Redox regulation in chloroplast thylakoid lumen: The pmf changes everything, again.

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Abstract

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Abstract

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Keywords: Photosynthesis, redox regulation, thiol-disulfide redox regulation, lumen, photoprotection, non-photochemical quenching (NPQ)

Introduction

Photosynthesis is a biological process that converts light energy into chemical energy, which is then used to fix CO_2 into organic compounds. This process is essential for life on Earth, as it provides the food and energy that all living things need. However, at the same time, photosynthesis can be a dangerous process if it is not finely tuned and regulated (Kanazawa et al. 2020; Kramer et al. 2004; Davis et al. 2017; Foyer 2018; Gururani et al. 2015; Murata et al. 2007; Aro et al. 1993; Raven 2011). For instance, the under stressful conditions, photosynthetic process can generate reactive oxygen species (ROS) which can ultimately damage numerous cellular components (Asada 1996; Nishiyama, Allakhverdiev & Murata 2006; Nawrocki *et al.*2021b).

To avoid photodamage, photosynthetic organisms have evolved a photoprotective mechanism, called non-photochemical quenching (NPQ) (Demmig-Adams & Adams 1996; Müller, Li & Niyogi 2001; Li *et al.* 2004). NPQ itself is composed of several distinctive components. Each component is defined by an underlying mechanism and rate of formation and relaxation (Bellafiore, Barneche, Peltier & Rochaix 2005). The most rapid responding form of NPQ, is qE (energy-dependent of quenching) which is activated by acidification of lumen (the ΔpH component of proton motive force (*pmf*)) via protonation of photosystem II subunit S (PsbS) (Li*et al.* 2004; Niyogi, Li, Rosenberg & Jung 2005) as well as activation of violaxanthin de-epoxidase (VDE), which catalyzes the conversion of violaxanthin (Vx) to antheraxanthin and then zeaxanthin (Zx) (Niyogi, Grossman & Björkman 1998). There are additionally, more slowly responding forms of NPQ, including sustained quenching (qH) (Malnoë*et al.* 2018), photoinhibition (qI) which is related to the photodamage of PSII centers and subsequent repair (Andersson & Aro 2004; Murata, Takahashi, Nishiyama & Allakhverdiev 2007; Nawrocki, Liu, Raber, Hu & De Vitry 2021a), zeaxanthin-dependent quenching (qZ) which involves the accumulation of Zx but not the PsbS (Demmig-Adams & Adams 1996; Müller *et al.* 2001; Li *et al.* 2004; Nilkens*et al.* 2010), and finally, qT, which involves antenna state transitions (where LHCII migrates to PSI) (Quick & Stitt 1989). qZ, qH, qI and qT are likely to be too slow to respond to rapid fluctuations in light but likely act as backup processes when qE fails.

It is important to note that the processes listed above are involved in balancing the tradeoffs between photoprotection and photosynthetic efficiency (Kramer*et al.* 2004; Zhu, Long & Ort 2010; Kromdijk *et al.* 2016; Davis *et al.* 2017; Kanazawa *et al.* 2020). For instance, slow onset of photoprotection causes photodamage when light intensity rapidly increases (Kramer*et al.* 2004; Davis *et al.* 2017; Kanazawa *et al.*2020), whereas slow recovery leads to losses of photosynthetic efficiency when light intensity suddenly decreases (Kramer*et al.* 2004; Zhu *et al.* 2010; Kromdijk *et al.* 2016; Davis *et al.* 2017; Kanazawa *et al.* 2020). Understanding such "fine-tuning" mechanisms that are involved in maintaining energy balance when plants are subjected to constantly changing environmental conditions will be required if one wants to achieve more robust and resilient photosynthesis, and thus, improve overall crop productivity.

So how do plants adjust to their constantly changing environmental conditions such as light availability and quality? One approach involves the regulation and/or fine-tuning of protein function using a thiol-disulfide redox mechanism (Buchanan & Balmer 2005; Waszczak *et al.* 2015). In this approach, we can exploit the fact that, under physiological conditions, pairs of the amino acid cysteine (Cys), exist in two forms: when reduced, the Cys residues will be in their (thiol, SH) forms, whereas when oxidized, may form a disulfide (S-S) form, linked by a covalent bond (Cremers & Jakob 2013). In the chloroplast, thiol-disulfide reactions are involved in numerous functions such as, in protein folding, in regulating the activity of countless enzymes, and in ROS detoxification (Buchanan and Balmer 2005; Kieselbach 2013; Balsera and Buchanan 2019; Yoshida and Hisabori 2016; Montrichard et al. 2009). These processes are mediated by a complex network of redox-sensing and redox-regulated enzymes. The reductive and oxidative activities of this intricate system are essential for achieving regulatory redox balance.

While many questions still remain, this general scheme for stromal redox control appears to explain much

of the known data on regulation of stromal enzymes as illustrated in Figure 1. In the light, electron flow from photochemistry reduces regulatory thiols through the ferredoxin/thioredoxin and NADPH-dependent thioredoxin reductase C (NTRC) systems in the stroma. This activation or deactivation of key enzymes is mediated by the redox state of the thiol groups. Meanwhile, H_2O_2 reoxidizes the regulatory thiol pools both in the light and dark (Cejudo, Ojeda, Delgado-Requerey, González & Pérez-Ruiz 2019).]

Recently, it has been recognized that reactions at the thylakoid membrane and within the lumen are also redox-regulated. However, the roles and mechanisms of these reactions are not well understood.

Several key proteins involved in photoprotection (e.g., violaxanthin de-epoxidase (VDE)), partitioning of *pmf* into $\Delta\psi$ and ΔpH (the H^+/K^+ antiporter KEA3), PSII stability and repair (Deg1 and PsbO), state transition (STN7) have redox-active thiol groups that are exposed to the thylakoid lumen (Ströher & Dietz 2008; Hall *et al.* 2010; Kieselbach 2013; Yu, Lu, Du, Peng & Wang 2014; Simionato *et al.* 2015; Hallin, Guo & Åkerlund 2015; Wang *et al.* 2017a; Wu *et al.* 2021). These thiol groups can undergo redox transitions, meaning that they can be oxidized or reduced. Interestingly, while the reduction of regulatory thiols in the stroma tends to activate enzymes involved in photosynthesis, the opposite seems to be true for lumenal proteins (e.g., VDE) (Yu*et al.* 2014; Simionato *et al.* 2015; Hallin *et al.*2015). This suggests that oxidative reactions may be critical for adjusting the activities of lumenal proteins and preventing the buildup of damaged PSII centers.

This review will discuss the possible important role(s) of redox regulation in photoprotection in preventing plants from photodamage, focusing on redox-regulated proteins in the thylakoid lumen, which is a unique environment with different properties than the stroma. Also, we will discuss the challenges of achieving redox regulation in the lumen and point out that it involves a distinct mode of regulation that links redox changes, reactive oxygen species generation, and stress responses.

2. Redox regulation in the stroma

In chloroplasts, thiol-disulfide redox regulation is essential for both the stroma and lumen, but redox regulation in the stroma has been more extensively investigated than in the lumen (Buchanan, Kalberer & Arnon 1967; Wolosiuk & Buchanan 1977; Meyer, Belin, Delorme-Hinoux, Reichheld & Riondet 2012; Buchanan, Holmgren, Jacquot & Scheibe 2012; Balsera, Uberegui, Schürmann & Buchanan 2014; Buchanan 2016a). However, in order to fully understand lumenal redox regulation, we need to have a good understanding of the stroma side as well. This is because the stroma and lumen are interconnected, and the redox status of the stroma can affect the redox status of the lumen.

During oxygenic photosynthesis, electrons are transported from photosystem I (PSI) to ferredoxin (Fd) in the stroma. Fd carries electrons between donor and acceptor pairs. In the stroma, the electrons from Fd along with ATP and NADPH derived from electron and proton transport reactions respectively are utilized to fix carbon dioxide through a series of enzymes that constitute the Calvin-Benson-Bassham (CBB) cycle. Redox regulation in the stroma is also essential for modulating the activity of various enzymes of the CBB as well as controlling numerous other metabolic pathways. Hence, the redox regulation system has been long considered an important means to synchronize photosynthetic reactions to respond to both light availability and quality (Pearcy, Krall & Sassenrath-Cole 2004; Kaiser *et al.* 2015). Indeed, several enzymes are known to be regulated by light-dependent reductive activation, for example, the gamma subunit of chloroplast ATP synthase (CF1-γ) and several key enzymes in the CBB cycle such as fructose-1,6 bisphosphatase (FBPase), sedoheptulose -1,7 bisphosphatase (SBPase), Phosphoribulokinase (PRK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and rubisco activase (Rca) (Michelet*et al.* 2013).

The following subsections will briefly discuss two current stroma reduction systems as well as a recently discovered new oxidation system. For more detailed information on these systems, the reader is encouraged to the following relevant papers (Nikkanen & Rintamäki 2014; Yoshida, Hara & Hisabori 2015; Cejudo *et al.*2019; Cejudo, González & Pérez-Ruiz 2021; Gurrieri, Fermani, Zaffagnini, Sparla & Trost 2021)

2.1 The stroma has two distinct thiol reduction systems (FTR-Trx and FNR-NTRC)

The ferredoxin-thioredoxin reductase (FTR)-thioredoxin (Trx) system is only active in light, as it requires

electrons from photosynthesis. The system works by transferring electrons from photosystem I (PSI) to ferredoxin (Fd), which then transfers these electrons to ferredoxin-thioredoxin reductase (FTR), which then transfers the electrons to thioredoxin (Trx), and then finally transfers these electrons to various target enzymes, such as FBPase and SBPase (Figure 1A) (Schürmann and Buchanan 2008; Michelet et al. 2013).

Trx contains cysteine residues that are redox-active and can reversibly transfer the reducing potentials from light reactions to thiol-regulated enzymes. Initially, two forms of Trx, -f and -m, were proposed to be involved in this redox process (Buchanan 1980) but later, more than 20 additional isoforms of Trx were found by sequencing of the *Arabidopsis thaliana* genome. The newly identified chloroplast Trxs were categorized into five classes, Trx-f, m, x, y and z (Collin*et al.* 2003; Yoshida, Matsuoka, Hara, Konno & Hisabori 2014; Geigenberger, Thormählen, Daloso & Fernie 2017). Essentially, all Trxs have the conserved sequence motif (WCGPC) which allows them to interact more specifically with subsets of target enzymes (Schürmann & Jacquot 2000; Collin *et al.* 2003; Yoshida *et al.* 2014; Geigenberger *et al.* 2017).

A second reduction system, NADPH-dependent thioredoxin reductase C (NTRC), was discovered relatively recently as a chloroplast thiol-regulatory system specifically in oxygenic organisms (Serrato, Pérez-Ruiz, Spínola & Cejudo 2004; Pulido *et al.* 2010; Carrillo, Froehlich, Cruz, Savage & Kramer 2016) (Fig.1B). In contrast to the FTR system, NTRC uses NADPH as a reducing power to deliver electrons to target enzymes. NTRC appears to be critical under lower light conditions, operating with even low LEF, or in the dark using NADPH generated from the oxidative pentose phosphate pathway (OPPP) (Neuhaus & Emes 2000).

2.2 The stroma has multiple sulfhydryl oxidation systems

The majority of research on redox regulation in chloroplasts has focused on light-dependent reduction of regulatory thiols in the light. More recently, attention has shifted to the mechanism of "thiol oxidation", the "reversal" of light-induced reduction important for determining the steady-state redox poise of regulatory thiols.

The thiol-modulated enzymes in the stroma have redox potentials relatively close to those of the major redox carrier, NADPH (see also below), so that the reaction should be thermodynamically reversible (Kramer et al. 1990). This means that a net reduction or oxidation of NADPH, should result in concomitant changes in the thiol regulatory status. For instance, relative changes in the rates of the light and assimilatory reactions should alter redox balance, in turn affecting the activation of a range of thiol-modulated enzymes (Kramer et al. 1990; Ort et al. 1990; Stitt 2004). The extent to which such modulation occurs, however, will depend on the relative redox potentials of the target enzymes. Some of these (e.g. FBPase) have relatively negative redox potentials and should be more rapidly reversed, whereas others, notably the chloroplast ATP synthase, have less negative potentials, and will only be reoxidized after extensive oxidation of NADPH.

This simple, redox quasi-equilibrium model has, in recent years, been challenged by the identification of redox proteins that specifically oxidize specific sets of regulatory thiols. For example, the newly discovered 2-Cys peroxiredoxin (2CP) is involved in oxidizing reductively activated proteins in the dark (Fig. 1B) (Yoshida, Hara, Sugiura, Fukaya & Hisabori 2018; Yoshida, Yokochi & Hisabori 2019; Vaseghi *et al.* 2018; Ojeda, Pérez-Ruiz & Cejudo 2018; Yokochi, Fukushi, Wakabayashi, Yoshida & Hisabori 2021). The proposed mediators for thiol oxidation of target proteins are Trx-f, Trx-like2 (TrxL2), atypical Cys His-rich Trx (ACHT), which have a less negative redox potential (more oxidizing) than the thiols on typical regulatory proteins (Eliyahu, Rog, Inbal & Danon 2015; Yoshida *et al.* 2018, 2019; Vaseghi*et al.* 2018; Ojeda *et al.* 2018; Yokochi *et al.*2019, 2021). Those mediators transfer reducing power from redox-regulated proteins to 2CP. Reduced 2CP then reduces H_2O_2 to H_2O. The protein-oxidizing activity of mediators such as TrxL2 is strongly dependent on 2CP and H_2O_2(Yoshida*et al.* 2018, 2019; Vaseghi *et al.* 2018; Ojeda *et al.* 2018; Yokochi *et al.* 2021).

Finally, other forms of peroxiredoxins, such as Prx IIE and Prx Q, did not show a similar role to 2CP in the dark transition. However, there are contradictory results for PrxQ (Yoshida*et al.* 2018, 2019; Vaseghi *et al.* 2018; Ojeda *et al.* 2018; Telman, Liebthal & Dietz 2020; Yokochi *et al.* 2021). It was also found that NTRC-modulated 2CP contributes to the control of chloroplast redox homeostasis (Pérez-Ruiz, Naranjo, Ojeda, Guinea & Cejudo 2017). Several thiol-regulated enzymes were revealed from these studies to be oxidized

by 2CP, including CF1-γ, FBPase, SBPase, Rca, and NADPH-malate dehydrogenase (MDH) (Yoshida*et al.* 2018, 2019; Vaseghi *et al.* 2018; Ojeda *et al.* 2018; Yokochi *et al.* 2021).

3. Connecting thiol regulation across the thylakoid membrane

Recent work has shown that the stromal redox pool communicates with redox-regulated proteins in the lumen, and there is strong evidence that several lumenal enzymes or proteins are modulated by thiol redox state (Ströher & Dietz 2008; Hall *et al.* 2010; Kieselbach 2013; Yu, Lu, Du, Peng & Wang 2014; Simionato *et al.* 2015; Hallin, Guo & Åkerlund 2015; Wang *et al.* 2017a; Wu *et al.* 2021). The special properties of the chloroplast compartments impose distinct requirements for the stromal and lumenal compartments. As with the stromal system, three functionalities are required to operate an effective redox regulatory network: 1) controlled injection of reducing equivalents (electrons, hydrogen); 2) balanced removal of electrons; and 3) adjustment of redox potentials and kinetics to match the specific regulatory requirements.

Upon illumination, the electron and proton transport reaction deposits protons into the lumen, generating a proton motive force (*pmf*), which is composed of two components, the electric field ($\Delta\psi$) and the proton gradient ([?]pH). Both *pmf* components drive the synthesis of ATP at the chloroplast ATP synthase (Kramer et al. 2003). The initial form of *pmf* is exclusively stored as the electric field component due to the lower capacity of electric capacitance and higher buffering capacity of [?]pH in the lumen (Kanazawa & Kramer 2002; Cruz *et al.* 2005; Takizawa, Kanazawa & Kramer 2008). As counterions move into the lumen, the proton gradient builds up, resulting in acidification of the lumen to values as low as about 5.5 (Kramer et al. 1999; Takizawa et al. 2007). By contrast, stromal pH tends to increase in the light to about 7.5-8.0 (Werdan and Heldt 1972; Heldt et al. 1973; Werdan et al. 1975; Reardon-Robinson 1985; Wu and Berkowitz 1992). In addition, lumen acidification is known to have crucial photoprotective regulatory roles, such as inducing PSII photoprotection mechanisms (section 4) and "photosynthetic control" of electron flow by slowing down the electron transfer rate at the Cytb_6f complex, thus preventing PSI photodamage.

However, each of these functions is strongly impacted by the specific properties of the lumen and its role in photosynthetic energy transduction, requiring the lumenal system to be distinct in several ways, as described in the following.

The need for transthylakoid redox exchange . To fulfill its role in chemiosmotic energy transduction, the thylakoid lumen must be an electrochemically sealed compartment, to prevent the leakage of energy stored in the *pmf* . There must then be machinery to allow for transmembrane thiol-disulfide exchange. While such systems have been extensively studied in bacteria, how this occurs in chloroplasts is only beginning to emerge. For example, in the well-characterized oxidative protein folding disulfide bond isomerization pathway in Gram-negative bacteria, the Dsb family (Ito & Inaba 2008; Reardon-Robinson & Ton-That 2015) protein has been imported into the periplasm by the Sec transport apparatus, is facilitated by protein oxidation by DsbA (Grauschopf*et al.* 1995). Subsequently, the reduced DsbA is oxidized by DsbB in the inner membrane (IM), which transfers reducing equivalents to quinones (Bader, Muse, Ballou, Gassner & Bardwell 1999). However, when a newly imported protein is subjected to oxidation, this can lead to misfolding and inactivation. To prevent or repair these situations, Trx in the cytoplasm transfers reducing power to DsbD in the inner membrane which then transfers this reducing power to DsbC, which maintains proteins in reduced (active or foldable) states (Krupp, Chan & Missiakas 2001; Herrmann, Kauff & Neuhaus 2009) (Fig 2A).

An analogous system likely operates in thylakoid membranes, but may also function in reversible thiol regulation of enzymes. The protein components needed to establish a functional thiol-disulfide exchange system across the thylakoid membrane, while not complete, are slowly being identified, as discussed in the following sections. Unlike the thiol-disulfide redox regulation in the stroma, stromal soluble electron carriers, such as Trx or Trx-like, proteins have not yet been identified in the lumen (Buchanan 2016b). However, several thiol-modulated enzymes or proteins have been identified (Ströher & Dietz 2008; Hall *et al.* 2010; Kieselbach 2013; Yu, Lu, Du, Peng & Wang 2014; Simionato *et al.* 2015; Hallin, Guo & Åkerlund 2015; Wang *et al.* 2017a; Wu *et al.* 2021) and multiple H-carriers (redox transporters) have been reported to transfer reducing equivalents from the stroma across the thylakoid membrane to the thylakoid lumen (Motohashi &

Hisabori 2006, 2010; Brooks, Jansson & Niyogi 2014; Motohashi & Hisabori 2006, 2010; Karamoko, Cline, Redding, Ruiz & Hamel 2011; Brooks *et al.* 2014).

3.1 HCF164, CcdA and SOQ1 transfer reducing equivalent from stroma to lumen

HCF164 was first identified by Meurer, Meierhoff & Westhoff as a gene that when mutated in plants resulted in high chlorophyll fluorescence phenotype (Meurer*et al.* 1996; Meurer, Plücken, Kowallik & Westhoff 1998). This phenotype was later found to be caused by a defect in the assembly of cytochrome b_6f (Lennartz*et al.* 2001). HCF164 is anchored in the thylakoid membrane via a single transmembrane domain (TMD) with the bulk of HCF164 orientated towards the thylakoid lumen (See Fig. 2B). HCF164 possesses a Trx-like domain, localized to the thylakoid lumen with disulfide reductase activity (Motohashi & Hisabori 2006, 2010). Further, HCF164 can interact with potential target proteins such as PSI-N, Cytf, and Rieske FeS (a subunit of the Cyt b_6f) through Trx affinity chromatography experiments (Motohashi & Hisabori 2006, 2010; Brooks *et al.* 2014). Intriguingly, it has also shown that Trx-m type is an electron donor for HCF164 (Motohashi & Hisabori 2006, 2010; Brooks *et al.* 2014). However, because HCF164 has its Cys residues on the lumenal side, and not on the transmembrane side, it was proposed that chloroplasts must possess a system for transferring redox equivalents across the thylakoid membrane from the stroma to the lumen. Motohashi & Hisabori found evidence that CcdA serves precisely this function: a thylakoid membrane protein that acts to transfer reducing equivalents from the stroma to the thylakoid lumen. CcdA is a homolog of the prokaryotic thiol-disulfide transporter, and it was previously reported to be required for the assembly of the Cyt$b_6f$complex (Page*et al.* 2004). Motohashi and Hisabori (2010) further showed that CcdA's redox state is modulated in the thylakoids by stromal m-type thioredoxins.

The suppressor of quenching (SOQ1) was identified during the process of a suppressor screening of non-photochemical quenching (NPQ) (Brooks, Sylak-Glassman, Fleming & Niyogi 2013). SOQ1 is anchored in the thylakoid membrane via a single transmembrane domain (TMD) with the bulk of SOQ1 localized within the thylakoid lumen (Fig. 2B). SOQ1 appears to transfer reducing equivalents from the stroma to the plastid lipocalin (LCNP), thereby suppressing the formation of a sustainable form of NPQ called qH (Malnoë*et al.* 2018) (see Section 4.3 for more details). The lumenal thioredoxin-like domain and a β-propeller NCL-1, HT2A, and LIN-41 (NHL) domain of SOQ1 have been shown to be required for the transfer of reducing equivalents from the stroma to the lumen (Slack & Ruvkun 1998; Brooks *et al.* 2013). Finally, another domain of SOQ1, the stromal-located halo-acid dehalogenase-like hydrolase (HAD) domain with a transmembrane helix, has been shown to be not involved in the suppression of qH (Brooks*et al.* 2013). Interestingly, recent work from Yu et al (Yu*et al.* 2022) showed that the independent β-stranded C-terminal domain (CTD), which has structural homology to the N-terminal domain of DsbD, is essential for the regulation of qH, suggesting that it is involved in transferring redox equivalents from the stroma side to the lumen (Yu*et al.* 2022). The electron donor to SOQ1 has not been specified yet, but it is possible that it is either CcdA or another unknown mediator.

Taken together, these examples suggest a mechanism for redox regulation of lumen proteins as illustrated in Fig. 2B in which disulfide-thiol redox control across the thylakoid membrane, in which reducing equivalents are transferred from the stroma (e.g., from Trx-m) to CcdA (or other, yet to be identified) carriers, and then to HCF164, which then delivers them to target proteins.

3.2 The acidification of the lumen by *pmf* alters the redox potentials of regulatory thiols, altering the mechanism of redox balancing

At physiological pH (less than about 8.0), the two-electron reduction of protein disulfide occurs with the uptake of 2 H^+, as in

$$\text{-S-S-} + 2e^- + 2H^+ = (\text{-SH})_2$$

The involvement of one proton per electron in the redox reaction implies that the redox midpoint potentials for the regulatory thiols will shift by -0.06 eV / pH unit, i.e., the lower the pH, the less reducing (more

oxidizing) the regulatory thiols will become. The redox potentials of the stromal and lumenal thiol couples will change in opposite directions. Interestingly, reducing the stromal NADP+/NADPH couple, involves only one proton per two electrons, so its redox potential will only shift by about -30 mV/pH unit.

Figure 4 illustrates what would happen if the lumen and stromal pH values started at about 7.0 in the dark and changed to 6.0 and 8.0 in the light. The midpoint potential of thiol couples in the stroma should become more negative relative to dark (more difficult to reduce) and in some cases (e.g., NADPH-MDH) will become more negative than the NADP+/NADPH couple. In this case, reversal of the electron transfer (oxidizing the thiols and reducing NADP+) should result in net oxidation of the regulatory thiols, as discussed above, which can account for at least some of the redox modulation (Kramer et al. 1990).

By contrast, the acidification of the lumen should shift the lumenal thiol couples to less reducing (more oxidizing) redox potentials, making the transfer of electrons from stromal carriers (like NADPH or Trx) strongly favorable, and inhibiting oxidation by reversal processes. Extrapolating from published values, we estimate that VDE and HCF164 will have redox midpoint potentials of -202 and -164 mV at pH = 6.0, reducing in a strongly favorable transfer of electrons from stromal NADPH or Trx, effectively making the transfer of electrons irreversible and preventing stromal carriers from acting as oxidants for lumenal thiol couples.

In the stroma, light-induced electron transport typically results in a net reduction of thiol modulation enzymes that activate assimilation (e.g., FBPase) and inactivate catabolism (e.g., glucose-6-phosphate dehydrogenase). In contrast to stromal thiol-modulated enzymes, which are activated by becoming more reduced, lumenal thiol-modulated enzymes are often activated by becoming more oxidized (compare Figure 3A to 3C, see section 4). For example, VDE is inactivated by the artificial treatment of dithiothreitol, a strong reducing agent (Bilger and Björkman 1990). This occurs despite the fact that acidification of the lumen should make thiol reduction more favorable, strongly implying that the kinetics of oxidative processes, rather than thermodynamic constraints, control the overall redox poise.

The question then is, how do electrons get out of the lumen in a chloroplast system? The above arguments imply that alternative, lumen-associated components are absolutely required for redox balancing and that their redox potentials must be more oxidizing than the least negative thiol component, i.e. midpoint potential at pH = 6 higher than about -160 mV. Molecular oxygen was proposed as an oxidant in the lumen (Buchanan and Luan 2005; Gopalan et al. 2004) (Figure 3B). However, this is unlikely, as the oxygen concentration does not change significantly in the lumen and O_2 is a relatively stable form. Obvious candidates include the plastoquinone (PQ) pool and some form of activated oxygen species, e.g. ROS species like H_2O_2, as seen in 2CP-mediated oxidation in the stroma.

In the Gram-positive bacterial system, thiol oxidation performed by DsbA (Fig. 2A) and a homologous proteins, 2CP, was proposed to oxidant in the stroma (Yoshida, Hara, Sugiura, Fukaya & Hisabori 2018; Yoshida, Yokochi & Hisabori 2019; Vaseghi *et al.* 2018; Ojeda, Pérez-Ruiz & Cejudo 2018; Yokochi, Fukushi, Wakabayashi, Yoshida & Hisabori 2021). However, 2CP is unlikely to have direct access to lumenal thiols.

LTO1 was proposed as a lumen-localized thiol oxidase by Karamoko et al (2011). LTO1 is an oxidoreductase that belongs to a distinct class of disulfide bond-forming enzymes in bacteria. It has two domains: a lumenal thioredoxin-like domain, which is functionally similar to DsbA, and functions to oxidize (forms disulfide bonds) proteins and a transmembrane domain, that has homology to the mammalian vitamin K epoxide reductase (VKOR) catalytic domain, which is also similar to DsbB (Karamoko*et al.* 2011; Onda 2013). LTO1 has been shown to oxidize lumenal proteins such as PsbO, STN7, and VDE (Kieselbach 2013; Lu *et al.* 2013, 2015; Yu *et al.* 2014; Wu *et al.* 2021) (see Section 4 for details). However, the final acceptor of LTO1 is still unknown (see Section 5).

Molecular oxygen was suggested as an oxidant in the lumen (Buchanan and Luan 2005; Gopalan et al. 2004) (Figure 3B). However, the oxygen concentration does not change significantly in the lumen and O_2 is a relatively stable form.

4. A model for the role of lumenal redox regulation in photoprotection

In the previous sections, we discussed redox regulation in the stroma, the trans-membrane mediators and the unique redox environments in the lumen, which are all affected by pH changes. In this section, we will explore the implications of the thylakoid *pmf* , not only on the canonical NPQ mechanisms, but on interactions with lumenal redox regulation, as summarized in Fig. 5.

There are several forms of NPQ, defined by their rates of activation and deactivation (Pinnola & Bassi 2018). These forms include the most rapid form, energy-dependent quenching (qE) (Li*et al.* 2004; Niyogi *et al.* 2005; Niyogi*et al.* 1998, 2005; Li *et al.* 2004), state-transition quenching (qT) (Quick & Stitt 1989), Z-dependent quenching (qZ) (Demmig-Adams & Adams 1996; Müller *et al.* 2001; Li *et al.* 2004; Nilkens*et al.* 2010), sustained quenching (qH) (Malnoë*et al.* 2018), and photoinhibitory quenching (qI) (Andersson & Aro 2004; Murata *et al.* 2007; Nawrocki *et al.* 2021a). In the following subsections, we will discuss the essential role of redox regulation on each NPQ form.

4.1 Energy-dependent quenching, qE

Energy-dependent quenching (qE) is the most rapidly-responding form of NPQ. It is triggered by the acidification of the lumen (proton gradient ([?]pH) component of proton motive force (*pmf*)) which results in the protonation of PsbS as well as the activation of violaxanthin de-epoxidase (VDE), which catalyzes the conversion of violaxanthin (Vx) to zeaxanthin (Zx). Zx subsequently dissipates excessive light energy and induces qE along with PsbS (Niyogi*et al.* 1998).

Recent studies have shown that both the proton gradient ([?]pH) and also the redox status of the thiol-disulfides within in VDE play a role in controlling its function (Arnoux, Morosinotto, Saga, Bassi & Pignol 2009; Yu *et al.* 2014; Hallin*et al.* 2015; Arnoux*et al.* 2009; Simionato *et al.* 2015). Specifically, the proton gradient ([?]pH) facilitates a structural change in VDE causing it to change from a monomer to a homo-dimer complex upon lumen acidification (Arnoux*et al.* 2009). In addition, to VDE undergoing a structural conformational change, VDE activity is also regulated in a thiol-disulfide-dependent manner (Hall*et al.* 2010; Kieselbach 2013; Yu *et al.* 2014; Simionato*et al.* 2015; Hallin *et al.* 2015). This elaborate regulation is achieved due to the unique structure of VDE. The VDE protein consists of three domains: a Cys-rich N-terminal domain, a lipocalin-like domain (predicted to bind violaxanthin) (Saga*et al.* 2010) and a glutamate-rich C-terminal domain. VDE has a total of 13 conserved Cys residues, 12 of which are at the N-terminal and these Cys residues play a major role in protein function. Consequently, VDE is active only when fully oxidized, with six disulfide bonds, is VDE active (Simionato*et al.* 2015), and this corresponds to a more compact, rigid, thermostable form of the protein (Hallin*et al.* 2015).

It has been proposed that the electron donor for VDE is Trx-m through CcdA and HCF164 (Motohashi & Hisabori 2006, 2010) and the oxidase is LTO1(Lu*et al.* 2013, 2015; Yu *et al.* 2014; Wu *et al.* 2021) (Fig. 4). Coincidently, an *lto1-2* mutant line showed a lower de-epoxidation state of xanthophyll cycle pigment de-epoxidase index (DEI), suggesting that de-epoxidation of Vx to Zx was suppressed in the*lto1-2* mutant line*(Yu et al. 2014; Lu et al. 2015).*

Another component that can affect the kinetics of qE is K^+ efflux antiporter3 (KEA3), a potassium/proton antiporter. KEA3 exchanges protons (out of the lumen) and potassium ions (into the lumen) (Armbruster*et al.* 2014). Consequently, when light transitions from high to low, the KEA3 antiporter function, helps relax qE. Furthermore, it has been recently reported that KEA3 has five Cys residues, but only one Cys residue is located at the N-terminal which extends into the lumen (Wang*et al.* 2017a). The location of this Cys residue suggests that KEA3 may be regulated by a redox mechanism, acting as adimer. However, the possible electron donors and oxidases involved in regulating KEA3 need further investigation.

4.2 Redistribution of excitation energy, qT

In response to light quality and intensity, the balance of excitation energy between the two photosystems is dynamically adjusted to avoid photodamage. This is achieved through serine/threonine-protein kinase (STN7) (Bellafiore*et al.* 2005). STN7 is a transmembrane protein having its catalytic domain extending

into the stroma. STN7 also has two conserved cysteines located on the lumen side of the thylakoid (Lemeille*et al.* 2009; Bergner *et al.* 2015).

The catalytic domain of STN7 is responsible for the phosphorylation of light-harvesting complex II (LHCII) (Puthiyaveetil 2011). Phosphorylation of LHCII changes its structure, which causes it to detach from PSII and migrate to PSI. This process is important for balancing the amount of light energy that is captured by each photosystem under dynamic environmental changes (Bellafiore et al. 2005).

The proposed model for STN7 functions is that plastoquinol (PQH_2) binds to the Qo site of Cytb_6f and activates STN7 by forming intermolecular disulfide bridges between the conserved cysteines in the lumenal domain of STN7. However, the dimer formed by these disulfide bridges is very transitory and can be easily converted back to a monomer (Lemeille*et al.* 2009; Wunder *et al.* 2013; Bergner *et al.*2015; Shapiguzov *et al.* 2016). Wu*et al.* (2021) showed that the lumenal cysteines of STN7 are maintained in the oxidized state by the Trx-like domain of LTO1, which then transfers reducing power to the VKOR domain (Wu*et al.* 2021). Under conditions where the PQ pool is oxidized, e.g. when PSI is preferentially excited by light, STN7 becomes inactivated, LTO1 no longer oxidizes STN7, and the antenna adopt the state 1 configuration (Fig. 5). However, it is noteworthy that state transitions are strongly decreased in the *lto1* mutant, but they are not completely abolished, unlike in the *stn7* mutant (Wu*et al.* 2021). This result suggests that other factors, in addition to LTO1, are likely involved in keeping STN7 oxidized and active in the acidic lumenal environments. In addition, it has shown that STN7 is inactivated by Trx-m, in coordination with HCF164 and CcdA (Ancin*et al.* 2019) (Fig. 5). In this scenario, Trx-m breaks the intermolecular disulfide bridges in STN7, leading to the formation of an intramolecular disulfide bond, which inactivates STN7 by monomerizing it. So once again, the function of STN7 is heavily impacted by its redox regulation However, the exact oxidative mechanism by which STN7 functions in the acidified lumen, where the redox midpoint potentials of the regulatory thiols increase, still needs further investigation.

4.3 Sustained quenching, qH

qH is a form of sustained and slowly reversible quenching that occurs in the peripheral antenna of PSII prior to photoinhibitory quenching (qI) (Malnoe*et al.* 2018). Bru*et al.* showed that the qH quenching site is a major trimeric antenna complex that consist of Lhcb1, Lhcb2, Lhcb3, which can form homotrimeric and heterotrimeric complexes (Bellottari et al 2012, Crepin et al 2018). Interestingly, Bru et al. also found specific isoforms of the major trimeric antenna complex that are not required for qH induction (Bru*et al.* 2021).

qH is known to be redox-regulated but independent of protonation of PsbS, accumulation of Zx, and phosphorylation by STN7 (Brooks*et al.* 2013; Malnoe *et al.* 2018; Bru *et al.* 2021). It has been shown that qH is induced by oxidative stress conditions such as cold and high light (Levesque-Tremblay, Havaux & Ouellet 2009; Brooks *et al.* 2013; Malnoe *et al.*2018; Bru *et al.* 2021).

The molecular players of qH are SOQ1 (Brooks*et al.* 2013; Malnoe *et al.* 2018; Malnoe 2018; Bru *et al.* 2021), plastid lipocalin (LCNP) (Brooks*et al.* 2013; Malnoe *et al.* 2018; Bru *et al.* 2021), and relaxation of qH1 (ROQH1) (Amstutz*et al.* 2020) (Fig. 5). LCNP is a major component that induces qH. SOQ1 is a trans-membrane protein (see section 3.1) that inhibits LCNP, while ROQH1 relaxes qH. LCNP is a soluble lumenal protein that induces qH by binding to LHCII and thus induces a conformational change within LHCII causing it to undergo a transformation from a light-harvesting state to a dissipated state (Yu*et al.* 2022). In addition, the redox status of the six conserved cysteines within LCNP play a critical role in regulating LCNP's activity (Malnoe*et al.* 2018).

Consequently, the reduced form of LCNP is inactive, while the oxidized form is active. Mechanistically, LCNP can be proposed to work as follows: SOQ1, transfers reducing equivalents to LCNP, which inhibits LCNP under non-stress conditions. However, under stress conditions (oxidative condition), SOQ1 no longer transfers reducing equivalents to LCNP, which keeps LCNP in an oxidized state and thus allows it to induce qH (Malnoe 2018). As a result, the function of LCNP and qH is being modulated by the redox state of the chloroplast (See Fig. 5).

4.4 PSII stability and repair of PSII, qI

Under stress conditions, such as high light intensity or extreme temperature, PSII is highly susceptible to photodamage of its core proteins, specifically the D1 protein. This photodamage can lead to the irreversible damage of PSII, which requires repair (Andersson & Aro 2004; Murata *et al.* 2007; Nawrocki *et al.* 2021a). However, PSII function can be maintained through an intricate mechanism involving the turnover and replacement of damaged D1 protein using an elaborate PSII repair cycle (Tikkanen, Mekala & Aro 2014). Accordingly, when the rate of damage of D1 exceeds the rate of repair of D1, this unbalance can ultimately lead to photoinhibition.

Briefly, the PSII repair cycle begins with the monomerization and detachment of the light-harvesting complex (LHCII) from the PSII core by phosphorylation through STN8 (STN7) kinase. The monomerized PSII core then migrates to the unstacked region of the thylakoid membrane, where it is dephosphorylated by the phosphatase, PBCP. The damaged D1 subunit is then degraded by the proteases Deg and FtsH. Once the damaged D1 subunit has been degraded, a new D1 subunit is synthesized and inserted into the PSII core (Kirchhoff 2014). Coincidently, the key proteases, Deg and FtsH, that are involved in D1 protein turnover are reported to be redox-regulated during this process.

By dynamic thiol-disulfide redox proteomics, Deg1 and Deg2 were found to be redox regulated (Stroher & Dietz 2008). Deg1 is peripherally attached to the thylakoid lumenal side (Chassin, Kapri-Pardes, Sinvany, Arad & Adam 2002) and is subsequently regulated by both the proton gradient ([?]pH) and redox state of the thylakoid membrane (Stroher & Dietz 2008; Knopf & Adam 2018). The formation of Deg1 homo-hexamers is dependent on [?]pH (Knopf & Adam 2018). Further, Deg1 showed maximal activity under reducing conditions and less activity under mild oxidative stress conditions (Stroher & Dietz 2008). Although Deg1 has only one Cys residue in its mature form (Table 1), the fact that it forms homo-hexamers and changes its activity depending on oxidizing/reducing condition suggests that is regulated by redox regulation upon formation of homo-hexamers, however this possible unique regulation needs to be studied further. Conversely, Deg2 is located in the stroma and has an opposite redox-dependent mechanism, showing higher proteolytic activity under oxidizing conditions (Haussuhl, Andersson & Adamska 2001). Other interacting factors of Deg protease, such as electron donors, and oxidizers have not yet been determined.

FtsH is an ATP-dependent zinc metalloprotease that exists as two types of subunits: A (FtsH1 and FtsH5) and B (FtsH2 and FtsH8) (Zaltsman, Ori & Adam 2005; Kato & Sakamoto 2018). Both types have a similar structure, with a C-terminal extension orientated towards the stroma and an N-terminal transmembrane domain (Lindahl*et al.* 1996). The C-terminal domain has ATP hydrolysis activity and conserved cysteines (Sauer & Baker 2011; Wang *et al.* 2017b). FtsH cooperates with Deg proteases to degrade the D1 protein in the PSII repair cycle. It has been suggested that FtsH degrades D1 after it has been cleaved by Deg-mediated proteolysis (Nishimura, Kato & Sakamoto 2016; Kato & Sakamoto 2018). FtsH is active under reducing conditions, and it has been proposed that the possible electron donors for FtsH are members of the stromal Trx family (Wang*et al.* 2017b). However, the oxidant for FtsH remains unknown.

Given that photoinhibition decreases the efficiency of photosynthesis and redox regulation directly affects protease activity and rate of repair, further research will be needed to fully understand the role that redox regulation has on these proteases.

Finally, besides proteases, PsbO, a subunit of the oxygen-evolving complex (OEC), is also associated with PSII stability and repair. The redox state of two cysteine residues in PsbO is the key determinant of its stability (Hall*et al.* 2010; Kieselbach 2013). The oxidized form of PsbO is stable, while the reduced form is unstable and becomes a target for proteolysis, leading to increased accessibility of PSII core proteins for degradation. For example, when D1 in PSII is damaged, PsbO in its stable oxidized form is less accessible to degradation, which further inhibits the repair cycle. Therefore, the redox state of PsbO must be tightly regulated. It has been shown that LTO1 oxidizes PsbO (Karamoko*et al.* 2011), but the reducing pathway for PsbO remains to be determined. PsbO was found to be a potential target of Trx (Lee*et al.* 2004; Marchand, Le Marechal, Meyer & Decottignies 2006; Hall *et al.* 2010), but given the location of Trx and PsbO in the

stroma and lumen respectively, a mediator such as CcdA and HCF164 or another unknown factor may be involved in the transfer of electrons to PsbO (Fig. 4).

5. *What are the missing pieces in the lumen thiol-disulfide regulation mechanism?*

So far, we have discussed the redox regulation from the stroma to the transmembrane region to the lumen and its photoprotective roles. The molecular players in the redox regulation and relevant photoprotective forms are summarized in Figure 5. In brief, reducing equivalents from the stroma are transferred through CcdA and HCF164 to VDE and STN7. These proteins are inactive in their reduced forms, as indicated by the arrows pointing in the opposite direction (-). Another reducing mediator, SOQ1, transfers reducing power to LCNP, which is inactivated in its reduced form. The electron donors for KEA3-1, PsbO, and Deg1 are still unknown, as indicated by the dashed arrows. The oxidizing system appears to be mediated by LTO1. LTO pulls electrons from VDE, STN7, and PsbO. The oxidizing mediators for KEA3-1, Deg1, and LCNP are not known. All of the redox-regulated proteins in the lumen, including VDE (qE, qZ), KEA3 (*pmf* partitioning into [?]ψ and [?]pH and effects on qE), STN7 (qT), LNCP (qH), Deg1, and PsbO (qI), orchestrate photoprotective mechanisms.

However, as seen in Figure 5, many of the pathways in lumenal redox regulation are still hypothetical (so many dashed lines!). Certainly, we have just begun to scratch the surface of this complex lumenal redox regulation network. While there are many unanswered questions regarding this complex redox network, there are two significant yet unresolved questions we would like to focus on:

How do electrons get out of the lumen? Recall that LTO1 may be involved in numerous oxidizing events but the final acceptor of LTO1 has not been identified (See Figure 5).

What keeps fine-tuning redox potentials and kinetics to achieve the appropriate regulatory levels in the "acidic lumen?" As we discussed in section 3.2, due to the [?]pH component of *pmf* , the lumen has quite a unique redox environment, where the redox midpoint potentials of the regulatory thiols substantially increase upon illumination (See Figure 4).

One hypothetical model that could answer both of these questions is that reactive oxygen species (ROS) act as a strong oxidant. It has been shown that in the stroma, the 2CP-mediated oxidation system pulls electrons from target enzymes and transfers them to the final acceptor hydrogen peroxide (H_2O_2), which is then reduced to water. In the hypothetical model for lumenal redox regulation, the Trx-domain of LTO1 would pull electrons from target enzymes, and these electrons would then be transferred to the VKOR domain of LTO1 in the thylakoid membrane. From there, the electrons could be transferred to an unknown oxidizing mediator (marked as "?" in the figure), which could further reduce H_2O_2. Another possibility is that there is another oxidoreductase in the thylakoid membrane that uses H_2O_2 as a final electron acceptor. This hypothesis is supported by the results of state transition experiments in the *lto1* mutant, which showed that state transitions were not completely abolished in this mutant (Wu et al 2021). This result strongly suggests that other factors are also involved in keeping STN7 oxidized and active. The other possible strong oxidants could be plastoquinone (PQ) pool. More research will be needed to determine the exact mechanisms involved.

Concluding remarks

Redox regulation is a key mechanism that enables plants to adapt to unpredictable changes in their environment, such as variations in light intensity or temperature. We have shown that numerous lumenal proteins involved in photoprotection are subject to regulation by their redox state, although there are still several unanswered questions regarding redox regulatory mechanisms in the lumen. It is critically important to keep in mind that **the *pmf* changes everything** . For instance, an acidified lumen makes it more challenging to maintain oxidizing environments in the lumen, as the redox midpoint potentials of regulatory thiols change and increase.

In future studies, it will be important to focus on the following aspects of redox regulation in the chloroplast thylakoid lumen:

- Study the effects of lower pH in the lumen that impacts the redox midpoint potentials of proteins.
- Identify the strong thiol-oxidizing system that extracts electrons from the thylakoid lumen in response to changes in ROS or redox status.
- Determine the final acceptor of the oxidation pathway that leads to the oxidation of lumenal proteins.
- Consider the possibility that there are more trans-thylakoid membrane thiol mediators that can reduce and oxidize lumenal proteins.
- Investigate the roles and interactions of target enzymes in the redox regulation network.

By considering these recommended focal points for future investigations, we should attain a better understanding of the complex mechanisms of redox regulation and photoprotection in the chloroplast. This new knowledge will contribute to our understanding of how plants survive and adapt to ever changing environments and it may also provide us with new insights as to how we can potentially improve photosynthesis.

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Conflict of interest statement

The authors declare no conflict of interest.

Data availability statement

Data sharing is not applicable to this article because no datasets were generated during the current study.

Figures

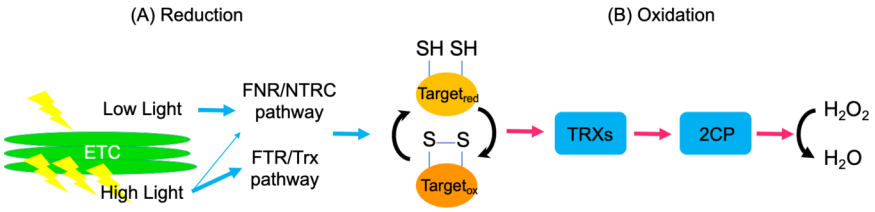

Figure 1. Scheme of chloroplast redox regulation in the stroma.

A schematic representation of two reduction pathways (A), FNR/NTRC and FTR/Trx under different light availability, and (B) oxidation pathway under dark is shown. (A) Under high light intensity, the FTR regulatory system dominantly reduces thiol-regulated target enzymes. Under limited light availability, the NTRC system mainly functions. (B) Under dark, 2CP mediates oxidizing the target enzymes. More details can be found in Section 2. The arrows indicate the flow of reducing equivalents, with blue representing reduction and pink representing oxidation.

Abbreviations: ETC, Electron transport chain; FNR, ferredoxin-NADP+ reductase; FTR, ferredoxin-thioredoxin reductase; NTRC, NADPH-dependent thioredoxin reductase C;

2CP, 2 Cys-peroxiredoxin

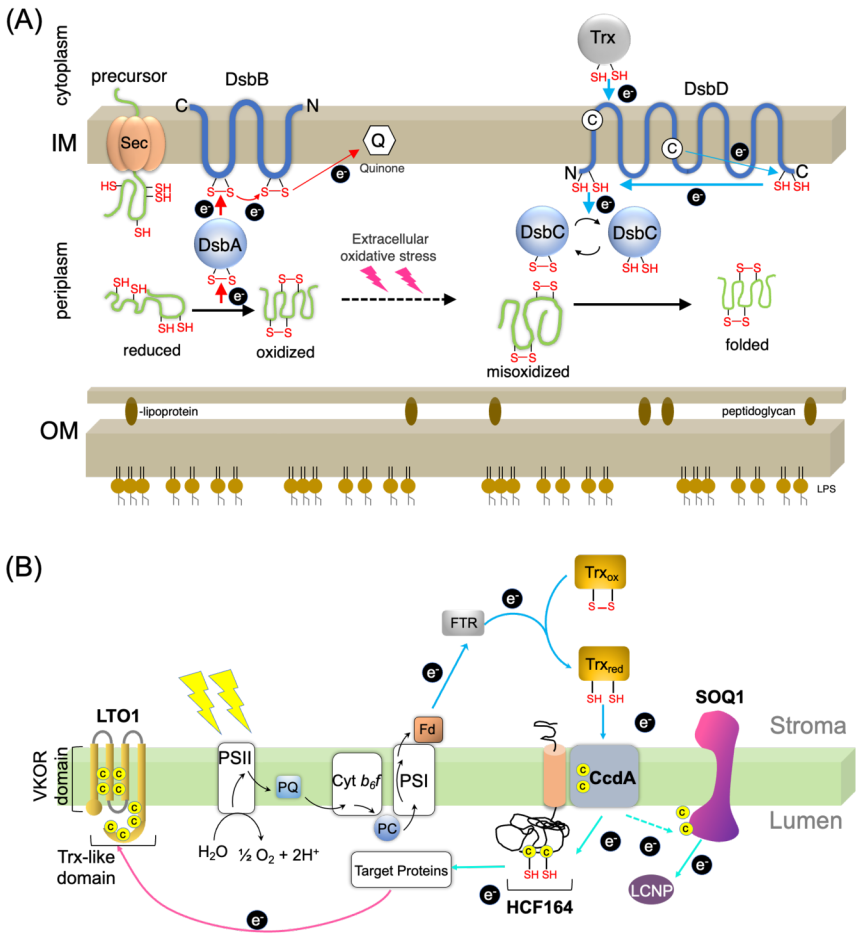

Figure 2. Comparison of the models of reducing equivalents transport pathway across (A) inner membrane (IM) of Gram-negative bacteria and (B) the thylakoid membranes chloroplast. (A) Hypothetical model of transferring reducing equivalents across the IM that involves protein folding in the periplasm. This oxidative protein folding disulfide bond isomerization pathway in Gram-negative bacteria through the Dsb family. The protein is transported to the periplasm by SecYEG, which is oxidized by DsbA. Reduced DsbA then transfers reducing equivalents to DsbB in the IM. Reduced DsbB then transfer reducing equivalents to quinone. When the protein is mis-oxidized, Trx in the cytoplasm transfers reducing power to DsbD in the cytoplasmic membrane, which then transfers it to DsbC for proper folding. (B) Hypothetical model of reducing equivalents across the thylakoid membrane that may be involved in possible protein folding and enzyme activity within the lumen. Reducing equivalents from the stroma are transferred through CcdA and HCF 164, followed by reduced redox-regulated proteins in the lumen. LTO1 oxidizes redox-regulated proteins. The arrows indicate the flow of reducing equivalents, blue for reduction and pink for oxidation. (Note: Figure A was adapted from From Melissa E. Reardon-Robinson, and Hung Ton-That J. Bacteriol. (2016) 198:746-754; while Figure B was adapted from Frontiers in Plant Sci (2017) doi: 10.3389/fpls.2017.01313)

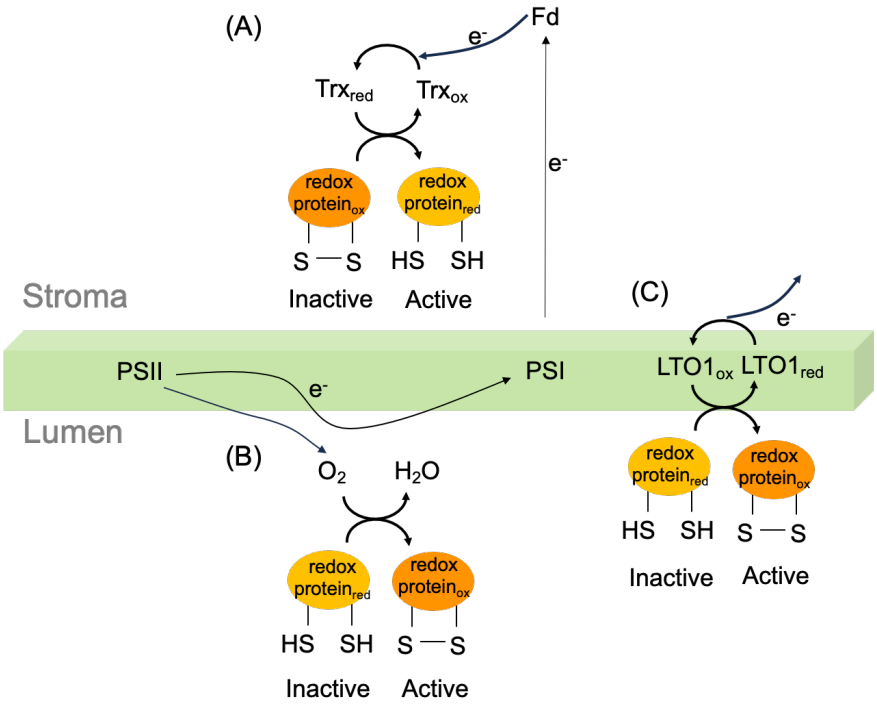

Figure 3. Distinct redox active statuses in the stroma and the lumen.

Light-dependent thiol/disulfide-regulated (A) stromal proteins are considered to be in their active form when they are reduced. Conversely, (B,C) lumenal proteins are considered to be in their active form when they become oxidized. The proposed oxidation models are: (B) oxygen (O_2) and (C) LTO1.

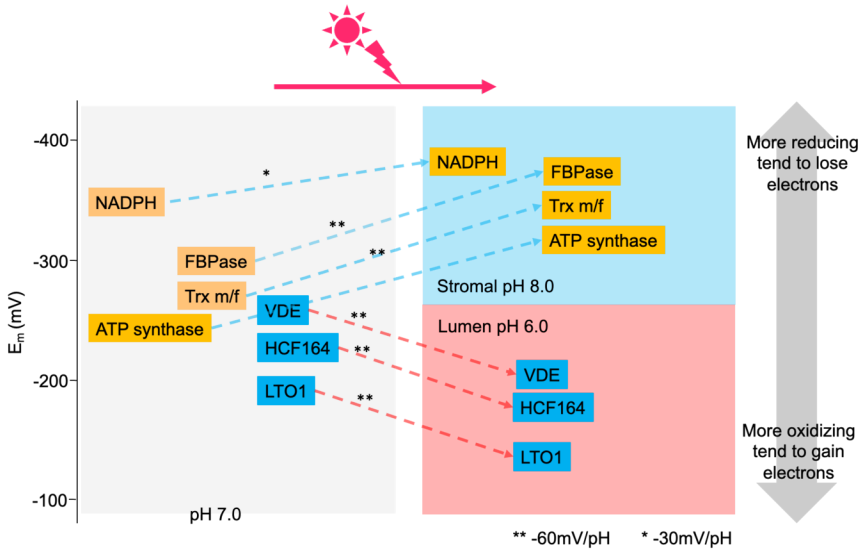

Figure 4. Scheme of hypothetical changes in redox potential of selected thiol/disulfide redox-regulation proteins upon the energized system by light, which induces increased and decreased pH in the stroma (blue box) and lumen (pink box) respectively . Redox-regulated proteins in the

stroma are shown in the orange box. Proteins that have the Cys residues on the lumenal side are shown in the blue box. Note that HCF164 and LTO1 is transmembrane protein (section 3), but the shown redox potentials here are for the soluble domain of those. The asterisk indicates indicates changes in redox potential, two asterisks (**) for a changes of -60mV/pH and one asterisk (*) for changes of -30mV/pH. Lumenal thiols tend to easily gain reducing equivalents upon pH changes (increased redox potential) while stromal thiols are the opposite.

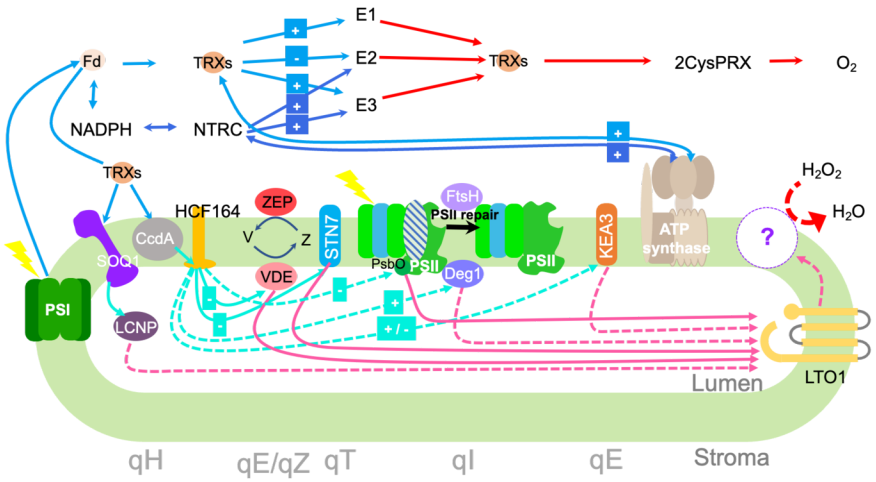

Figure 5. Hypothetical model for redox regulation in the stroma and the lumen.

The redox-regulated stromal enzymes are shown in the upper panel (for details, see section 2) and the redox-regulated thylakoid membrane and lumenal proteins (section 4) are at the bottom of the panel. The arrows indicate the flow of reducing equivalents. Hypothetical electron flow is shown as dashed lines. The reductive pathway is indicated by the blue and cyan arrows for stromal, and lumenal respectively, and oxidative pathways are shown in red and pink arrows for stromal, and lumenal respectively. To clarify, the use of a plus (+) sign in reductive arrows denotes that the reduced state of the protein in the direction of the arrow is in an active or stable form. On the other hand, the presence of a minus (-) sign in the reductive arrow indicates that the reduced state of the protein in the direction of the arrow is in an inactive or unstable form. In summary, reducing equivalents from the stroma are transferred to the lumen via CcdA and HCF164 to VDE and STN7. These proteins are inactive when in their reduced forms, as indicated by the minus (-) sign in the arrows. Additionally, SOQ1 also serves as a reducing mediator, transferring reducing power to LCNP, which is deactivated when reduced. The oxidizing system is mediated by LTO1, which pulls electrons from VDE, STN7, and PsbO. However, the oxidizing mediators for KEA3, Deg1, and LCNP are still not known. LTO1 mediates the oxidizing system, extracting electrons from VDE, STN7, and PsbO. Nevertheless, the oxidizing mediators for KEA3, Deg1, and LCNP remain unknown. All of these redox-regulated proteins in the lumen, including VDE (qE, qZ), KEA3 (qE), STN7 (qT), LNCP (qH), Deg1, and PsbO (qI), are cooperating to induce photoprotective mechanisms. The hypothetical model for removing electrons from the lumen is shown that reactive oxygen species (ROS) act as a strong oxidant via unknown strong oxidant. The Trx-domain of LTO1 would pull electrons from target enzymes, and these electrons would then be transferred to the VKOR domain of LTO1 in the thylakoid membrane. From there, the electrons could be transferred to an unknown oxidizing mediator (marked as "?" in the figure), which could further reduce H_2O_2. See more details in Table 1 and section 3 and 4.

Table 1.

Summary of redox-regulated proteins that contribute to photoprotection in the thylakoid lumen and transmembrane

Categorized role	Protein	Accession	Redox potential	Localization	Known function	Roles in redox pathway or Redox status of Active form / Stable form	Interactions (Trx, HCF164, LTO1)	number of cysteines in mature proteins	ref
Trans-thylakoid thiol mediators	CCDA	AT5G54290	Unknown	thylakoid membrane	Assembly of Cytb_6f	transfer redox equivalents	Trx, HCF164	4	Pa 200 Mo & His 201
	HCF164	AT5G23120	-224mV (pH 7.0)	thylakoid membrane	Assembly of Cytb_6f	transfer redox equivalents	Trx, CCDA	3	Le et 200 Mo & His 200
	SOQ1	AT1G56500	Unknown	thylakoid membrane	Photoprotection	transfer redox equivalents	LCNP	2	Br et 201
	LTO1	AT4G35760	-180mV (pH 7.0)	thylakoid membrane	Assembly of PSII	oxidant	VDE, STN7, PsbO	8	Ka Cli Re Ru Ha 201 et 201 Lu Pe Wa 201
Photoprotection (qE)	KEA3-1	AT4G04850	Unknown	thylakoid membrane	*pmf* partitioning	Unknown	Unknown	5	Wa et 201
	VDE	AT1G08550	-317.3mV (pH 7.9)	Lumen	catalyzes the conversion of violax-anthin (Vx) to zeaxan-thin (Zx)	oxidized state is active	LTO1	13	Ar al. Yu Ha 201 Sir 201

Sustained quenching (qH)	LCNP	AT3G47860	Unknown	Lumen	photoprotection	oxidized state is active	SOQ1	6	Ma al. 20
State transition (qT)	STN7	AT1G68830	Unknown	thylakoid membrane	state transition	oxidized state is active	Trx, LTO1	2	Ri Ma suc Pu mo 200 Pe al. Le et 200 et
PSII stability and repair (qI)	PsbO1	AT5G66570	Unknown	Lumen	PSII subunit, OEC	oxidized state is stable	Trx, LTO1	2	Ha 20 Ka et
	PsbO2	AT3G50820	Unknown	Lumen	PSII subunit, OEC	oxidized state is stable	Trx, LTO1	2	Lu Ha son Sch Ve & Sp 200 Ka et 20
	DEG1	AT3G27925	Unknown	Lumen	protease, degradation of D1	reduced state is stable	Trx	1	Ch al. Str Di 200 et

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