Review

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Thiol switches in redox regulation of chloroplasts: balancing redox state, metabolism and oxidative stress

Abstract: In photosynthesizing chloroplasts, rapidly changing energy input, intermediate generation of strong reductants as well as oxidants and multiple participating physicochemical processes and pathways, call for efficient regulation. Coupling redox information to protein function via thiol modifications offers a powerful mechanism to activate, down-regulate and coordinate interdependent processes. Efficient thiol switching of target proteins involves the thiol-disulfide redox regulatory network, which is highly elaborated in chloroplasts. This review addresses the features of this network. Its conditional function depends on specificity of reduction and oxidation reactions and pathways, thiol redox buffering, but also formation of heterogeneous milieus by microdomains, metabolite gradients and macromolecular assemblies. One major player is glutathione. Its synthesis and function is under feedback redox control. The number of thiol-controlled processes and involved thiol switched proteins is steadily increasing, e.g., in tetrapyrrole biosynthesis, plastid transcription and plastid translation. Thus chloroplasts utilize an intricate and versatile redox regulatory network for intraorganellar and retrograde communication.

Keywords: Arabidopsis thaliana; chloroplast; glutathione; redox regulation; thiol switch.

Introduction: the challenge – regulation in photosynthesizing chloroplasts

Photosynthesis consists of a complex series of redox reactions. The balance between light harvesting, physicochemical energy conversion and metabolic consumption of chemical energy determines the redox state of participating reactions. Thus the redox milieu of chloroplasts rapidly changes in dependence on the photosynthetic state, which, in turn, depends on environmental conditions such as light intensity, temperature and CO₂ availability. Thereby the redox milieu provides important information on the metabolic state and thus the systems performance of photosynthesis (Scheibe and Dietz, 2012). Chloroplasts, unlike any other organelle, alter their energization state and reduction potential in immediate response to environmental cues on shortest time scales of less than 1 s. Adjustment of photosynthesis to changing photon flux densities serves as the best example because light intensities drop or rise by shading in combination with moving sun flecks or wind. Maximum photosynthetic CO₂-fixation rates reach 400 μmol CO₂/mg chlorophyll h corresponding to linear electron transport rates from water to NADP⁺ of 12 mmol electron/ls or 6 mmol NADPH/ls (calculated based on 40 μl chloroplast volume/mg chlorophyll). Considering the total NADPH concentration of 50–100 μM, such rates correspond to 60- to 120-fold turnover per second. Light intensities change within seconds in the natural environment and instantaneously in the laboratory or growth chamber. A small NADPH pool with tremendous turnover represents a sensitive redox hub in the chloroplast. But plants efficiently cope with these rapid metabolic challenges. Obviously, they are able to coordinate light harvesting and light-driven generation of reducing power with its consumption in metabolism under most conditions almost instantaneously (Puthiyaveetil et al., 2012; Shikanai, 2014).
The redox milieu of the chloroplast

Excessively accumulating reducing power in the photosynthesizing chloroplast strongly affects redox homeostasis and normal cell functions. Reactive oxygen species (ROS) or other reactive intermediates are generated by transfer of electrons to oxygen if the final electron acceptor NADP⁺ is unavailable. Thus over-reduction is linked to ROS-mediated oxidative switching of redox sensors and redox signaling, but may also cause oxidative damage. Non-aqueous chloroplasts isolated from Beta vulgaris or Spinacia oleracea leaves reflect the specific metabolic situation of the chloroplasts in vivo and revealed that the NADPH/(NADP⁺NADPH) ratio in chloroplasts increases from 0.22 to 0.32 in the dark to 0.33–0.52 after 15 s of illumination with 700 μmol quanta m⁻² s⁻¹. Reaching a slightly higher maximum after 30 s of illumination, the ratio returned to the dark levels after about 3 min in the light (Heber and Santarius, 1965). This rapid activation testifies the efficient coordination of light and dark reactions of photosynthesis. These data also imply that the system usually adjusts to a rather oxidizing NADPH/NADP redox state.

Over-reduction of the photosynthetic electron transport chain (PET) describes a state of accumulated reduced electron carriers with very negative redox potential. Several non-invasive methods allow for determination of PET redox and energization state, e.g., redox state of the primary acceptor of photosystem II Qa, reduction state of photosystem I, the electrochromic shift measured near 520 nm and light scattering of leaves (Kobayashi et al., 1982; Takizawa et al., 2007). Such measurements with leaves in vivo as well as with isolated chloroplasts and thylakoids show a relationship between PET over-reduction and enhanced production of reactive molecular species, namely singlet oxygen, superoxide, hydrogen peroxide, lipid peroxides and other reactive species. Such reactive species oxidize macromolecules of the cell interfering with their normal function. Such redox changes are used for tuning protein activity and thus metabolic rates, in other cases these changes trigger degradation of damaged macromolecules, or they initiate cell death program. Monitoring and controlling the redox state of the PET and the chloroplast stroma is central to counteract over-reduction and avoid the development of threatening oxidative stress.

In technical devices that need accurate process regulation, monitoring mechanisms sense the state of important elements within the system. Deviations from norm levels initiate feedback to adjust the desired condition. In contrast to simple circuitries often utilized in technology biological systems commonly use multiple control mechanisms. Regulation of photosynthesis employs multiple inputs and tuning mechanisms. In fact the superior performance of sophisticated cross-talking control networks is nowadays realized also in technology where board computers control the entire system.

The catchest in vivo and thus 'on line'-observation of regulation involved in photosynthesis was reported and studied in particular by Walker’s group (Sheffield, UK). They explored oscillations in photosynthetic parameters that can be observed in many plant species under conditions, such as low phosphate supply, low temperature, high CO₂-concentrations, or re-illumination after a short dark period. Photosynthetic gas exchange, chlorophyll fluorescence, but also biochemical parameters start to oscillate with initially high amplitude and subsequent dampening to the new steady state (Figure 1). Oscillations testify overshooting responses of regulation (Walker, 1992). In fact Laisk proposed that understanding

![Figure 1: Oscillations in photosynthesis witness regulation and limitations.](image-url)
oscillations would be equivalent to understanding regulation of photosynthesis (Laisk et al., 1991; Walker, 1992). Figure 1 shows that each measured parameter showed a specific pattern of consecutive maxima and minima. Following a minimum in energization as indicated by light scattering, there occurred a minimum in thylakoid membrane potential (electrochromic shift at 518 nm). This was paralleled by increased reduction of photosystem I and high fluorescence emission. With slight delay CO₂ fixation reached a minimum. Subsequently, all the changes were mirrored in opposite direction, i.e., maximum in energization, maximum in membrane potential, minima in PSI reduction and fluorescence followed by maximal CO₂ fixation (Sivak et al., 1985; Walker, 1992). Determination of NADPH and NADP⁺, as well as ATP and ADP (Laisk et al., 1991) revealed the surprising fact that maximal CO₂ fixation coincided with high ATP/ADP ratios but with minima in NADPH/NADP⁺ (Figure 1). Maxima in NADPH/NADP⁺ were preceded by maximal reduction of PSI. Therefore, Laisk et al. (1991) concluded that imbalanced supply of reducing power in form of NADPH and phosphorylation potential represent the cause for oscillations in photosynthesis. Apparently, redox processes, e.g., alterations between high and low electron pressure (PSI, 518 nm shift, NADPH/NADP⁺) participate in this phenomenon. Oscillations depend on delays in regulatory response time. It is tempting to speculate that thiol-dependent activation of the Calvin cycle is crucially involved in triggering the oscillations, namely if high reduction state of PSI diverts electrons into the thioredoxin system which in turn ‘over’-activates committed enzymes of photosynthetic metabolism. ‘Over-activation’ may subsequently cause a collapse in energization and inhibition of thiol-regulated target proteins below the needed rates in balance with light energy input. Thus in the next step energization will increase and reach the next maximum.

**Thiol regulation in chloroplasts**

The thiol redox state constitutes one of the most important process parameters in chloroplasts. Several redox players serve as hubs in the redox network of the chloroplast and thus act as information-rich agents in chloroplast signaling (Figure 2). (i) the plastoquinone (PQ) redox state provides integrated information on light conversion into excitation and activity of reaction centers of photosystem I relative to photosystem II (Rochaix, 2013). (ii) Chloroplast ferredoxin (Fd) functions as distributor of electrons between Fd-dependent NADP reductase, Fd-dependent thioredoxin reductase, nitrite reductase, sulfite reductase, Fd-dependent glutamine-oxoglutarate amidotransferase (Fd-GOGAT), cyclic electron transport and others (Blanco et al., 2013). (iii) NADPH delivers reducing power to metabolic reactions. (iv) glutathione buffers thiol-disulfide redox state and is present in millimolar concentrations. Glutathione has additional functions as electron donor for reduction of dehydroascorbate in the water/water cycle, which detoxifies hydrogen peroxide (Miyake, 2010). Likewise Trx, glutathione/glutaredoxins and NADPH-dependent thioredoxin reductase C (NTRC) feed electrons into the peroxiredoxin system for detoxifying hydrogen peroxide, alkylhydroperoxides and peroxinitrite (Dietz, 2011).

Each of these redox hubs is linked to sensory systems. Figure 2 schematically links PQ redox state to the protein kinases STN7/8, which control the phosphorylation state of photosynthetic proteins. Electron channeling from Fd to multiple Trx isoforms controls specifically the redox state of diverse dithiol-disulfide-target proteins. Targets such as sedoheptulose-1,7-bisphosphatase (SBPase) and fructose-1,6-bisphosphatase (FBPase) are classical thiol switches in the chloroplast (Schürmann and Wolosiuk, 1978; Yoshida et al., 2014). NADPH feeds electrons into...
diverse metabolic reactions and also into the NTRC system, which will be discussed below. Glutathione concentration and redox state control S-glutathionylation of chloroplast target proteins in tight interaction with chloroplast glutaredoxins (Zaffagnini et al., 2012). Applying this concept of regulation to the example of photosynthetic oscillations described above, one has to consider the relative concentrations of redox transmitters, in particular thioredoxins, the concentration of target proteins and the kinetics of activation in order to address the hypothesis that thiol redox regulation might be involved as delay factor triggering phase separation of processes and thus oscillations: Proteomics studies have revealed that thioredoxins are low abundant proteins relative to their target proteins (Peltier et al., 2006). Thus the concentrations of SBPase and FBPase appear to exceed the concentration of thioredoxin f1 by a factor of more than 1000. This unfavorable ratio contrasts the conditions of in vitro enzyme assays where Trx f1 is typically added in excess and maximum activation is only seen at high molar ratios of Trx f1/FBPase (de Lamotte-Guery et al., 1991). Knowledge on reoxidation of target enzymes is not available. Interestingly, SBPase was among the targets easily oxidized in vivo upon methylviologen treatment (Muthuramalingam et al., 2013). This result shows that oxidation occurs, but involved mechanisms such as oxidation by Trx-disulfide, direct oxidation by H2O2, proximity based oxidation or other reactions still need to be identified (König et al., 2012).

Modulation of the glutathione hub in chloroplasts

Glutathione in plants is associated with many functions in addition to ROS scavenging and redox regulation (Noctor et al., 2012). Its role is therefore intertwined with metabolism, in particular the assimilation of sulfate in chloroplasts. The relevant parameters for the redox potential of glutathione are its concentration and the GSH to GSSG ratio according to the Nernst equation. Synthesis, intracellular exchange and adjustment of reducing capacity of glutathione are decisive factors for understanding modulation of downstream targets of photosynthesis and stress acclimation (Ball et al., 2004; Meyer and Rausch, 2008). However, as glutathione appears to be present in most compartments of plant cells, the analysis and interpretation of glutathione functions still suffer from technical limitations that enable analyses of whole organ or tissue levels but impede differentiation between cell types and subcellular compartments. This limitation can currently only be overcome by non-aqueous fractionation and live cell imaging but most of the existing datasets are predominately derived from whole-tissue analyses. According to these reports, disturbances of redox homeostasis based on the total content of glutathione, of the ratio of GSH to GSSG towards oxidation and increased expression or enzyme activity of glutathione reductase (GR) have frequently been reported in response to stress factors, such as high light, osmotic factors and pathogen infection, all causing enhanced formation of ROS [see reviews by Klapheck et al. (1987); May et al. (1998); Tausz et al. (2004); Rausch and Wachter (2005); Hell and Kruse (2007); Noctor et al. (2011, 2012)].

An estimated one-third of total glutathione content of leaves can be attributed to chloroplasts (Klapheck et al., 1987) with estimations ranging from 1 to 4.5 mM in chloroplasts (Bergmann and Rennenberg, 1993; Noctor and Foyer, 1998). Arabidopsis leaves contain an averaged glutathione pool of 0.7 mM. Non-aqueous fractionation revealed nearly equal glutathione pools with 3–4 mM concentrations in the cytosol and chloroplasts (Krüger et al., 2009). For comparison, the overall cysteine concentration was 17 μM but only 9 μM of cysteine was determined in chloroplasts and 3 μM in the cytosol (Krüger et al., 2009). GSH/GSSG ratios have not been determined by non-aqueous fractionation nor potential changes in the distribution of glutathione within cells in response to stress. However, with respect to glutathione these results (Krüger et al., 2009) suggest equilibrium between the pools in both compartments at least under non-stressed conditions due to exchange processes as outlined below. Thus, significant changes of total glutathione pools may similarly affect both compartments and therefore also redox processes triggered by glutathione in the chloroplasts. Whether this assumption also holds for short-term disturbances of glutathione homeostasis is unclear. In fact most reports focus on time points several hours or even days after onset of stress. A time resolved analysis in response to high light revealed an increase in GSH levels after 30 min in normal light grown Arabidopsis thaliana that was exposed to 10-fold light increase and 1 h, respectively, in shade-acclimated plants transferred to a 100-fold higher photon flux density (Alsharafa et al., 2014).

The promise of redox probes and cell imaging in plants

The contribution of chloroplast glutathione pools to stress defense and the cell-specificity of responses are difficult to assess owing to the analytical limitation mentioned
above. A significant improvement is provided by life cell imaging using fluorescent probes. The introduction of the GSH-specific fluorescent dye monochlorobimane allows researchers to monitor cellular glutathione levels, changes of glutathione abundance and cellular transport between cytosol and vacuole (Meyer et al., 2001; Grzam et al., 2007). Unfortunately, this approach for plastids is hampered by the lack of glutathione-S-transferase activity that is necessary to conjugate the dye with glutathione to the fluorescent form. A major breakthrough was therefore the development of glutathione redox sensors that consist of a fusion of glutaredoxin with redox-sensitive GFP (roGFP) (Gutschner et al., 2008). This probe operates not only in the cytosol but also in the ER (Birk et al., 2013), mitochondria (Albrecht et al., 2014) and plastids (Maughan et al., 2010). Within the limits of the redox potential range the roGFP probe reports the redox potential of the glutathione pool of a given compartment. Remarkably, the in vivo measurement of the glutathione redox state indicates its almost complete reduction under non-challenged growth (Meyer et al., 2007). Using this approach the glutathione pool in Arabidopsis mutants lacking a glutathione transporter in the chloroplast membrane could be visualized (Maughan et al., 2010). Expression of roGFP in mitochondria or chloroplasts of Arabidopsis revealed that oxidative shifts in mitochondria stimulate intercellular transport via plasmodesmata. In a converse manner, chloroplast thiol oxidation inhibits intercellular transport (Stonebloom et al., 2012).

### Thiol switches in chloroplast glutathione homeostasis

The general response of the glutathione pool to oxidative stress is characterized by an initial change to more oxidized states followed by acclimation based on increased glutathione concentrations and related enzyme activities, both leading to enhanced regeneration of glutathione levels and readjustment of redox state (Tausz et al., 2004). The initial phase initiates signal transduction via oxidation of protein-thiol relays, whereas the following phase requires cysteine for glutathione synthesis and NADPH for reduction of GSSG. Assimilatory reduction of sulfate and the synthesis of cysteine and glutathione are best characterized in A. thaliana. Remarkably, the key integrator of external and internal signals for flux control in sulfate reduction, adenosine 5’-phosphosulfate reductase (APR) in plastids, is suggested to be redox controlled via disulfide bridges (Bick et al., 2001). Thus the thiol redox state feeds back to the committed step of sulfur assimilation via a thiol switch (Figure 3).

A peculiar subcellular distribution of involved enzymes controls the synthesis of cysteine from assimilatory sulfide. Cysteine synthesis is accomplished by the enzymes serine acetyltransferase (SAT) and O-acetylserine (OAS) thiol lyase via the intermediate O-acetylserine. Both enzymes are present in the cytosol, plastids and mitochondria and together form the cysteine synthase complex that acts as sulfide sensor and regulator [overview in Hell and Wirtz (2011)]. Enzyme activities of SAT and OAS-TL are unevenly distributed between the three compartments. Systematic analysis of null mutants indicates that chloroplasts synthesize little cysteine under control conditions while mitochondria provide the bulk of the precursor OAS and the cytosol generates most of the cysteine, and that OAS and cysteine are freely exchangeable between the compartments (Heeg et al., 2008; Birke et al., 2013). Nevertheless, deletion of the plastid isoform OASTL-B significantly reduced biomass production by about 25%. The significance of plastid cysteine synthesis may further increase conditionally.

In contrast, the first step of glutathione synthesis leading to γ-glutamylcysteine is catalyzed by γ-glutamylcysteine ligase (GCL) exclusively in the plastids and the second step, catalyzed by glutathione synthetase (GS), is present in plastids and the cytosol in Arabidopsis (Wachter et al., 2005). GCL catalyzes the regulatory step and is subject to transcriptional control in response to certain stresses, e.g., imposed by cadmium and jasmonic acid, but scarcely in response to H₂O₂ (Xiang and Oliver, 1998). GCL structure and activity are

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**Figure 3:** Subcellular organisation of the glutathione redox hub and reactions leading to glutathione synthesis. APR, adenosine-5’-phosphosulfate reductase; APS, adenosine-5’-phosphosulfate; CLT, CRT-like transporter; CSC, cysteine synthase complex; γGC, γ-glutamylcysteine; GCL, γ-glutamylcysteine ligase; GR, glutathione reductase; GS, glutathione synthetase; NTR, NADPH-dependent thioredoxin reductase A, B.
Balancing reduction and oxidation defines activity

Effective control of functional activity depends on reversibility, which requires mechanisms of activation and deactivation (Figure 4). The PQ redox sensing is linked to the kinases STATE TRANSITION7 (STN7) and STN8 (Samol et al., 2012). STN7 affects relative activities of photosystem I and photosystem II by phosphorylating light harvesting proteins of PSII. STN8 phosphorylates core polypeptides of PSII and thereby affects thylakoid assembly and the repair cycle of PSII. PHOTOSYSTEM II CORE PHOSPHATASE (PBCP) dephosphorylates, e.g., CP43, D1, D2, and PsbH of PSII proteins and antagonizes STN8, while PPH1/TAP38 phosphatase is the antagonist of STN7 kinase. Likewise activation by, for example, disulfide reduction, requires a mechanism for oxidation. While reduction processes are well described for decades, the oxidation of dithiols is little understood. Figure 4 presents three thiol switch mechanisms that interfere with signaling. Thiol peroxidases such as peroxiredoxins and glutathione peroxidases react with peroxides with high affinity. The two catalytic thiols, the peroxidatic thiol and the resolving thiol, reduce H₂O₂ to water, and organic peroxides to the

![Diagram](https://via.placeholder.com/150)

**Figure 4:** Mechanisms of reversible thiol switching in chloroplasts. (A) The plastoquinone (PQ) redox state activates the state transition kinases STN7 and STN8. STN8 phosphorylates, for example, the thylakoid protein PsbH. Dephosphorylation is catalyzed by the photosystem II core phosphatase PBCP. (B) Adjustment of peroxiredoxin (Prx) redox state by oxidation with H₂O₂ or organic peroxides and reduction by NTRC or thioredoxin. Here, the sulfenic acid derivative of Cys₉ of the peroxiredoxin oxidizes the thiol of the protein in near proximity. The sulfenic acid on the protein reacts with a second thiol to form a disulfide. (C) Thiol/disulfide control of a target protein (TP) by proximity based oxidation through Prx and reduction by NTRC or thioredoxin. Here, the sulfenic acid derivative of Cys₉ of the peroxiredoxin oxidizes the thiol of the protein in near proximity. The sulfenic acid on the protein reacts with a second thiol to form a disulfide. (D) Oxidation and reduction of a TP by equilibration with the redox transmitter, e.g., NTRC, whose redox state in turn is controlled by NADPH and drainage of electrons into the Prx pathway.
Thiol switches in oligomeric assemblies and microdomains

The number of described thiol switches in chloroplasts increases rapidly. Interestingly many of the redox regulatory agents are associated with specific macromolecular assemblies or suborganellar structures such as 2-CysPrx with PSII, PrxQ with thylakoid stacks or Trx-z with transcriptionally active chromosomes (TACs) (Arsova et al., 2010). The specific localization of these components indicates functions in defined molecular environment, despite the fact that the strong phenotype of Trx-z-deficient mutants can be complemented with thiol-mutated Trx-z excluding an exclusive redox function of Trx-z (Wimmelbach and Börnke, 2014). Significant heterogeneity in terms of spreading can be expected from different ROS signals. Important factors are the rate of ROS generation, ROS reactivity and half life time and activity of the antioxidant defense system. Singlet oxygen likely acts in the thylakoid membrane and initiates oxylipin signaling, which in turn activates Cys synthesis by oxophytodienoic acid (OPDA) binding to cyclophilin Cyp20-3 (Park et al., 2013). The Cyp20-3/OPDA complex binds the cysteine synthase complex consisting of OAS thiol lyase and serine acetyltransferase and is suggested to stimulate OAS synthesis as precursor of cysteine synthesis. Superoxide and H₂O₂ can diffuse, but their concentration is controlled by efficient antioxidant defense systems (Figure 5). Thus, if thylakoid-bound superoxide dismutases, ascorbate peroxidases and thiol peroxidases are active, O₂⁻ and H₂O₂ only

Figure 5: Sources and gradients in redox stimulus propagation and signaling.

The schematics depict a chloroplast with a stack of thylakoid membranes as site of photosynthetic electron transport. ROS gradients are labeled as red areas and show singlet oxygen initiating lipid signaling, a site of efficient antioxidant defense (upper part) where ferredoxin- (Fd) and plastoquinone (PQ)-dependent redox signaling affect thiol regulation and protein kinase activity. A site of weakened antioxidant defense (lower part) allows for spreading of ROS and reaction with ROS sensors which then trigger ROS-related signaling.
can act locally similar as discussed for retrograde signaling by Baier and Dietz (2005). If the antioxidant defense is weakened, then ROS signals may spread and even leave the chloroplast. It has been proposed that the antioxidant activity is regulated. Such mechanisms would allow for control of local signaling in macromolecular assemblies on the scale of nanometers vs. more distant signaling on the submicrometer to micrometer scale by diffusion (Heyno et al., 2014). Buffering of ROS and redox disequilibrium is not only achieved by the enzymatic and non-enzymatic antioxidants, but also by the huge concentration of protein thiols, which exceeds the glutathione pool more than 10-fold (Muthuramalingam et al., 2013). Ribulose-1,5-bisphosphate carboxylase/oxygenase is the predominant protein in the stroma with estimated concentrations of 0.5 mM. Considering the hexadecameric structure with eight large (9 Cys) and eight small subunits (4 Cys) the potential contribution of Rubisco Cys amounts to 52 mM (Muthuramalingam et al., 2013). Many RibuloseCYS Cys have been identified as targets of posttranslational modifications with effects on activity and turnover (Moreno et al., 2008).

**Redox control of chloroplast pathways and activities**

Tetrapyrrole biosynthesis, chloroplast transcription and translation may serve as three examples where knowledge on redox regulation and thiol switches in chloroplasts has advanced tremendously in recent years. NTRC has emerged as a central player in chloroplast thiol redox homeostasis (Toivola et al., 2013). NTRC contains the functional domain of a thioredoxin reductase and a thioredoxin domain. Genetic ntrc:GFP fusions distribute equally in the functional domain of a thioredoxin reductase and a thioredoxin domain. Genetic NTRC:GFP fusions distribute equally in the chloroplast (Kirchsteiger et al., 2012). ntrc-knock out *A. thaliana* plants grow slowly and display a chlorotic phenotype. Complementation with an NTRC containing the functional thioredoxin reductase domain but inactive Trx domain restored growth indicating that the TR domain likely interacts with other chloroplast Trx (Toivola et al., 2013). NTRC controls components of tetrapyrrole biosynthesis (Richter et al., 2013). Detailed analysis of metabolite levels and enzyme activities of tetrapyrrole biosynthesis in *ntrc* lines provides evidence that NTRC controls the activity of glutamyl-transfer RNA reductase 1 and MgP methyltransferase by thiol switching (Richter et al., 2013). Additional steps of the tetrapyrrole pathway like Glu-1-semialdehyde aminotransferase (GSAT) or 5-aminolevulinic acid dehydratase (ALAD) are suggested to be under control of the ferredoxin-thioredoxin pathway (see review by Richter and Grimm, 2013). The precise mechanisms including involved thiol switches still need to be investigated in detail.

Transcriptionally active chromosomes (TAC) represent DNA-protein assemblies of high complexity with more than 30 different constituents including RNA polymerase (Pfalz et al., 2006). Some components are linked to redox and ROS such as Fe superoxide dismutase 3 (FSD3) and possibly Trx-z as still has to be shown (Arsova et al., 2010; see above). Recently, AtECB1/MRL7, was identified as another component of the TAC complex. It possesses a typical thioredoxin protein fold and exhibits activity of disulfide reductase. The albino phenotype of *ecb1*-knock out plants indicates its function in plastid gene expression and chloroplast biogenesis (Yua et al., 2014). The mutant plants lack immunodetectable amounts of photosynthetic proteins such as LSU of ribulose-1,5-bisphosphate carboxylase/oxygenase, PsAD, PetC and PsBA. Reliable molecular interaction of AtECB1 with Trx-z and FSD3 were taken as circumstantial evidence that AtECB1 might regulate plastid transcription by redox-related mechanisms (Yua et al., 2014). WHIRLY1 and possibly WHIRLY3 (WHY1, WHY3) are components of the plastid nucleoid and were suggested to function as major scaffold for nucleoid assembly (Krupinska et al., 2014). WHY1 and WHY3 have conserved Cys residues that could regulate WHY-function. WHY3 was identified as redox-sensitive target in a redox proteomic approach (Ströher and Dietz, 2008). Interestingly WHY 1 is unique because it is the only transcription factor described so far that is dually localized to the plastids and nucleus and thus may serve as retrograde signal. It will be interesting to determine the significance of redox regulation for WHIRLY distribution and function.

Redox regulation of chloroplast translation allows for rapid adjustment of protein synthesis to the demand for their assembly in photosynthetic complexes and of ribulose-1,5-bisphosphate carboxylase/oxygenase (Marín-Navarro et al., 2007). In yeast extracts prepared for *in vitro* translation studies, addition of thioredoxin or glutaredoxin in combination with dithiothreitol enhanced synthesis of luciferase mRNA (Jun et al., 2009). Likewise translation of *PsbA*-mRNA in illuminated chloroplasts was stimulated by light. The light stimulus activated translation through the ferredoxin-thioredoxin system (Trebitsh and Danon, 2001). Recently, the protein disulfide isomerase AtPDI6 could be localized to the chloroplast (Wittenberg et al., 2014). *AtPdi6*-plants when exposed to a 10-fold light increase showed less photoinhibition and higher rates of D1-protein turnover. The authors suggest that AtPDI6 may act as attenuator of translation, controlling D1 synthesis in response to light.
and redox cues (Wittenberg et al., 2014). Thus mounting evidence ties translation into the redox regulatory network of the chloroplast.

Conclusions

Thiol switches are fundamental part of cell redox regulation. The photosynthesizing chloroplast experiences significant redox changes. Considering the structure of the redox regulatory thiol-network consisting of redox input elements, redox transmitters, redox targets and redox sensors the following scenario needs to be considered: metabolism including PET chain feeds electrons via the redox input elements to the targets. Thus electron input by increasing electron pressure reduces targets. Conversely, ROS act as final electron acceptors (Dietz, 2008). The balance between electron input and electron drainage defines the redox state of the targets and controls metabolism, transcription, translation or export. Both, decreasing the reductive pressure or increasing the oxidative drainage of electrons will have similar effects. As described in this review, only part of redox regulation and thiol switching occurs via the bulk phase. As alternative mechanism, redox information is exploited in microdomains and supramolecular assemblies. To fully assess the significance of the thiol/disulfide redox proteome for chloroplast function, much additional ex vivo work will have to link environmental conditions and metabolic state to specific patterns of the redox proteome.

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