J.* 195: 93–101.
- Page, M.L.D., Hamel, P.P., Gabilly, S.T., Zegzouti, H., Perea, J.V., Alonso, J.M., Ecker, J.R., Theg, S.M., Christensen, S.K., and Merchant, S. (2004). A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the Cytochrome b6/f complex in *Arabidopsis* chloroplasts. *J. Biol. Chem.* 279: 32474–32482.
- Pérez-Ruiz, J.M., Guinea, M., Puerto-Galán, L., and Cejudo, F.J. (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.
- Pérez-Pérez, M.E., Mauriès, A., Maes, A., Tourasse, N.J., Hamon, M., Lemaire, S.D., and Marchand, C.H. (2017). The deep thioredoxome in *Chlamydomonas reinhardtii*: new insights into redox regulation. *Mol. Plant* 10: 1107–1125.
- 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.
- 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.
- Reinbothe, C., Buhr, F., Bartsch, S., Desvignes, C., Quigley, F., and Pesey, H., Reinbothe, S. (2006). In vitro-mutagenesis of NADPH: protochlorophyllide oxidoreductase B: two distinctive protochlorophyllide binding sites participate in enzyme catalysis and assembly. *Mol. Genet. Genom.* 275: 540–552.
- Reinbothe, S., Reinbothe, C., Holtorf, H., and Apel, K. (1995b). Two NADPH:protochlorophyllide oxidoreductases in barley: evidence for the selective disappearance of PORA during the light-induced greening of etiolated seedlings. *Plant Cell* 7: 1933–1940.
- Reinbothe, S., Reinbothe, C., Lebedev, N., and Apel, K. (1996b). PORA and PORB, two light-dependent protochlorophyllide-reducing enzymes of angiosperm chlorophyll biosynthesis. *Plant Cell* 8: 763–769.
- Reinbothe, S., Reinbothe, C., Neumann, D., and Apel, K. (1996a). A plastid enzyme arrested in the step of precursor translocation *in vivo*. *Proc. Natl. Acad. Sci. Unit. States Am.* 93: 12026–12034.
- Reinbothe, S., Runge, S., Reinbothe, C., van Cleve, B., and Apel, K. (1995a). Substrate-dependent transport of the NADPH: protochlorophyllide oxidoreductase into isolated plastids. *Plant Cell* 7: 161–172.
- 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.
- Richter, A.S., Banse, C., and Grimm, B. (2019). The GluTR-binding protein is the heme-binding factor for feedback control of glutamyl-tRNA reductase. *eLife* 8, <https://doi.org/10.7554/elife.46300>.
- Richter, A.S. and Grimm, B. (2013). Thiol-based redox control of enzymes involved in the tetrapyrrole biosynthesis pathway in plants. *Front. Plant Sci.* 4: 371.
- 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.

- Richter, A.S., Peter, E., Rothbart, M., Schlicke, H., Toivola, J., Rintamäki, E., and Grimm, B. (2013). Posttranslational influence of NADPH-dependent thioredoxin reductase C on enzymes in tetrapyrrole synthesis. *Plant Physiol.* 162: 63–73.
- Richter, A.S., Wang, P., and Grimm, B. (2016). *Arabidopsis* Mg-protoporphyrin IX methyltransferase activity and redox regulation depend on conserved cysteines. *Plant Cell Physiol.* 57: 519–527.
- Schauer, S., Chaturvedi, S., Randau, L., Moser, J., Kitabatake, M., Lorenz, S., Verkamp, E., Schubert, W.-D., Nakayashiki, T., Murai, M., et al (2002). *Escherichia coli* Glutamyl-tRNA Reductase. *J. Biol. Chem.* 277: 48657–48663.
- Schmied, J., Hou, Z., Hedtke, B., and Grimm, B. (2018). Controlled partitioning of glutamyl-tRNA reductase in stroma- and membrane-associated fractions affects the synthesis of 5-aminolevulinic acid. *Plant Cell Physiol.* 59: 2204–2213.
- Schubert, W.-D., Moser, J., Schauer, S., Heinz, D.W., and Jahn, D. (2002). Structure and function of glutamyl-tRNA reductase, the first enzyme of tetrapyrrole biosynthesis in plants and prokaryotes. *Photosynth. Res.* 74: 205–215.
- Schulz, R., Steinmüller, K., Klaas, M., Forreiter, C., Rasmussen, S., Hiller, C., and Apel, K. (1989). Nucleotide sequence of a cDNA coding for the NADPH-protochlorophyllide oxidoreductase (PCR) of barley (*Hordeum vulgare* L.) and its expression in *Escherichia coli*. *Mol. Gen. Genet.* 217: 355–361.
- Serrato, A.J., Fernandez-Trijuque, J., Barajas-Lopez, J.D., Chueca, A., and Sahrway, M. (2013). Plastid thioredoxins: a “one-for-all” redox-signaling system in plants. *Front. Plant Sci.* 4: 463.
- 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.
- Seung, D., Thalmann, M., Sparla, F., Abou Hachem, M., Lee, S.K., Issakidis-Bourguet, E., Svensson, B., Zeeman, S.C., and Santelia, D. (2013). *Arabidopsis thaliana* AMY3 is a unique redox-regulated chloroplastic α -amylase. *J. Biol. Chem.* 288: 33620–33633.
- Skryhan, K., Cuesta-Seijo, J.A., Nielsen, M.M., Marri, L., Mellor, S.B., Glaring, M.A., Jensen, P.E., Palcic, M.M., and Blennow, A. (2015). The role of cysteine residues in redox regulation and protein stability of *Arabidopsis thaliana* starch synthase 1. *PLoS One* 10: e0136997.
- Song, Y., Pu, H., Jiang, T., Zhang, L., and Ouyang, M. (2016). Crystal structure of glutamate-1-semialdehyde-2,1-aminomutase from *Arabidopsis thaliana*. *Acta Crystallogr. F* 72: 448–456.
- Sparla, F., Costa, A., Lo Schiavo, F., Pupillo, P., and Trost, P. (2006). Redox regulation of a novel plastid-targeted β -amylase of *Arabidopsis*. *Plant Physiol.* 141: 840–850.
- Stenbaek, A. and Jensen, P. E. (2010). Redox regulation of chlorophyll biosynthesis. *Phytochemistry* 71: 853–859.
- Tanaka, R., Kobayashi, K., and Masuda, T. (2011). Tetrapyrrole metabolism in *Arabidopsis thaliana*. *Arabidopsis Book* 9: e0145.
- Tanaka, R. and Tanaka, A. (2007). Tetrapyrrole biosynthesis in higher plants. *Annu. Rev. Plant Biol.* 58: 321–346.
- Tang, W., Wang, W., Chen, D., Ji, Q., Jing, Y., Wang, H., and Lin, R. (2012). Transposase-derived proteins FHY3/FAR1 interact with PHYTOCHROME-INTERACTING FACTOR1 to regulate chlorophyll biosynthesis by modulating HEMB1 during deetiolation in *Arabidopsis*. *Plant Cell* 24: 1984–2000.
- Tanou, G., Job, C., Rajjou, L.C., Arc, E., Belghazi, M., Diamantidis, G., Molassiotis, A., and Job, D. (2009). Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. *Plant J.* 60: 795–804.
- Tarrago, L., Laugier, E., Zaffagnini, M., Marchand, C.H., Le Maréchal, P., Lemaire, S.D., and Rey, P. (2010). Plant thioredoxin CDSP32 regenerates 1-cys methionine sulfoxide reductase B activity through the direct reduction of sulfenic acid. *J. Biol. Chem.* 285: 14964–14972.
- Thormählen, I., Meitzel, T., Groysman, J., Öchsner, AB, von Roepenack-Lahaye, E., Naranjo, B., Cejudo, FJ, and Geigenberger, P. (2015). Thioredoxin f1 and NADPH-dependent thioredoxin reductase C have overlapping functions in regulating photosynthetic metabolism and plant growth in response to varying light conditions. *Plant Physiol.* 169: 1766–86.
- Thormählen, I., Ruber, J., von Roepenack-Lahaye, E., Ehrlich, S.-M., Massot, V., Hümmer, C., Tezycka, J., Issakidis-Bourguet, E., and Geigenberger, P. (2013). Inactivation of thioredoxin f1 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.
- Townley, H.E., Sessions, R.B., Clarke, A.R., Dafforn, T.R., and Griffiths, W.T. (2001). Protochlorophyllide oxidoreductase: a homology model examined by site-directed mutagenesis. *Proteins* 44: 329–335.
- Tripathy, B.C., Sherameti, I., and Oelmüller, R. (2010). Siroheme. *Plant Signal. Behav.* 5: 14–20.
- Trost, P., Fermani, S., Marri, L., Zaffagnini, M., Falini, G., Scagliarini, S., Pupillo, P., and Sparla, F. (2006). Thioredoxin-dependent regulation of photosynthetic glyceraldehyde-3-phosphate dehydrogenase: autonomous vs. CP12-dependent mechanisms. *Photosynth. Res.* 89: 263–275.
- Vanacker, H., Guichard, M., Bohrer, A.-S., and Issakidis-Bourguet, E. (2018). Redox regulation of monodehydroascorbate reductase by thioredoxin y in plastids revealed in the context of water stress. *Antioxidants* 7: 183.
- Vaseghi, M.J., Chibani, K., Telman, W., Liebthal, M.F., Gerken, M., Schnitzler, H., Mueller, S.M., and Dietz, K.J. (2018). The chloroplast 2-cysteine peroxiredoxin functions as thioredoxin oxidase in redox regulation of chloroplast metabolism. *eLife* 7: e38194.
- Vieira Dos Santos, C., Laugier, E., Tarrago, L., Massot, V., Issakidis-Bourguet, E., Rouhier, N., and Rey, P. (2007). Specificity of thioredoxins and glutaredoxins as electron donors to two distinct classes of *Arabidopsis* plastidial methionine sulfoxide reductases B. *FEBS Lett.* 581: 4371–4376.
- Wang, P., Liang, F.-C., Wittmann, D., Siegel, A., Shan, S.-O., and Grimm, B. (2018). Chloroplast SRP43 acts as a chaperone for glutamyl-tRNA reductase, the rate-limiting enzyme in tetrapyrrole biosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* 115: E3588–E3596.
- Wang, P., Liu, J., Liu, B., Feng, D., Da, Q., Wang, P., Shu, S., Su, J., Zhang, Y., Wang, J., 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.
- Wimmelbacher, M. and Börnke, F. (2014). Redox activity of thioredoxin z and fructokinase-like protein 1 is dispensable

- for autotrophic growth of *Arabidopsis thaliana*. J. Exp. Bot. 65: 2405–2413.
- Wittmann, D., Kløve, S., Wang, P., and Grimm, B. (2018). Towards initial indications for a thiol-based redox control of *Arabidopsis* 5-aminolevulinic acid dehydratase. Antioxidants 7: 152.
- 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.
- Yoshida, K., Hara, S., and Hisabori, T. (2015). Thioredoxin selectivity for thiol-based redox regulation of target proteins in chloroplasts. J. Biol. Chem. 290: 14278–14288.
- 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.
- Yoshida, K. and Hisabori, T. (2017). Distinct electron transfer from ferredoxin-thioredoxin reductase to multiple thioredoxin isoforms in chloroplasts. Biochem. J. 474: 1347–1360.
- Yoshida, K., Uchikoshi, E., Hara, S., and Hisabori, T. (2019). Thioredoxin-like2/2-Cys peroxiredoxin redox cascade acts as oxidative activator of glucose-6-phosphate dehydrogenase in chloroplasts. Biochem. J. 476: 1781–1790.
- Zaffagnini, M., Bedhomme, M., Groni, H., Marchand, C.H., Puppo, C., Gontero, B., Cassier-Chauvat, C., Decottignies, P., and Lemaire, S.D. (2012). Glutathionylation in the photosynthetic model Organism *Chlamydomonas reinhardtii*: a proteomic Survey. Mol. Cell. Proteomics 11: M111.014142: 014142.
- Zhang, M., Zhang, F., Fang, Y., Chen, X., Chen, Y., Zhang, W., Dai, H.-E., Lin, R., and Liu, L. (2015). The non-canonical tetratricopeptide repeat (TPR) domain of fluorescent (FLU) mediates complex formation with glutamyl-tRNA reductase. J. Biol. Chem. 290: 17559–17565.
- Zhao, A., Fang, Y., Chen, X., Zhao, S., Dong, W., Lin, Y., Gong, W., and Liu, L. (2014). Crystal structure of *Arabidopsis* glutamyl-tRNA reductase in complex with its stimulator protein. Proc. Natl. Acad. Sci. Unit. States Am. 111: 6630–6635.
- Zhao, A. and Han, F. (2018). Crystal structure of *Arabidopsis thaliana* glutamyl-tRNA^{Glu} reductase in complex with NADPH and glutamyl-tRNA^{Glu} reductase binding protein. Photosynth. Res. 137: 443–452.
- Zhu, M., Zhu, N., Song, W.-Y., Harmon, A.C., Assmann, S.M., and Chen, S. (2014). Thiol-based redox proteins in abscisic acid and methyl jasmonate signaling in *Brassica napus* guard cells. Plant J. 78: 491–515.