Novel Regulators in Photosynthetic Redox Control of Plant Metabolism and Gene Expression[1]

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Reduction-oxidation (redox) reactions are an essential part of cell metabolism and represent a major fraction of all catabolic and anabolic reactions. Their dominant characteristic is that they generate and consume compounds with in part highly negative redox potential. Redox reactions occur at many sites in the cell, e.g. in membranes such as thylakoids, plastid envelope, and plasma membrane and in aqueous cell phases such as the stroma, thylakoid lumen, and cytosol. Electron transport systems in cell membranes, particularly in the photosynthetic and respiratory electron transport chains, employ diverse redox cofactors such as iron-sulfur (FeS) clusters and quinones and also excitable systems in photosynthesis that all can generate reactive oxygen species (ROS). The redox state of the aqueous phase is dominated by soluble redox metabolites, which include NAD(P)H, glutathione, and e.g. metabolite pairs such as malate and oxaloacetate, and in addition thiol/disulfide proteins (Foyer and Noctor, 2009). The redox potential of a compound is a relative attribute and defines its propensity to donate electrons to another compound within a given redox couple. The process of electron transfer is directed from the compound with more to that with the less negative redox potential. By this means redox reactions largely determine the thermodynamics of the energetic fluxes in living cells. However, the electron transfer within a redox couple needs to be strictly controlled to avoid the unintended electron transfer to other substrates with a relative positive redox potential. Oxygen represents such a compound and electron transfer to it can generate potentially harmful ROS.

To balance redox metabolism and minimize ROS or reactive nitrogen species (RNS) formation, cells operate a redox signaling network. The network senses environmentally induced redox imbalances and initiates compensatory responses either to readjust redox homeostasis or to repair oxidative damage. Basically, the network consists of redox input elements, redox transmitters, redox targets, and redox sensors (Dietz, 2008). The basic structure and many components of the thiol-disulfide redox regulatory network are conserved among all cells and most cell compartments. The significance of this network is well established for some pathways, but still emergent for additional functions due to the ongoing identification of novel redox targets. Lindahl and Kieselbach provided a comprehensive inventory of the experimentally identified disulfide proteomes of the chloroplast (Lindahl and Kieselbach, 2009). As part of the *Plant Physiology* Focus Issue on Plastid Biology, this Update focuses on plastid redox regulation as an example for the basic principles of redox regulation in metabolism. In addition, the function of recently identified new players in plastid redox regulation is described.

SUPPLY OF REDUCTION POWER BY PHOTOSYNTHETIC LIGHT REACTIONS AND ITS DISTRIBUTION

All reducing power in plant cells ultimately originates from the light-driven electron transfer from water to $NADP^+$, which is performed by the photosynthetic apparatus in the thylakoid membrane system of chloroplasts (Fig. 1). In linear electron transport, the reaction center of PSII is excited, creating a high energetic potential form of it (P680 → P680*). This is strong enough to initiate a charge separation that allows an electron to move via pheophytin to the first stable electron acceptor Q_A, a plastoquinone (PQ) bound to the PSII subunit D2. The resulting electron gap within the reaction center is closed by electrons from water delivered by the manganese cluster of the water-splitting complex. As the by-product of this reaction, molecular oxygen (O_2) is released. The electrons of Q_A are then transferred to a second PQ (Q_B) bound to the reaction center protein D1. After receiving two electrons the reduced PQ (PQH_2) is released from PSII and carries them to another membrane complex, the cytochrome b_6f complex (Cytb_6f). Here, PQH_2 becomes oxidized. This is the slowest step within photosynthetic electron transport turning the PQ pool into an ideal sensor for unbalanced excitation of the two photosystems. The electrons are transferred by the Cytb_6f complex to a lumenal electron carrier, plastocyanin, which transports the electrons to PSI. In PSI the electrons are excited to a reduction potential sufficient to reduce (via a number of redox steps) ferredoxin (Fd) on the stromal side of PSI. From here most electrons are transferred by the enzyme Fd-NADP-oxido-reductase to $NADP^+$ generating NADPH + H^+. By this means the electrons cross a

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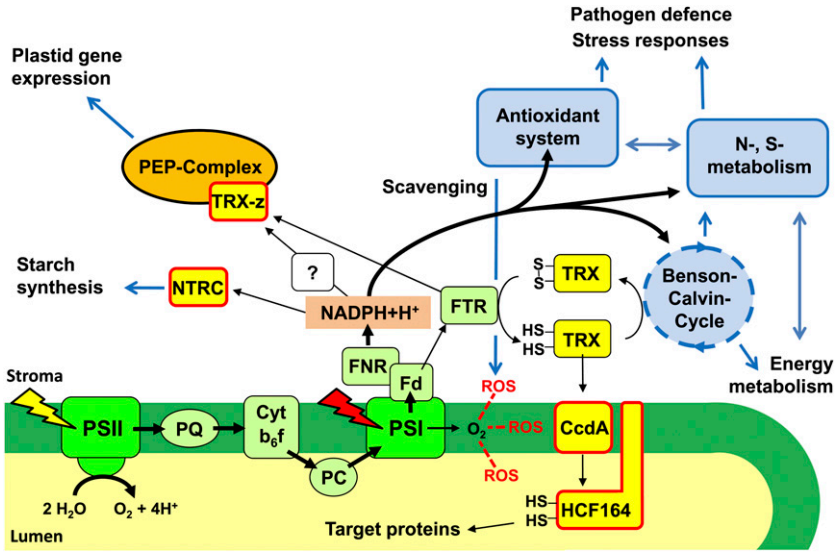

Figure 1. Redox chemistry of photosynthetic electron transport chain and associated redox regulators. The sketch displays thylakoid membrane compartments, intrinsic and extrinsic protein complexes of the photosynthetic electron transport chain, and associated redox mediators (green) and various redox transmitters (yellow; red contours mark novel ones described in the text in more detail) coupled to it. The yellow and red flashes indicate light-dependent charge separation in the reaction centers of PSII and PSI as well as their different absorption maxima (680 and 700 nm, respectively). Thick black arrows represent the main electron flow from water to NADP and the subsequent cellular processes (blue). Thin black arrows indicate the flow of a minor proportion of electrons used for regulatory redox reactions. Blue arrows mark the influence of regulators on distinct cellular processes as well as potential interactions. For detailed explanations, see the text.

redox potential difference of about 1.13 V in total that is strong enough to fuel all subsequent redox-dependent reactions in the cell.

The reducing power delivered from the electron transport chain is distributed to mainly three categories of processes that are connected to each other. These are (1) anabolic reactions of metabolism, (2) the antioxidant systems, and (3) redox regulatory systems.

In metabolism the reduction equivalent NADPH is often directly used as cofactor in enzymatic reactions, mainly in anabolic reactions synthesizing molecules of higher complexity or energetic content, for instance carbohydrates in the Benson-Calvin cycle or reduced intermediates in sulfur or nitrogen metabolism. Reduced substrates can be used to generate reduction equivalents in the dark or in nonphotosynthetic tissues, thereby allowing the plant to uncouple redox-dependent reactions from a direct connection to the light reactions of photosynthesis (see below).

Reduction of soluble antioxidants such as ascorbate or glutathione is used to defend cellular components against oxidative damage by ROS. These are unavoidable by-products of oxygenic photosynthesis and are generated by uncontrolled electron leakage mainly at PSI. ROS can generate deleterious effects and must be detoxified. Thereby, ROS function as important sinks for reducing power. But ROS also perform important signaling functions and trigger cellular processes including stress responses, pathogen defense, and targeted cell death (Apel and Hirt, 2004; Foyer and Noctor, 2005; Mullineaux and Baker, 2010) that represent important research fields in plant science of its own, however, due to space constraints are not covered in this Update here.

The redox regulatory system of a plant cell appears to be the most complex one among the three given categories of redox metabolism, antioxidant defense, and redox regulation. The redox system consists of a great number of various components that generate a hierarchical and highly interconnected network. The components and their relationships and functional connections are described in more detail in the following section.

THE STRUCTURE OF THE REDOX REGULATORY DITHIOL-DISULFIDE NETWORK

Four main routes of redox regulation exist in chloroplasts, namely via (1) Fd directly, (2) NADPH, (3) thioredoxin (Trx) system, and (4) glutathione/glutaredoxin (Grx; Fig. 2; Dietz, 2008). Trxs are reduced by a specific enzyme, the Fd-Trx-oxido-reductase (FTR) catalyzing the reduction of a conserved dithiol group in the Trx that is then used by the Trx molecule to transfer the reductive power to its specific target protein(s). Thus, they function as redox transmitters. The redox targets also possess intra- or intermolecular dithiol groups and their reduction most commonly leads to the activation of the enzyme. By this means many Benson-Calvin cycle enzymes are activated in the light when the photosynthetic light reaction is

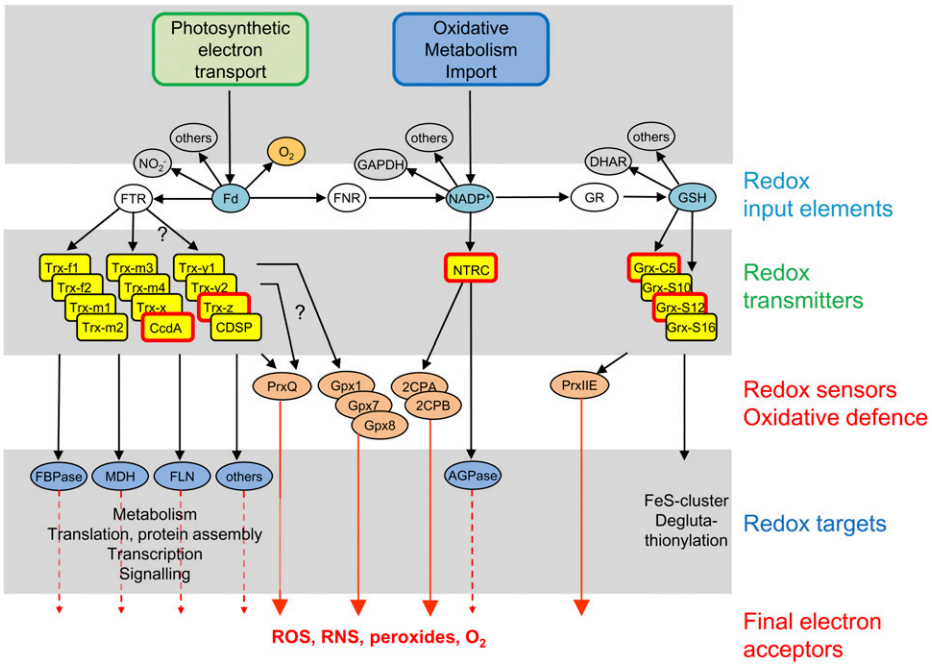

Figure 2. Schematics of the dithiol-disulfide network of the chloroplast. The top layer depicts the supply pathways feeding electrons to the input elements Fd, NADPH, and GSH. In competition with other electron-draining reactions these input elements donate electrons to a set of redox transmitters. These adjust the redox state of a large set of target proteins, here represented by FBPase, malate dehydrogenase (MDH), FLN, and AGPase or donate electrons to thiol-dependent peroxidases (Prx, glutathione peroxidase [Gpx]). ROS, RNS, peroxides, and O_2 serve as final electron acceptors maintaining electron flux through the network by reoxidizing protein thiols. Some Grx function in deglutathionylation, FeS-cluster assembly/delivery, or as redox sensors and seem to lack disulfide reduction activity.

running while they are inactivated in the dark. Glutathione reductase links reduction of oxidized glutathione to NADPH oxidation to yield reduced glutathione (GSH). Each of the four pathways fuels, targets, or regulates specific processes. Recently, novel players in the plastid redox regulatory network were identified, namely NADPH-dependent Trx reductase C (NTRC) in metabolic control and antioxidant defense (Serrato et al., 2004; Michalska et al., 2009), Trx-z in chloroplast development and transcription (Arsova et al., 2010; Schröter et al., 2010), and Grxs in disulfide reduction, deglutathionylation, and FeS-cluster assembly (Rouhier, 2010). The plastid Trx and Grx families in Arabidopsis (*Arabidopsis thaliana*) are comprised of 11 and four members, respectively, namely Trxf1, f2, m1 to m4, x, y1, y2, z, and CDSP32 (Meyer et al., 2005), and GrxC5, S12, S14, and S16 (Rouhier, 2010). The redox network basically represents an electron flow system where photosynthesis or nonphotosynthetic metabolic reactions provide electrons to input elements such as Fd, NADPH, and GSH. Transmitters mediate electron transfer from input elements to the targets or fulfill special functions. Electrons finally are drained by ROS, RNS, peroxides, and O_2. Generation of ROS is stimulated upon metabolic imbalances that usually are induced by sudden environmental changes. Unquenched excited states of chlorophyll and overreduction of specific energetic redox couples may cause ROS production as described above. In chloroplasts high-reduction states of the PQ pool, Fd, and NADP systems indicate excess excitation energy and thus an imbalance between energy supply and demand. Under such conditions singlet oxygen, O_2^-, and hydrogen peroxide are released. Within the network O_2 and ROS maintain electron flow by either controlled reaction with peroxiredoxin (Prx) or glutathione peroxidase proteins or in an uncontrolled reaction by oxidizing sensitive protein thiols that in many cases display regulatory function. Well-known examples are the thiol-disulfide activation of Benson-Calvin cycle enzymes such as Fru-1,6-bisphosphatase (FBPase) and the reductive activation of the malate valve (Scheibe et al., 2005). It is important to note that in addition to photosynthetic electron transport, other routes also provide reductive power to plastids and the thiol/disulfide network, e.g. the oxidative pentose phosphate pathway and the malate/oxaloacetate shuttle (malate valve) across the plastid envelope. These pathways are especially important at night and in nonphotosynthetic plastids. The oxidative pentose phosphate pathway operates within chloroplasts and, in principle, reverses the reductive pentose phosphate pathway. Since both pathways share essential metabolites and even a number of enzymes this would create a futile cycle. Regulation via reduced Trx prevents such a waste of energy by activation of FBPase and seduheptulose-bisphosphatase in the reductive cycle and parallel inactivation of the Glc-6-P dehydrogenase in the oxidative cycle. This directs FBP into the reductive cycle in the light. In the dark Trx becomes oxidized and the opposite situation becomes predominant. By this means the reduction state of Trx creates a conditional separation of metabolic fluxes within the same compartment. It should be noted that the dominant Trx regulation is supported by fine-tuning mechanisms including other parameters such as ATP/ADP ratio, Mg^{2+} ion, and pH gradients as well as the NADPH/NADP ratio.

NOVEL TARGETS OF REDOX REGULATION IN PLASTID METABOLISM

Proteomics-Based Identification of Novel Targets

Redox proteomics is an emerging technology aimed at defining the redox protein inventory of the cells and cell compartments and analyzing the redox state of target proteins on a broad scale. Both gel- and chromatography-based redox protein screening systems have been applied to plant and chloroplast protein fractions and resulted in lists of thylakoid lumenal, stromal, and chloroplast membrane-bound candidate redox proteins that undergo thiol modifications, most commonly dithiol-disulfide transitions (Meyer et al., 2005; Rouhier et al., 2005; Ströher and Dietz, 2008; Lindahl and Kieselbach, 2009; Hall et al., 2010). With increasing sensitivity these approaches allow for proteome-wide identification of proteins potentially subjected to thiol-disulfide or nitrosothiol transitions in vivo but face some drawbacks: (1) Low abundance proteins still are underrepresented in the target lists, thus mainly dominant and metabolic proteins are identified, while regulatory proteins escape from the identification. (2) Despite specific methods such as Trx and Grx trapping or Trx-dependent reduction of previously oxidized targets, the results from in vitro methods lack specificity and cannot be translated into mechanisms in vivo. (3) The functional significance of intramolecular or intermolecular thiol-disulfide transitions or nitrosylation of proteins needs to be explored in each case by time-intensive biochemical and molecular biological studies. Only in few and selected cases, the experimental verification has been attempted. (4) Information is needed on the critical cell redox conditions that activate the regulatory redox switches. Often, rather extremely reducing, with dithiothreitol (DTT), or oxidizing conditions, with hydrogen peroxide, have been employed to identify redox proteins. To assess the physiological significance, fine-scaled redox buffers must be used to identify the redox environment that activates the redox switch of the redox proteins.

NTRC

FTR accepts electrons from photosynthetic electron transport and donates them to the various Trxs as redox transmitters. In addition to this conventional pathway, NTRC was identified in a genome-wide screen for Trx reductase (NTR)-like proteins that are enzymes found in the cytosol and mitochondrion. The chloroplast NTRC consists of an N-terminal NTR domain with NADPH and FAD binding as well as double Cys active sites and a C-terminal Trx domain. When separately expressed in *Escherichia coli*, both domains reveal the respective activity (Serrato et al., 2004). Homologous genes are found in plants, algae, and some cyanobacteria. The 2-CysPrx was identified as the first substrate that is efficiently reduced by NTRC. Recently ADP-Glc pyrophosphorylase (AGPase) was recognized as another target of NTRC-dependent redox regulation (Michalska et al., 2009). Thus NTRC is a novel player in the thiol-dependent plastid redox network and allows for reduction of disulfides at the expense of NADPH as electron donor and also functions in darkness and nonphotosynthetic plastids. NTRC and Trx activate starch synthesis under reducing conditions (see below).

Redox Regulation of Metabolism

Thiol regulation of Benson-Calvin cycle enzyme activities links light-dependent electron pressure in photosynthetic light reactions to ATP and NADPH consumption in reductive carbohydrate metabolism. The regulatory mechanism may be considered as a prototypic feed-forward activation loop. In fact thiol state-dependent regulation of carbon fluxes through the Benson-Calvin cycle and their link to Trx (Trx-f)-mediated activation of chloroplast FBPase marked the starting point of more than 30 years of successful research on redox regulation in metabolism (Buchanan and Balmer, 2005). In addition to FBPase, sedoheptulose-1,7-bisphosphatase, activities of ribulose-5-P kinase, glyceraldehyde-3-P dehydrogenase, and Rubisco activase are controlled by Trx. Trx-f donates electrons to target proteins that have a broad range of redox midpoint potentials E_m (Hutchison et al., 2000). Differential inactivation of target proteins, e.g. in the Calvin cycle, is unrelated to the value of E_m but highly relevant for photoinhibition under nonoptimal environmental conditions such as chilling temperatures (Hutchison et al., 2000). This complexity is partly explained by the fact that thiol modulation is tied into additional metabolic control systems, e.g. the presence of Fru-1,6-BP is needed for FBPase thiol activation (Reichert et al., 2000). Two main carbon pathways drain carbon from the Benson-Calvin cycle, namely Suc synthesis following export of triose phosphate to the cytosol and starch synthesis in the plastids. The committed step of starch synthesis is catalyzed by AGPase (Fig. 3). AGPase is activated by reduction of a disulfide bridge between the two slightly smaller subunits of the tetrameric holoenzyme in vitro (Ballicora et al., 2000) and in vivo (Tiessen et al., 2002). Reduction is achieved by Trx-f and Trx-m in vitro and allows for a 4-fold stimulation of ADP-Glc synthesis (Ballicora et al., 2000). A good correlation exists between Suc concentration, reduction state of the chloroplast, and starch synthesis (Tiessen et al., 2002; Geigenberger et al., 2005). NTRC also reductively activates AGPase. NTRC-deficient Arabidopsis show less redox-dependent stimulation of AGPase activity and lower starch synthesis rates in high light and upon external feeding of Suc. Inhibition in *ntrc* knockout plants ranges between 40% and 60% in leaf chloroplasts and reaches 90% in nonphotosynthetic amyloplasts (Michalska et al., 2009).

In addition of redox regulation in carbohydrate metabolism, proteomic and biochemical data indicate that thiol modifications also control other major metabolic pathways such as nitrogen assimilation, tetrapyrrole

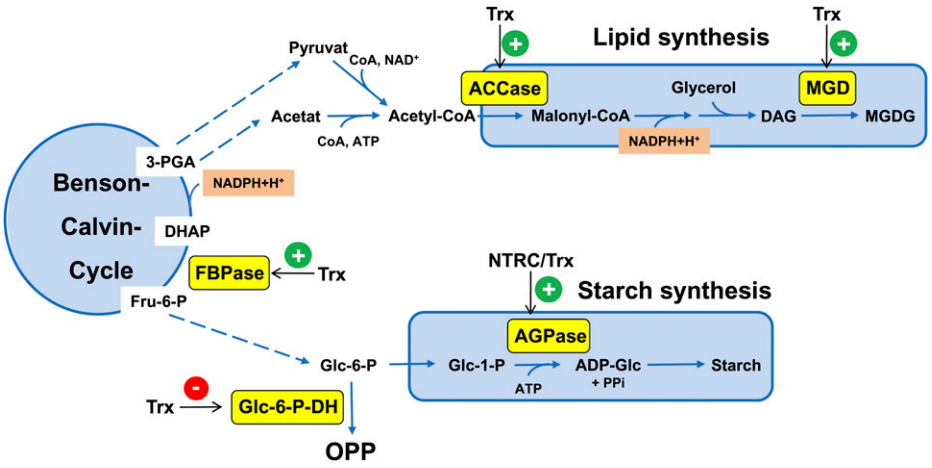

Figure 3. Redox regulation in plastid starch and lipid synthesis. AGPase as committed step of starch synthesis, and ACCase and MGD in plastid lipid synthesis are activated by reduction of regulatory thiols, either by Trx or in case of AGPase by NTRC as described in the text in more detail.

synthesis, and lipid synthesis (see above; Lindahl and Kieselbach, 2009). Here we only discuss recent advance in understanding regulation of lipid metabolism.

Lipid synthesis that occurs in the plastids is a strong sink for electrons. Synthesis of palmitic acid (C16) from acetyl-CoA requires 14 molecules of NADPH and seven molecules of ATP. Plastid redox state affects lipid metabolism (Fig. 3). Acetyl-CoA carboxylase (ACCase) catalyzes the committed step of malonyl-CoA production in plastid lipid synthesis. Isolated ACCase in vitro is inactive without reductant and activated after addition of DTT or reduced Trx-f1 or Trx-m (Sasaki et al., 1997). Reductive activation is supported by pH shift to alkalinization and by increasing Mg^{2+} concentrations. The chloroplast ACCase consists of four polypeptides, the biotin carboxylase, biotin carboxyl carrier protein, transcarboxylase α-subunit, and transcarboxylase β-subunit with three, one, two, and five Cys residues, respectively (Sasaki et al., 1997). One of the α- or β-subunits is suggested to mediate the redox regulation (Kozaki and Sasaki, 1999). Biotin carboxyl carrier subunit of ACCase in *Chlamydomonas reinhardtii* is subjected to *S*-thiolation with glutathione (Michelet et al., 2008). Biotin carboxylase is target of glutathionylation in Arabidopsis cell culture (Dixon et al., 2005). Thus, each of the subunits of ACCase is potentially controlled by redox regulation using diverse mechanisms. This fact underlines the link between redox state and lipid metabolism. Envelope-bound monogalactosyldiacylglycerol synthase (MGD) synthesizes monogalactosyldiacylglycerol from diacylglycerol and UDP-Gal. Monogalactosyldiacylglycerol is a major lipid component of chloroplasts. In vitro MGD activity depends on the presence of reductants such as DTT, is inhibited by thiol-alkylating agents, and is modulated by Trx acting on intramolecular disulfide bonds (Yamaryo et al., 2006). Plant MGD possesses nine conserved Cys residues. Its regulation by thiol redox state is suggested to enable galactolipid synthesis along with photosynthetic activity and to foster replacement of eventually oxidized lipids under conditions that cause oxidative stress (Yamaryo et al., 2006).

Control of Lumenal Redox Environment

Redox information is intensively used in metabolic regulation in the stroma. Recent evidence reveals that also lumenal proteins undergo dithiol-disulfide transitions and that lumenal redox processes are critical for normal development. Thus, a set of 15 lumenal proteins has been found in the compilation from published data of the chloroplast disulfide proteome (Lindahl and Kieselbach, 2009). The *hcf164* mutant was identified in a screen for high chlorophyll fluorescence phenotype. HCF164 encodes a lumenal Trx-like protein involved in functional assembly of Cytb_6f complex (Lennartz et al., 2001) and targets proteins such as subunit N of PSI (PsaN). Thylakoid-bound cytochrome c defective A (CcdA) is a homolog of prokaryotic thiol-disulfide transporters and required for efficient electron transfer from the stroma to the lumen (Motohashi and Hisabori, 2010). The tentative model suggests that thylakoid-associated CcdA receives electrons from stromal Trx-m and donates them to HCF164 that in turn reduces target proteins. Electron transfer processes are not only involved in complex assembly but also in lumenal antioxidant defense since the Prx Q that is a thiol-dependent peroxidase was at least partly assigned to the lumenal proteome (Petersson et al., 2006). Prxs detoxify a broad range of peroxide substrates but must be regenerated by thiol donors in each catalytic cycle. Other lumenal thiol targets include the components of the water-splitting complex PsbO1, O2, P1, and Q, peptidyl prolyl cis/trans isomerases of the FKBP type (FK506 binding protein) and the Deg1 protease (Ströher and Dietz, 2008; Lindahl and Kieselbach, 2009).

Adjustment of Plastid and Leaf Cell Metabolism by Redox Stimuli

Exogenous and endogenous factors such as light quantity and quality, CO_2 availability in combination with low or high temperature, and other stresses modify the balance between energy supply and demand, alter

cellular and chloroplast redox milieu, and affect the state of the dithiol/disulfide redox network. The significance of redox-dependent readjustment of global metabolism under nonstressed conditions has been revealed by two types of experiments. Kolbe et al. (2006) manipulated the redox state of Arabidopsis leaf discs by applying the reductant DTT in the light. A combination of transcript profiling, metabolome, and ^{14}C-flux analyses revealed a profound redirection of metabolism upon shifting from control conditions in light to light plus DTT. Absolute figures of ^{14}C-Glc uptake and flux into Suc decreased by 25% and 56%, respectively. In contrast synthesis of cell wall constituents increased to 860% that of proteins to 430%, amino acids 280%, starch 260%, and organic acid 230% (Fig. 4). The changes in flux were accompanied by congruent changes in metabolite levels. Metabolites of central carbohydrate metabolism decreased while end products such as the amino acids Asn, Cys, Ile, Pro, Tyr, and Val accumulated (Kolbe et al., 2006). Redox proteomics has identified several enzymes of amino acid synthesis as potential targets of Trx-dependent regulation, e.g. Ala aminotransferase, Asp aminotransferase, argininosuccinate synthase, dihydroxyacid dehydratase, ketolactid reductoisomerase, branched chain ketoacid decarboxylase, 3-isopropylmalate dehyrogenase, and Cys synthase (Lindahl and Kieselbach, 2009).

In a physiological approach it could be demonstrated that such readjustments of metabolism are of direct relevance for plant responses to environmental cues. Arabidopsis plants grown on soil were subjected to defined light quality shifts known to generate distinct redox signals within the PQ pool by uneven excitation of the two photosystems. The acclimation responses of the plants that counterbalance this uneven excitation were observed by transcriptomics and metabolomics as well as further physiological experiments including the acclimation mutant *stn7* (Fey et al., 2005; Wagner et al., 2008; Bräutigam et al., 2009). Besides the expected structural and functional reconfiguration of the photosynthetic apparatus a strong impact on metabolism genes and metabolites was observed. Bioinformatic analyses uncovered that as part of the light acclimation response the plants redirect their metabolism between two distinct states and that these have great importance for plant growth efficiency. One metabolic state is characterized by decreases in primary photosynthetic metabolite levels and increases in important intermediates of subsequent metabolic pathways, while the second metabolic state is characterized by a down-regulation of many subsequent metabolites, including amino acids and organic acids. Thus, environmentally induced redox signals within the photosynthetic electron transport chain trigger two adjustment loops that coordinate metabolic states and/or fluxes with the efficiency of the photosynthetic light reaction. This is in line with the above-mentioned study and underlines the importance of redox signaling networks for the global adjustment of plant metabolism.

REDOX REGULATION OF PLASTID GENE TRANSCRIPTION

Plastids possess their own genome, the plastome, and a complete machinery to express the genetic information on it. Although this plastome encodes just approximately 120 genes (in vascular plants) the expression mechanisms appear to be rather complex and highly regulated. This includes a number of redox control mechanisms that influence regulatory proteins at all important levels of gene expression, i.e. transcription, posttranscriptional mechanisms, and translation initiation (Pfannschmidt and Liere, 2005).

A major target of photosynthetic redox signals is the plastid-encoded RNA polymerase (PEP; Fig. 5). Unbalanced excitation of the two photosystems generates either a reduced or oxidized PQ pool that act as signals that control the phosphorylation of the light-harvesting complexes of PSII via the thylakoid-associated kinase STN7 (Lemeille and Rochaix, 2010; Pesaresi et al., 2010). The same signals also trigger a phosphorylation cascade toward the PEP enzyme that results in changes of

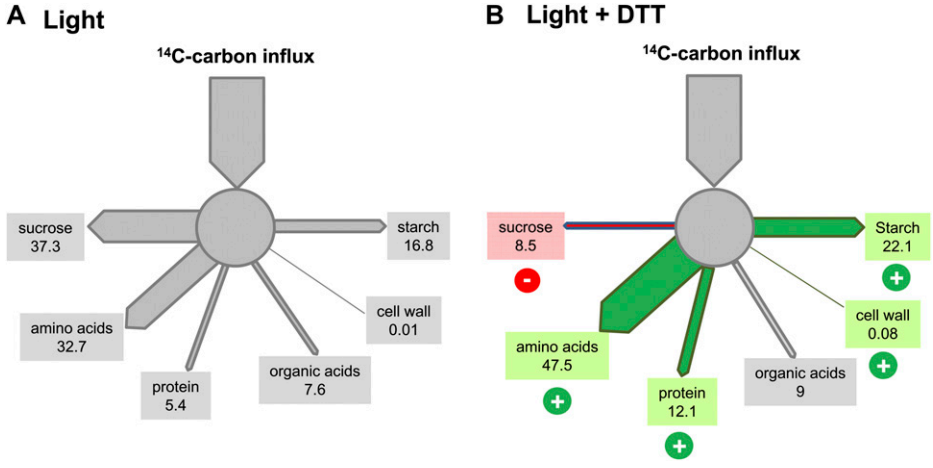

Figure 4. Redirection of leaf metabolism under highly reducing conditions. Leaf discs were illuminated in the absence or presence of the reductant DTT and supplied with ^{14}C-Glc. Distribution of ^{14}C label in different metabolite pools was investigated after 1 h (Kolbe et al., 2006). The diagrams compare the distribution patterns under control conditions (A) and after DTT treatment (B). The numbers give the relative distribution in percent of total. Green, Up-regulation; red, down-regulation.

photosynthesis gene expression (Allen and Pfannschmidt, 2000). Both processes aim to counteract the unbalanced excitation to maintain photosynthesis efficiency as high as possible. The phosphorylation cascade likely includes the action of a number of further kinases (STN8, an ortholog of STN7; CSK, the chloroplast sensor kinase; PTK, the plastid transcription kinase), generating a phosphorylation network. In a simplified view the reduction of the PQ pool activates STN7, which provides an input signal for the subsequent kinase network. This controls the phosphorylation state of the sigma factor Sig1 that in turn regulates the relative transcription of the photosynthesis reaction center genes *psbA* (encoding the D1 protein of PSII) and *psaA/B* (encoding the P700 apoprotein of PSI; Shimizu et al., 2010). This view coincides with the observation that CSK, PTK, and Sig1 were able to interact with each other in the yeast (*Saccharomyces cerevisiae*) two-hybrid system (Puthiyaveetil et al., 2010). In organello transcription experiments in presence of kinase inhibitors and/or the reductant DTT, however, indicated that this phosphorylation-dependent signal interacts with a second, thiol-dependent signal (Steiner et al., 2009). PTK, a casein-kinase 2 type enzyme has been reported to be under control of the redox state of glutathione (Ogrzewalla et al., 2002), but its activity could not be modulated with DTT. This suggested the involvement of a further regulator. Recently, two independent studies identified a novel Trx-like protein that likely represents this additional player (Arsova et al., 2010; Schröter et al., 2010). The novel Trx was named Trx-z because of its distinct evolutionary position in relation to Trx-x and Trx-y. In a yeast two-hybrid screen it was identified as interacting protein of two chloroplast-located phosphofructokinase-like proteins called FLN1 and FLN2. The Arabidopsis knockout mutant line of Trx-z exhibited pale-white leaves and was viable only on Suc-supplemented medium, a unique phenotype since the Trx system is highly redundant and can easily compensate for the loss of single components. Gene expression analyses indicated the same plastid gene expression profiles as in PEP-deficient mutants, pointing to an important role of Trx-z in plastid development and gene expression (Arsova et al., 2010). These observations were complemented by mass spectrometry results that demonstrated that both the Trx-like protein and the FLN2 kinase are intrinsic subunits of the PEP enzyme of chloroplasts (Schröter et al., 2010). This provides an immediate explanation for the phenotype and the expression profiles in the knockout mutant. A lack in Trx-z prevents a proper assembly of the PEP enzyme and, consequently, the developmental transition from the nuclear-encoded RNA polymerase-driven transcription to the PEP-dependent transcription does not take place. The precise functional role of Trx-z within the PEP complex, its relation to FLN1 and FLN2, as well as its regulatory impact remains to be

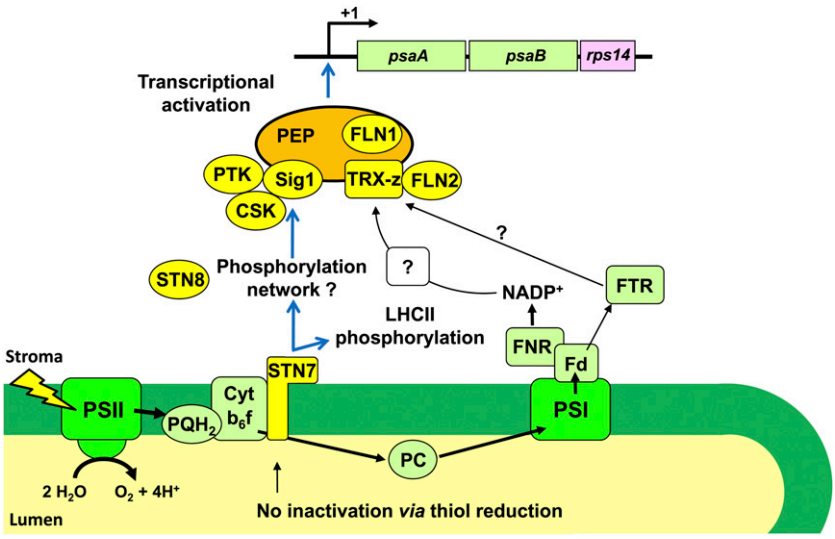

Figure 5. Protein factors and signaling pathways involved in photosynthetic redox control of plastid transcription. The photosynthetic electron transport chain and its protein complexes are depicted as in Figure 1. The sketch refers to light conditions which preferentially excite PSII. Under these conditions the PQ pool becomes reduced, hence, activating the thylakoid-bound kinase STN7. This kinase is oppositely regulated at its lumenal side by a reduction signal from the Trx system that is not active under these conditions (Bräutigam et al., 2009). STN7 provides an input signal that presumably is integrated within a subsequent phosphorylation network targeted to the control of the PEP. PEP-associated proteins (PAPs) are given in yellow and described in text. One group of PAPs is mediating the phosphorylation signal, another one appears to integrate a second signal originating from the Trx system. Both signals together contribute to the transcriptional activation of the *psaA/B* operon (a mixed operon encoding a ribosomal subunit at its end [*rps14*, given in light pink]). The pathway by which Trx-z is reduced is unknown to date (question mark).

elucidated. Furthermore, it is completely open how it relates to the other known redox regulators mentioned above. In summary, our understanding of photosynthetic redox signal transduction toward the level of gene expression is still at the beginning. The increasing number of identified regulatory components unravels step by step a complex mechanistic redox tool box enabling chloroplasts to respond to a wide range of environmental conditions in a dynamic and flexible manner.

CONCLUSION AND OUTLOOK

The cellular redox environment has global significance in regulating most plastid processes, namely carbohydrate, lipid, amino acid, and tetrapyrrole metabolism as well as gene transcription, protein synthesis, and also e.g. protein import via modulating the activity of the translocons of the inner and outer chloroplast membranes (TIC, TOC; Balsera et al., 2010). Tentative experimental evidence and theoretical considerations (Fig. 2) suggest that redox regulation in plastids is a tightly interlinked phenomenon. However, it is still characterized by dispersed knowledge on (1) redox effects on only single processes that have been characterized in detail, (2) unsurpassed complexity of involved players particularly redox transmitters, (3) limited knowledge on linkages among the components, and (4) poor quantitative understanding of network function. These shortcomings direct us to future research: The predictions on redox targets from proteomics approaches need to be addressed by biochemical studies (Ströher and Dietz, 2008; Lindahl and Kieselbach, 2009). Most indicated linkages in the network are still hypothetical, e.g. it is not clear whether FTR is able to reduce all Trxs or how Trx-z as integral component of the transcriptional complex is reduced. The interactions between the network components need to be qualitatively and quantitatively assessed by in vitro and in vivo methods such as isothermal titration calorimetry, kinetic assays, and fluorescence resonance energy transfer similar as done for the chloroplast 2-CysPrx (Barranco-Medina et al., 2008; Muthuramalingam et al., 2009). Models of partial networks and simulations of electron fluxes and redox states will help to test our knowledge and predict regulatory states that can be validated in further experiments (Kemp et al., 2008). In the end this will help to understand how the fluctuating environment is reflected by distinct changes in the cellular redox signaling networks and paves the avenue for a systematic research of plant acclimation in response to environmental challenges.

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