Thiol-disulfide chemistry refers to the oxidation of thiols (sulfhydryls) into disulfides and also the reversible reaction, i.e., the reduction of the disulfides to free thiols. In vivo, both chemical reactions are catalyzed by dedicated enzymes in cellular compartments where disulfide bonds and reduced thiols in protein targets need to be maintained at all times. Thiol-disulfide chemistry has been best studied in bacteria where it is required for the biogenesis of the periplasmic compartment (Denoncin and Collet, 2013). It has now become apparent that catalyzed thiol-disulfide reactions operate in the mitochondrial intermembrane space (IMS) and the thylakoid lumen, which are topologically equivalent to the bacterial periplasm. In mitochondria, the Mia40/Erv1 proteases were discovered to be key enzymes of a disulfide relay system driving the import of cysteine-rich proteins into the IMS by an oxidative folding mechanism (Herrmann and Riemer, 2012). The thylakoid lumen has long been viewed as a vacant compartment where cysteine-containing proteins are oxidized and some of the redox carriers (e.g., cytochrome c, Rieske, and plastocyanin or its substitute cytochrome c3) involved in electron transfer during the light reactions of photosynthesis. This dogma has now been revised with the realization that numerous molecules reside in the thylakoid lumen in addition to the previously known photosynthetic proteins. Proteomics studies revealed that the lumenal proteome (~80–200 proteins) is far more complicated than initially anticipated and includes proteases, chaperones, isomerases, redox enzymes, and other proteins of unknown activities (Kieselbach and Schröder, 2003). It is possible that the proteins revealed by proteomics regulate photosynthesis or other yet-to-be-discovered processes unrelated to photosynthesis.

A recent study demonstrated that lumenal PPFDs is not involved in photosynthetic electron transfer reactions, but rather controls the synthesis of strigolactone, a plant hormone regulating axillary bud formation (Roose et al., 2011). There is mounting evidence that several lumenal proteins, including components of the photosynthetic chain, contain one or several disulfide bonds (Kieselbach, 2013, Table 1). However, the functional importance of the disulfide(s) has only been examined for a small number of these proteins. The molecular mass of disulfide bonded proteins in the thylakoid lumen range from 10 to 55 kDa and the disulfide bond forming cysteines do not appear to occur in a motif. This is at contrast with most of the disulfide bonded molecules in the mitochondrial IMS which are small proteins (6–18 kDa) containing two disulfide bonds in either a C(X)2C or a C(X)2C motif (Sideris and Tokatlidis, 2010). Interestingly, while disulfide bond reduction in stroma-localized targets serves to activate several enzymes (Meyer et al., 2009), it appears that it is the reverse reaction, i.e., disulfide bond formation, that is required for the activities of several lumen-resident proteins (Buchanan and Luan, 2005).

In bacteria, the requirement for disulfide bond reduction in the periplasmic space was established mainly through studies of cytochrome c maturation. Cytochromes c are metalloproteins with one or several hemes covalently linked to a CXXCH motif on the protein (Thony-Meyer, 1997; Hamel et al., 2009). The need for a disulfide-reducing activity in the context of bacterial cytochrome c assembly seemed obvious because the periplasmic space is also the compartment where cysteine-containing proteins are oxidized by dedicated catalysts (Denoncin and Collet, 2013). The current

**Keywords:** thylakoid lumen, photosynthesis, disulfide, thioredoxin

Thiol oxidation to disulfides and the reverse reaction, i.e., disulfide reduction to free thiols, are under the control of catalysts in vivo. Enzymatically assisted thiol-disulfide chemistry is required for the biogenesis of all energy-transducing membrane systems. However, until recently, this had only been demonstrated for the bacterial plasma membrane. Long considered to be vacant, the thylakoid lumen has now moved to the forefront of photosynthesis research with the realization that its proteome is far more complicated than initially anticipated. Several lumenal proteins are known to be disulfide bonded in Arabidopsis, highlighting the importance of sulfhydryl oxidation in the thylakoid lumen. While disulfide reduction in the plastid stroma is known to activate several enzymatic activities, it appears that it is the reverse reaction, i.e., thiol oxidation that is required for the activity of several lumen-resident proteins. This paradigm for redox regulation in the thylakoid lumen has opened a new frontier for research in the field of photosynthesis. Of particular significance in this context is the discovery of trans-thylakoid redox pathways controlling disulfide bond formation and reduction, which are required for photosynthesis.

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**Table 1**

| Protein | Disulfide Bonds | Function |
|---------|----------------|----------|
| Mia40   | C(X)2C         | Import   |
| Erv1    | C(X)2C         | Import   |
| Thony-Meyer | CXXCH      | Redox    |

**References**

1. Denoncin, J. and Collet, E. (2013). "Thiol Disulfide Chemistry: From Bacteria to Plants."._Front. Plant Sci._ **4**, 476. doi: 10.3389/fpls.2013.00476
Table 1 | Lumenal disulfide bonded proteins in Arabidopsis.

| Protein       | Gene locus      | Classification          | Function                      |
|---------------|-----------------|-------------------------|-------------------------------|
| PsbO1 (2 cys, 1 SS) | AT5G66570     | PSI subunit             | Oxygen evolution              |
| PsbO2 (2 cys, 1 SS) | AT3G50920     | PSI subunit             | Oxygen evolution              |
| PSII N (4 cys, 2 SS) | AT5G64040     | PSI subunit             | Regulation of PSI activity?   |
| Rieske (2 cys, 1 SS) | AT4G03280     | Cytochrome b$_{5'}$ subunit | Electron transfer in b$_{5'}$ complex |
| Cyt-c (2 cys, 1 SS) | AT5G64040     | c-type cytochrome       | Unknown                        |
| STT7/STN7 (2 cys, 1 SS) | AT1G66830     | Kinase                  | State transition              |
| FHBP13 (4 cys, 2 SS) | AT5G65680     | Peptidyl-prolyl cis-trans isomerase | Unknown                       |
| FHBP20 (2 cys, 1 SS) | AT5G45040     | Protease                | Processing of D1 subunit      |
| PrxQ (2 cys, 1 SS) | AT2G6060      | Peroxiredoxin           | Unknown                        |
| VDE (13 cys, 4 SS) | AT1G9855      | Violaxanthin de-epoxidase | Photoprotection               |
| PP06 (2 cys, 1 SS) | AT3G65650     | PsbP domain protein     | Unknown                        |
| Tl15 (2 cys, 1 SS) | AT2G44920     | Pentapeptide repeat      | Unknown                        |
| Tl17 (4 cys, 2 SS) | AT5G63490     | Pentapeptide repeat      | Unknown                        |
| Tl20.3 (4 cys, 1 SS) | AT1G12250     | Pentapeptide repeat      | Unknown                        |
| Tl29.2 (2 cys, 1 SS) | AT4G09510     | Ascorbate peroxidase     | Unknown                        |
| CDD1 (5 cys, 2 SS) | AT4G1790      | Protease                | Processing of D1 subunit      |
| CDD1-like (4 cys, 2 SS) | AT5G46990     | Protease                | Unknown                        |
| Degl (2 cys, 1 SS) | AT4G16970     | Protease                | Degradation of lumen proteins |

The number of lumenal cysteines (cys) that can form an intramolecular disulfide and the number of disulfides (SS) are indicated in parenthesis. The number of disulfide groups experimentally determined. Bolded font indicates that the protein contains at least one disulfide. Experimental evidence for the presence of disulfide bonds comes from a combination of thiol trapping labeling experiments, structure determination of the protein and/or enzymatic activity sensitive to disulfide reducing agents such as DTT or abolished by site-directed mutagenesis of conserved cysteine residues.

Several single cysteine containing lumenal proteins (not indicated here) were recovered in thiol trapping experiments, suggesting that intermolecular disulfide chemistry can be formed. See references (Hall et al., 2010; Hal, 2012; Kanello, 2013).

The view is that the CXXC motif is oxidized after translocation of apocytochromes c to the periplasmic space and then reduced by a reducing pathway to provide free sulfhydryls for ligation with heme (Bonnard et al., 2010; Sanders et al., 2010). This question had received little attention in the context of plastid cytochromes c because thiol-disulfide chemistry as a catalyzed process was not believed to take place in the thylakoid lumen, the compartment where heme attachment to apocytochromes c occurs. The discovery of trans-thylakoid redox pathways controlling disulfide bond formation and reduction in Chloramydomonas and Arabidopsis has now changed this perception.

**TWO DISULFIDE-REDUCING PATHWAYS OPERATE IN THE LUMEN**

A central component of the bacterial disulfide-reducing pathways is the thiol-disulfide reductase of the DsbD family (Cho and Collet, 2013). In bacteria, members of this family (CddA, DsbD, ScsB) are cytoplasmic membrane proteins conveying reducing power from the cytosol to the active sites of several target molecules in the periplasm. Reducing power is transferred across the cytoplasmic membrane through sequential thiol-disulfide exchanges (Cho and Collet, 2013). One target required for cytochrome c assembly is the membrane-bound, periplasm-facing, thioredoxin-like protein (CcmG/ResA/CcsX). CcmG/ResA/CcsX is postulated to reduce a disulfide in the CXXC heme-binding site of apocytochrome c prior to heme ligation to the cytochromes (Bonnard et al., 2010; Sanders et al., 2010). In the plastid lumen, the involvement of a disulfide-reducing pathway was first suspected based on the presence of CcdA, an ortholog of bacterial CcdA from the DsbD family, at the thylakoid membrane (Page et al., 2004; Motohashi and Hisabori, 2010; Figure 1). Another component in the plastid is HCF164 (High Chlorophyll Fluorescence), a membrane-anchored, lumina-facing, thioredoxin-like protein with similarity to bacterial CcmG/ResA/CcsX (Lennartz et al., 2001; Figure 1). The disulfide-reducing activity of HCF164 was inferred from the fact that a recombinant form of its luminal domain displays disulfide reductase activity in vitro (Lennartz et al., 2001; Motohashi and Hisabori, 2006). Loss of function of CcdA or HCF164 in Arabidopsis produces a photosynthetically-deficient phenotype due to a defect in cytochrome b$_{5'}$ complex assembly (Lennartz et al., 2001; Page et al., 2004). However, the biochemical activity and site of action of CcdA and HCF164 in the assembly process remained unknown until the characterization of the Chloramydomonas ccs4 and ccs5 mutants (Xie et al., 1998). The ccs4 and ccs5 mutants (ccs4 for cytochrome c synthesis), which are partially photosynthetically deficient, display a block in the conversion of apo to holoform of lumen resident cytochromes c, namely membrane-bound cytochrome b$_{5'}$.
f and soluble cytochrome c₆. The ccs mutants are deficient for cytochrome b₆f assembly, which is dependent on cytochrome f, the thylakoid membrane-bound cytochrome c. This assembly defect is at the step of heme attachment to apocytochrome c, a chemical reaction taking place in the thylakoid lumen (Howe and Merchant, 1992, 1993, 1994; Xie et al., 1998). The Chlamydomonas CCS gene was cloned and shown to encode a thioredoxin-like protein with similarity to CCS5 gene was cloned and shown to encode a thioredoxin-like protein (Motohashi and Hisabori, 2006; Gabilly et al., 2011; Figure 1). The CCS4 protein contains an N-terminal hydrophobic stretch that could serve as a membrane anchor and a C-terminal domain rich in charged residues. On the basis of the positive-inside rule that governs the topology of bacterial and thylakoid membrane proteins (von Heijne, 1989; Gavril et al., 1993), the C-terminal domain of CCS4 is predicted to be exposed to the stromal side of the thylakoid membrane. Surprisingly, CCS4 does not display any motif or residue (such as cysteines) suggesting a role in thyl-based redox chemistry. Yet, the thyl-dependent photo-synthetic rescue of the cc6 mutant and the suppression of the cc6 phenotype by ectopic expression of CCDA, the thyl disulfide oxido-reductase of the DsbD family, at the thylakoid membrane, confirms the activity of CCS4 in a disulfide-reducing pathway for cytochrome c assembly. Moreover, the CCS4-dependent suppression of the cc6 mutant confirms the placement role of CCDA in plastid cytochrome c maturation. Indeed, earlier studies in Arabidopsis supported, but did not establish, the requirement of plastid CCDA in the conversion of apo- to holocytochromes c (Page et al., 1997; Motohashi and Hisabori, 2010). Interestingly, the cc6-null cc5-null double mutant displays a synthetic phenotype characterized by a complete loss of b₆f assembly; an indication that CCS4 and CCS5 are redundant. This functional redundancy suggests that CCS4 might control a different disulfide-reducing pathway than the CCS5/HCF164 dependent one (Figure 1). The CCS4-dependent suppression of the cc6 mutant can be explained by a compensatory effect due to enhanced expression of the third disulfide oxido-reductase CCDA. At the present time, the activity of CCS4 in such a pathway remains elusive. While the CCDA-dependent pathway relies on the transfer of reducing agents through sequential thyl-disulfide exchanges, it is conceivable that CCS4 controls the transport across the thylakoid membrane of a molecule acting as a reducing agent (Figure 1). In bacteria, operation of such redundant routes for export of reductant to the periplasm have been postulated (Pittman et al., 2005). The nature of the exported reductant can only be speculated upon but glutathione or ascorbate is an obvious candidate. While the presence of glutathione in the thylakoid lumen remains to be established, ascorbate is known to function in this compartment as an alternative electron donor to PSII and a co-factor for violaxanthin de-epoxidase (VDE) dependent photoprotection (Yoth et al., 2013).

**FIGURE 1** | Thylakoid thiol metabolizing pathways. The disulfide-reducing (CCDA, CCS4/HCF153 and thiol-oxidizing pathways) STO1 are represented in dark and light grey, respectively. Cysteines are represented as blue balls. Electron (e⁻) routes are indicated by arrows. The topology of CCDA/HCF164 is hypothetical but the positive-inside rule predicts a stromal localization for the C-terminal domain of the protein. CCS4/HCF164 is a membrane anchored lumen-facing thioredoxin-like protein. LTO1 contains a VKOR-like membrane domain and a lumen-facing thiol-disulphide domain. Stromal reductants for the disulfide reducing pathways are indicated in red. The electron acceptor for the thiol-oxidizing pathway is indicated in orange. Thiol-disulphide (Trx-m) is the possible reductant for the CCDA:CCSA/HCF164 pathway. The CCS4/HCF164 pathway might be involved in the transport of a reductant such as glutathione or ascorbate (X?). The final electron acceptor of the LTO1-dependent pathway is not known and is postulated to be a plastid protein (Gabilly et al., 2011). LTO1 topology was deduced using PhoA/LacZ topological reporters (Page et al., 2004; Feng et al., 2011; Karamoko et al., 2011). The thylakoid membrane is shown in light grey.
DIscOVery of a diSuFlide-foRmInG eNzyme in the tHyllaKoid lumEn

In the periplasmic space of most protocell eukaryotes, the thiol-oxidizing pathway consists of a di sulfide bond catalytic system defined by soluble DsbA and membrane-bound DsbB (Denixon and Collet, 2013). DsbA catalyzes di sulfide bridge formation in cysteine-containing subunits that are translocated across the membrane into the periplasmic space. DsbB operates by recycling reduced DsbA to its oxidized form with transfer of electrons to quinones, which are membrane-soluble redox carriers in the respiratory chain. The fact that no DsbA or Dsb-like enzymes can be detected in the genomes of photosynthetic eukaryotes or cyanobacteria, the presumed ancestors of the chloroplast, reinforces the view that di sulfide bond formation did not take place in the thylakoid lumen. However, the operation of catalyzed di sulfide bond formation in the lumen is supported by the finding that bacterial alkaline phosphatase (PhoA), an enzyme requiring two di sulfide bonds for activity, and basic pancreatic trypsin inhibitor BPTI (aprotinin), a molecule containing three di sulfide bonds, are active when targeted to this compartment in tobacco (Stone et al., 1997; Bally et al., 2008; Tissot et al., 2008). A novel class of di sulfide-forming enzymes with similarity to VKOR (vitamin K epoxide Oxidoreductase) was recently recognized in some bacterial phyla lacking the typical DsbAB components (including cyanobacteria) and in all photosynthetic eukaryotes (Dutton et al., 2008; Singh et al., 2008; Grossman et al., 2010). VKOR is well studied for its involvement in the reduction of vitamin K, a phylloquinone required as a co-factor for the γ-carboxylation of clotting factors in blood (Tie and Stafford, 2008). Recent work shows that the enzymatic activity of VKOR is also linked to oxidative folding of proteins in the ER lumen (Rishavy et al., 2011; Schulman et al., 2011). A first indication that the thylakoid lumen houses a thiol-oxidizing pathway came from the identification of a VKOR-like protein in cyanobacteria (Singh et al., 2008). In vitro reconstitution of di sulfide bond formation with the purified cyanobacterial enzyme demonstrated the sulfhydryl oxidase activity of the protein (Li et al., 2010). However, the localization of the protein at the thylakoid membrane and its relevant targets of action in the lumen were not documented (Singh et al., 2008). The identity of the thiol-oxidizing catalyst in the thylakoid lumen of plastids has now emerged through the discovery of LTO1 (Lumen Thiol Oxidoreductase 1), a thylakoid membrane protein containing a VKOR domain fused to a thioredoxin-like moiety (Figure 1; Furt et al., 2010; Feng et al., 2011; Karamoko et al., 2011). Topological studies using bacterial topological reporters established a luminal location for the LTO1 domains carrying the redox motifs and conserved cysteines (Figure 1). Previous studies with bacterial VKOR-like proteins have demonstrated that the thiodioxidine-like domain carries a DsbA-like activity while the VKOR-like central domain is functionally equivalent to DsbB (Singh et al., 2008; Dutton et al., 2010; Wang et al., 2011). In Arabidopsis, loss of LTO1 function is associated with a severe photo- morphic growth defect (Karamoko et al., 2011; Lu et al., 2013). Measurements of the photosynthetic activity indicate that lto1 mutants display a limitation in the electron flow from Photosystem II (PSII). In accord with these measurements, lto1 mutants show a severe depletion of several of the structural subunits of PSII (including subunits of the OEC) but no change in the accumulation of the cytochrome b6/f complex or PSI and no defect in the activity of ATP synthase. In a yeast two-hybrid assay, the lumens-facing thiodioxidine-like domain of LTO1 was shown to interact with PsbO, a luminal PSI subunit in the OEC known to be di sulfide bonded (Table 1). In vitro, the thiodioxidine-like domain of LTO1 is able to introduce a di sulfide bond in the PsbO target when recombinant forms of the molecules are used. Because the redox state of the sulfhydryls in PsbO was shown to be a determinant for the stability of this subunit and also for PSI assembly defect. It is not known if the ability of LTO1 to form a di sulfide bond in PsbO is linked to the import of this protein into the lumen, similarly to the Mia40-dependent pathway in mitochondria (Herrmann and Riemer, 2012). In organello import experiments showed that PsbO translocates at a different site than PsbP, another subunit of the PSI OEC (Hashimoto et al., 1997). It is plausible that this translocation step is assisted by LTO1 but this awaits experimental testing.

The final electron acceptor of the LTO1-dependent di sulfide bond forming pathway is currently unknown (Figure 1). It is likely to be a phylloquinone based on the fact that the Arabidopsis protein reduces phylloquinone in an in vitro enzymatic assay (Furt et al., 2010). The role of phylloquinone as a structural cofactor tightly bound to PSI is well documented (Brepet, 1997). However, the occurrence of a pool of phylloquinone that is not bound to PSI suggests phylloquinone might participate in redox processes in addition to the known electron transfer reactions through PSI (Gross et al., 2006; Lehmann et al., 2006). This pool may act as an electron acceptor for the LTO1-dependent di sulfide bond forming pathway in vivo.

OUTLOOK

It is conceivable that catalyzed thiol-oxidation in the lumen extends to other di sulfide-bond containing targets in addition to PsbO (Table 1). In vitro experiments suggest that the thiodioxidine-like domain of LTO1 is also able to catalyze the formation of the two di sulfide bonds in FKBP13 (Lu et al., 2013), a peptidyl-prolyl cis-trans isomerase whose activity is dependent upon sulfhydryl oxidation (Gopalan et al., 2004; Table 1). It is not known if additional enzymes with sulfhydryl oxidase activity besides LTO1 also operate in the thylakoid lumen. In bacteria, the di sulfide-reducing pathway is also required to maintain the reduction state of periplasmic oxido-reductases that shuffle di sulfide bonds that are incorrectly formed (Kadokura and Beckwith, 2010; Depuydt et al., 2011). The need for di sulfide bond isomerization is critical for proteins containing more than two cysteines such as VDE, an enzyme involved in photoprotection whose activity depends on sulfhydryl oxidation (Kanervo et al., 2005; Table 1). Interestingly, the activity of recombinant VDE was initially reported to be low, presumably because of improper protein folding due to incorrect di sulfide linkages (Bogus and Yamamoto, 1996; Heiber et al., 2002). Expression in the cytosol of a bacterial strain engineered for di sulfide bond formation and isomerization resulted in a high level of VDE activity for the purified enzyme, an indication that VDE can fold properly in the cytosol.
that isomerization of disulfide bond in the lumen is also likely to be required for yielding an active enzyme (Saga et al., 2010). It is possible that LTO1 exhibits this activity based on the finding that its thioredoxin-like domain is active as a disulfide-bond isomerase. Another thylakoid membrane protein displaying disulfide-bond isomerase activity in vitro is LQY1 (Bally et al., 2008). Both LQY1 and LTO1 are required for critical reading of the manuscript.

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Karamoko et al. Thiol based chemistry in the thylakoid lumen

In this study, the role of thiol-based chemistry in the thylakoid lumen of land plants is explored. The authors discuss the importance of thiol-based reactions in the context of thylakoid thiol-metabolizing pathways in photosynthesis. Front. Plant Sci. 4:476. doi: 10.3389/fpls.2013.00476

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