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Chloroplasts as source and target of cellular redox regulation: a discussion on chloroplast redox signals in the context of plant physiology

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Abstract
During the evolution of plants, chloroplasts have lost the exclusive genetic control over redox regulation and antioxidant gene expression. Together with many other genes, all genes encoding antioxidant enzymes and enzymes involved in the biosynthesis of low molecular weight antioxidants were transferred to the nucleus. On the other hand, photosynthesis bears a high risk for photo-oxidative damage. Concomitantly, an intricate network for mutual regulation by anthero- and retrograde signals has emerged to co-ordinate the activities of the different genetic and metabolic compartments. A major focus of recent research in chloroplast regulation addressed the mechanisms of redox sensing and signal transmission, the identification of regulatory targets, and the understanding of adaptation mechanisms. In addition to redox signals communicated through signalling cascades also used in pathogen and wounding responses, specific chloroplast signals control nuclear gene expression. Signalling pathways are triggered by the redox state of the plastoquinone pool, the thioredoxin system, and the acceptor availability at photosystem I, in addition to control by oxolipins, tetrapyrroles, carbohydrates, and abscisic acid. The signalling function is discussed in the context of regulatory circuitries that control the expression of antioxidant enzymes and redox modulators, demonstrating the principal role of chloroplasts as the source and target of redox regulation.

Key words: Abscisic acid, antioxidants, chloroplast, gene expression, oxolipin, peroxiredoxin, photosynthesis, redox regulation, signalling, stress.

Introduction
In plants, photosynthesis generates redox intermediates with extraordinarily negative redox potentials. Light-driven electron transport transfers electrons from the acceptor site of photosystem I ($E_m < -900$ mV) to various acceptors including oxygen ($E_m = 815$ mV; Blankenship, 2002). The redox intermediates cover an exceptionally wide range of mid-point redox potentials (Dietz, 2003) with a significant risk for electron transfer to oxygen and other appropriate targets. Among the best known examples for chloroplast redox chemistry is the direct electron transfer from reduced ferredoxin to O$_2$ in the so-called Mehler reaction (Mehler, 1951). The superoxide radicals formed can be quickly converted into H$_2$O$_2$ and highly reactive hydroxyl radicals (HO') (Elstner, 1990). Together with reactive oxygen species (ROS) generated by other sources, they are a continuous threat to cellular constituents for uncontrolled oxidation.

In the context of (i) the physiologically bivalent oxygen chemistry, (ii) the demand for reductive power, and (iii) the peril of excess photosynthetic electron pressure, chloroplasts are prone to oxidative damage like no other organelle (Foyer et al., 1997). Consequently, photosynthetic organisms have evolved defence mechanisms to control the redox poise of the electron transport chain and the redox environment of the stroma. They range from the suppression of...
ROS generation and avoidance of uncontrolled oxidation of essential biomolecules by accumulating high concentrations of low-molecular weight antioxidants to repair mechanisms by reduction and de novo synthesis of damaged molecules. In the illuminated chloroplast, the reduction energy for the regeneration of oxidized metabolites is mainly taken from the photosynthetic electron transport (PET). Depending on the photo-oxidative strain, up to almost 100% of the photosynthetically transported electrons can be diverted into the antioxidant defence system (Asada, 2000). In the dark, when the strong reductive power of photosynthesis is missing, the redox environment of chloroplasts probably adjusts to redox potentials similar to those measured in the cytoplasm of heterotrophic cells (i.e. below −300 mV to approximately −240 mV; Dietz, 2003). Under these conditions, chloroplasts stabilize their redox poise by metabolism of starch and import of reduction energy.

Studies with various transgenic plant lines and mutants demonstrated the importance of a high antioxidant capacity as realized by low-molecular weight antioxidants (Pastori et al., 2003; Ball et al., 2004) and antioxidant enzymes (Willekens et al., 1997; Baier et al., 2000; Davletova et al., 2005). The antioxidant defence system responds to redox imbalances. The pool size of low molecular weight antioxidants and the expression of chloroplast antioxidant enzymes increases if the photo-oxidative strain on the system is high (Foyer et al., 1997). Interestingly, oxidizing conditions correlate with a high reduction state, since, as outlined above, excess electrons are transferred to O2 that, in turn, abstract electrons from organic targets. This apparent contradiction (‘high reducing activity results in oxidizing conditions’) will be important for understanding redox regulation, as for example in the peroxiredoxin function, in the context of photosynthesis (see below). However, all chloroplast antioxidant enzymes and all enzymes involved in the biosynthesis of low-molecular-weight antioxidants are nuclear encoded. The spatial separation of expression and function demands for signal transduction over the chloroplast envelope. Deviations from regular redox homeostasis can be sensed in the chloroplast and transmitted to the nucleus by retrograde signalling cascades. Alternatively, redox imbalances in the chloroplast could be transmitted into the cytoplasm by metabolic coupling (Fig. 1). The sensor then resides in the cytoplasm. Transmission of redox signals suffers from the drawback that cytoplasmic redox homeostasis and antioxidant defence will strongly damp the spreading signal.

Another terminology to be clarified is the distinction of signalling and regulation. Both terms are used with a broad meaning here. Regulation describes any adjustment of activity, i.e. of enzymes or transcription factors, while signalling refers to any transport of information from one site to another via a signalling molecule that, by itself, may have additional functions, for example, as a metabolic intermediate.

**Redox coupling**

All redox active compounds together define the cellular redox poise of a cell (Schafer and Buettner, 2001). In addition to the redox energetics defined by the mid-point potentials and the concentrations of the various antioxidants, the redox state of a particular compound is under the control of kinetic constraints defining the reaction constants. Enzymes lower the kinetic barriers and couple redox pairs. Recycling of oxidized intermediates, as well as regeneration of the reductants, affect the redox state of specific redox pairs (Dietz, 2003). Different drainage rates of electrons from antioxidants and weak enzymatic linkages can uncouple the redox states of individual components of the antioxidant network, as has been observed for ascorbate and glutathione. While specific oxidation of the glutathione pool took place in catalase-deficient barley (Willekens et al., 1997), reduced 2-Cys peroxiredoxin levels led preferentially to the oxidation of the ascorbate pool in Arabidopsis thaliana (Baier et al., 2000). The favoured oxidation of glutathione in the catalase-lines is proposed to be caused by higher cytosolic dehydroascorbate reductase activity rather than glutathione reductase activity, while the redox shift in the ascorbate pool in the peroxiredoxin antisense plants might be caused by insufficient dehydroascorbate reductase activity in the chloroplasts (Noctor et al., 2000). For cellular redox homeostasis and redox metabolism, the nature of a particular antioxidant employed in a specific detoxification reaction and, within limits, the relative redox state of an
individual antioxidant may be of minor importance. However, deviations from redox normality can serve in signal generation and signal transmission.

**Defining redox signals in the context of photosynthesis**

The nature of the redox signals perceived, the sensory systems, and the classification of the respective responses have received increasing attention in recent years. In plant biology special focus has been given to redox signal transduction in chloroplasts as well as between chloroplasts and the nucleus for experimental and thematic reasons. (i) The metabolic state of chloroplast metabolism can easily be manipulated by altering exogenous parameters such as light, CO₂, and temperature. (ii) Tools such as chlorophyll a fluorescence-based technology to estimate photosynthetic efficiency and redox state, methods at virtually all levels of molecular biology and biochemistry, and a vast accumulated body of knowledge on structure, function, regulation, and assembly facilitate interpretation of the results. (iii) Photosynthesis exerts a strong impact on the cellular redox signature and nuclear gene expression and regulates the photosynthetic capacity during long-term adaptation (Dietz, 2003). However, the hypothesis, on retrograde redox signalling originating inside chloroplasts, is generally accepted and despite these systematic advantages, the precise nature of the signals, their specificity and interaction are only recently becoming tangible.

Theoretical considerations and experimental data have produced a long list of potential signals, of which a selection is discussed here in the context of the regulation of genes involved in the control of the cellular redox poise. Due to the complexity of the possible chemical nature and origin of the signals, defining redox signals depends on the line of vision and the intention. For example, Dietz (2003) grouped redox signals based on their origin and mode of action in signal transmission. According to his definition, type-I signals derive specifically from single pathways, while type-II signals integrate redox information from various pathways; type-III redox signals indicate more extreme redox imbalances and their transmission depends on crosstalk with other signalling cascades. Other definitions for redox signals are based on the threshold of induction relative to photosynthetic electron pressure (Pfannschmidt et al., 2001a) or on their putative initiation sites (Pfannschmidt et al., 2003). For chloroplast-to-nucleus signalling, which depends on passing the chloroplast envelope, a classification based on the chemical nature of the signalling compound leaving the chloroplast has been proposed (Baier and Dietz, 1998). Based on this chemical classification, a concept is followed here in which signals transmitted by redox shifts in cellular redox components or by ROS are distinguished from signals transmitted by second messengers synthesized inside chloroplasts (Fig. 1).

**Sensing the cellular redox poise**

Compelling experimental evidence for redox regulation of nuclear gene expression is available for redox signalling induced by the application of ROS, ROS-inducing stressors, low availability of antioxidants, and by antioxidant feeding, respectively. The common feature of all these treatments is that the cytosolic redox environment specifically or generally is shifted to a more oxidized or reduced state. In the context of signal transmission, special attention has been given to H₂O₂ (Desikan et al., 2001; Mittler et al., 2004; Vandenabeele et al., 2004). Alternatively, monitoring the cellular redox environment by sensing the redox state of certain metabolites, for example, glutathione and ascorbate, has been postulated (Foyer et al., 1997; Dietz, 2003; Pastori et al., 2003; Ball et al., 2004). Both types of metabolites, ROS and low-molecular-weight antioxidants, closely interfere. Consequently, in most experiments it is hardly possible to distinguish between signalling that is possibly originating from H₂O₂ and that from shifts in the redox poise of low-molecular-weight antioxidants. Therefore, they are discussed here together.

Recently cDNA array hybridization experiments were performed and indicate a strong regulatory function of redox signals on nuclear gene expression. For example, Desikan et al. (2001) analysed the response of Arabidopsis thaliana to H₂O₂ application and Mahalingham et al. (2003) to ozone. Pastori et al. (2003) investigated the regulation of transcript amounts in the low-ascorbate mutant vct1 and Vandenabeele et al. (2004) that in a catalase-deficient tobacco line, as well as Davletova et al. (2005) that of a knock-out mutant of cytosolic ascorbate peroxidase APx1, and Ball et al. (2004) determined the transcriptional response of the allelic glutathione biosynthetic mutants rax1-1 and cad2-1. Typical target genes with altered transcript accumulation upon treatment or in the mutants are those for PR proteins, e.g. PR1 (At2g14610), chitinase (At2g43570), and PAL (encoding phenylalanine ammonium lyase; At3g53260; At5g04230), and antioxidant enzymes as peroxidases (e.g. At4g11290 and At4g21960), dehydroascorbate reductase (At1g19570) and CuZn-superoxide dismutase (At5g18100). The sets of target genes widely overlap with the transcripts induced by pathogens and wounding (Mahalingam et al., 2003; Cheong et al., 2002).

The transcription factors involved in these responses were tentatively identified either by being up-regulated at the transcriptional level (Mahalingam et al., 2003; Pastori et al., 2003; Ball et al., 2004; Davletova et al., 2005) or through the bioinformatic comparison of promoters for stress-induced transcripts (Chen et al., 2002). Candidate transcription factors are WRKY (W-box: TTGACY; Eulglen et al., 2000), WRKY-like (BBWGCAYT; Chen et al., 2002), SA (ACGTCA; Lebel et al., 1998), b-ZIP of the TGA1-type (TGACG; Schindler et al., 1992), GBF-type
(CACGTG; Schindler et al., 1992) and ABRE-type (BACGTGKM; Shinozaki and Yamaguchi-Shinozaki, 2000), Myb (AtMyb1: MTCCWACC; AtMyb2: TAACS-GTT; AtMyb3: TAACCTAC; AtMyb4: AMCWAMC; Martin and Paz-Ares, 1997; Rushton and Somssich, 1998), and AP2-like transcription factors (GCCGCC for GCC-box binding AP2s or DRCCGACNW for DRE-binding AP2s; Shinozaki and Yamaguchi-Shinozaki, 2000).

In response to the many stressors inducing shifts in the cellular redox poise, for example, wounding, pathogens, ozone, UV-B, and cadmium application, mitogen activated protein kinases (MAPK) play a critical role in signal transduction (Karpinski et al., 1997) and (over-) expression of catalase or ascorbate peroxidase in chloroplasts (Yabuta et al., 2004) suppress the high-light-dependent induction of apx2. Constitutive expression of apx2 in the rax1-1 mutant, which is affected in chloroplast glutathione biosynthesis by decreased γ-glutamyl cysteine synthetase activity (Ball et al., 2004), also points at signal initiation depending on oxidative stimuli. Transcripts for cytosolic Apx2 accumulated in parallel to a decrease in the photochemical quench (qP), prior to the accumulation of H2O2, and responded differentially to DCMU and DBMIB, which block PET before and after the PQ pool, respectively. Therefore, Yabuta et al. (2004) reinstated the hypothesis, originally presented by Karpinski et al. (1997), that a redox change in PET, presumably the redox state of the plastoquinone pool, controls nuclear expression of cytosolic Apx2.

Apx2 is regulated by a heat shock factor, namely HSF3 (Panchuk et al., 2002). In the regulation of apx1, which is also H2O2-responsive, the heat-shock factor HSF21 is involved in H2O2-sensing (Davletova et al., 2005). HSF21 transmits the redox signal to the zinc-finger protein Zat12 (Rizhsky et al., 2004), whose expression is under the control of HSF21 (Davletova et al., 2005). Results from apx1 knock-out mutants of Arabidopsis thaliana suggests that cytosolic ascorbate peroxidase activity is involved in signal transmission in excess light, including ROS-based signal transmission between the chloroplasts and the nucleus (Davletova et al., 2005). The sensitivity of ascorbate peroxidases to inhibition by ROS (Miyake and Asada, 1996), may facilitate signal transmission by micro-bursts.

For the regulation of nuclear-encoded chloroplast antioxidant enzymes, transcriptome analysis (Kirimura et al., 2003; Davletova et al., 2005), the analysis of transcript amount for the regulation of chloroplast ascorbate peroxidase (Yoshimura et al., 2000), the chloroplast peroxiredoxins (Baier and Dietz, 1997; Horling et al., 2003), chloroplast superoxide dismutases (csd2,fsd1; Kliebenstein et al., 1998), and glutathione peroxidase gpx1 (Milla et al., 2003) revealed a lower sensitivity of gene expression to shifts in the cytosolic redox poise compared with non-plastidic antioxidant enzymes. However, in 2-Cys peroxiredoxin antisense lines of Arabidopsis thaliana, in which a specific component of the chloroplast enzymatic redox defence was suppressed, the transcripts of chloroplast monodehydroascorbate reductase and stromal and thylakoid ascorbate peroxidase were selectively induced (Baier et al., 2000). Loss of 2-Cys peroxiredoxin function led to slight photo-inhibition, damage of the photosynthetic membrane, and oxidation of the ascorbate pool, suggesting photo-oxidative stress (Baier and Dietz, 1999b). The fact that three transcripts for chloroplast antioxidants accumulated, while the transcript levels for various other antioxidant
enzymes did not respond, indicates chloroplast-specific signals triggering nuclear expression (Baier et al., 2000).

**Chloroplast-to-nucleus redox signals for transmission of moderate redox imbalances**

Efficient transmission of a signal depends on a low threshold concentration. Accumulation of the signal, in turn, is controlled by its stability within the metabolic network. Therefore, messengers that are metabolically more inert and cannot or can only slowly be inactivated are more efficient than redox compounds such as ROS and oxidized antioxidants that are decomposed or reduced, respectively, during diffusion through the cell. In respect of chloroplast-to-nucleus signalling, several putative signals have been suggested, of which some will be discussed here.

**Tetrapyrrole signals**

Indications for tetrapyrrole signals come from the analysis of cab gene expression during de-etiolation of Arabidopsis seedlings (Susek et al., 1993). The arrest of chloroplast development by a norflurazonemediated block of carotenoid biosynthesis suppressed the expression of various nuclear-encoded chloroplast proteins, for example, Lhcb1, PsbR, RbcS, PetH, and PetE, during early seedling development (Harpster et al., 1984; Mayfield and Taylor, 1984; Bolle et al., 1994; Gray et al., 1995). Based on these observations, retrograde chloroplast signals were hypothesized for the control of nuclear transcription (Taylor, 1989). A first insight into signalling was provided by Susek et al. (1993), who isolated Arabidopsis mutants (gun), that express cab3, encoding Lhcb1-2, although chloroplast development had been arrested by norflurazon. Mapping of the mutations showed defects in haem oxidase (gun2), phytochromobilin synthase (gun3), a regulator of Mg-chelatase (gun4), and the H-subunit of Mg-chelatase (gun5) (Mochizuki et al., 2001; Larkin et al., 2003; Strand et al., 2003) indicating a role of tetrapyrrole biosynthesis in the regulation of nuclear transcription (Strand et al., 2003).

Either Mg-protoporphyrin-IX, haem or a haem precursor were assumed to be released from chloroplasts and to modify nuclear gene expression by binding to a regulatory protein, which interacts with the CUF1-element found in several promoters of 70 genes mis-regulated in gun2 and gun5 (Strand et al., 2003; Strand, 2004).

In higher plants, the pathways of chlorophyll and haem biosynthesis are tightly regulated at an early step. Haem triggers feed-back inhibition of Glu-tRNA reductase, which catalyses biosynthesis of the tetrapyrrole precursor δ-aminolevulinic acid (ALA) (Beale, 1999). While haem binds to the N-terminus of the enzyme (Vothknecht et al., 1998), FLU, which is a negative regulator of chlorophyll biosynthesis (Meskauskien et al., 2001), regulates GlutRNA reductase at the C-terminus (Goslings et al., 2004). Characterization of the mutant ulf3, which is allelic to gun2 (Susek et al., 1993), demonstrated that tetrapyrrole biosynthesis is concurrently regulated by FLU mediating the feedback from the Mg-2+ branch and ulf3/gun2 controlling the haem branch (Goslings et al., 2004), which makes tetrapyrroles unlikely to accumulate in mature tissues. In addition, Keetman et al. (2002) showed, in tobacco coproporphyrinogen oxidase antisense lines, which accumulate protoporphyrin-IX, that imbalances in tetrapyrrole biosynthesis primarily lead to modulation of gene expression by photosensitization of the pigments. Redox signals, for example, H2O2 accumulation or shifts in the redox state of low-molecular-weight antioxidants, may be involved in the suppression of nuclear gene expression in norflurazonetreated seedlings, although Strand et al. (2003) excluded severe differences in the steady-state levels of superoxide for the gun mutants by semi-quantitative NBT-staining. In mature leaves, expression of cab3, which was used as a target gene in the gun-screen, is regulated by photosynthetic electron transport (Sullivan and Gray, 2002), presumably by the acceptor availability of photosystem I (Pursiheimo et al., 2001). Photo-damaged tetrapyrroles may regulate nuclear gene expression, as for example, photo-oxidized haem controls the capping of catalase mRNA in rye (Schmidt et al., 2002).

**Oxolipin signals**

Besides oxidatively damaged tetrapyrroles, oxolipins are another type of putative redox-related signals in chloroplast-dependent redox regulation. In pathogen response, for example, they modulate oxidative bursts (Rao et al., 2000). Their biosynthesis initiates from alkyl hydroperoxides, which are formed preferentially under unfavourable conditions by oxidation of unsaturated fatty acids either mediated by ROS (Blée and Joyard, 1996), lipoxygenase (LOX) (Feussner and Wasternack, 2002) or fatty acid dioxygenases (DOX) (de Leon et al., 2002). Detailed time-resolved mass spectrometric analysis of lipids and lipid hydroperoxides (Montillet et al., 2004) revealed early activation of the 13-LOX pathway in response to various kinds of stresses. By contrast, ROS-mediated peroxidation, which is stimulated by excess excitation energy, appears to be a late process (Montillet et al., 2004) when the antioxidant defence is close to oxidative collapse. Various plant signalling molecules are synthesized from alkyl hydroperoxides (Blée and Joyard, 1996). Jasmonates, which are a group of 12-carbon fatty acid cyclopentanones and dinor-oxo-phytodienoic acids, and 2(R)-hydroperoxide fatty acids, and are well known from pathogen and wounding responses, protect plant cells from oxidative stress and cell death (Farmer et al., 1998; Mauch et al., 2001; Hamberg et al., 2003).

Inside the chloroplast, accumulation of lipid peroxides is suppressed by glutathione peroxidases and peroxiredoxins.
Both enzymes are haem-free peroxidases reducing alkyl hydroperoxides by a thiol-based reaction mechanism (Baier and Dietz, 1999a). The genome of Arabidopsis thaliana contains two open reading frames for chloroplast glutathione peroxidases (gpx1 and gpx7), of which only gpx1 is expressed (Milla et al., 2003), and four open reading frames for chloroplast peroxiredoxins (Horling et al., 2003). Peroxiredoxins and gpx1 are induced by H₂O₂ and butylhydroperoxide (Horling et al., 2003; Avsian-Kretchmer et al., 2004) indicating that their expression possibly antagonizes oxolipin signal formation. However, expression of gpx1 and 2CPA, which are the only isogenes for which the analysis has been performed so far, are not responsive to jasmonates or salicylates (Milla et al., 2003; Baier et al., 2004) suggesting primary control of oxolipin biosynthesis by the relative rates of fatty acid peroxidation. Peroxide formation and reduction may be further uncoupled due to the sensitivity of the active site cysteine residues to over-oxidation. 2-Cys Prx have been suggested to function as flood gates that normally keep peroxides at a low level (Wood et al., 2003; König et al., 2003). Following a sudden increase in peroxides they are over-oxidized and inactivated. Subsequently, the oxolipin signal can spread freely as shown for mammalian cells (Wood et al., 2003). Glutathione peroxidases, which show only low activities in plant cells, and the ascorbate peroxidases, which are highly specific for H₂O₂ and sensitive to inactivation by low ascorbate availability, are very likely not able to compensate for decreased Prx activity. Plant 2-Cys Prx is efficiently inactivated especially by bulky peroxides, like lipid peroxides (König et al., 2003). Therefore, with the accumulation of alkyl hydroperoxides, oxolipin biosynthesis may get increasingly dependent on LOX-, DOX-, and ROS-stimulation.

In recent years various mutants with decreased sensitivity to jasmonates (and salicylates) and high sensitivity to ROS have been isolated, for example, the ity to jasmonates (and salicylates) and high sensitivity to and ROS-stimulation. This may get increasingly dependent on LOX-, DOX-, and ROS-stimulation.

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### Photosynthetically controlled signals

The redox state of the plastoquinone pool: Efficient regulation of gene expression in relation to photosynthesis should directly respond to photosynthetic activity. In recent years, photosynthetic control of both nuclear as well as plastid gene expression has been linked to the redox state of the plastoquinone (PQ) pool which regulates expression of, for instance, petE2 (Pfannschmidt et al., 2001b), encoding a plastocyanin, in sugar-starved cells (Oswald et al., 2001) and under low light conditions (Pfannschmidt et al., 2001b). A small pool of 7–10 PQ molecules per photosystem II mediates electron transfer between photosystem II and the cytochrome b₆f-complex, making plastoquinol diffusion the rate limiting step (Haehnel et al., 1984). The relative activities of photosystem II as electron input and photosysytem I as drainage, cyclic electron transport and chlororespiration adjust the redox state of PQ (Allen, 1992; Heber, 2002). Depending on the PQ redox state presumably by binding of plastoquinol to the cytochrome b₆f-complex (Zito et al., 1999), the kinase TAK1 is activated and initiates processes like state transition (Snyders and Kohorn, 2001). Whether this kinase also transmits other kinds of PQ-dependent responses, for example, in the regulation of psaAB transcription (Pfannschmidt et al., 1999) and nuclear expression of petE2, or whether PQ triggers several independent signal transduction cascades in parallel is still open for discussion.

Regarding expression of other nuclear-encoded chloroplast proteins, co-regulation with the redox state of the PQ pool has been demonstrated for cab genes in unicellular green algae (Escoubas et al., 1995; Maxwell et al., 1995). A more detailed recent study (Chen et al., 2004) demonstrates that, in the case of Dunaliella tertiolecta, PQ-dependent modulation of lhcb1 expression starts only 8 h after modifying the PQ redox state. Immediately after inducing the redox shift in the PQ pool the trans-thylakoid membrane potential is the predominant regulator of gene expression. The long lag phase suggests that expression of signal transduction elements and proteins involved in the co-ordination of a regulatory network is necessary for establishing the correlation between gene activity and the redox state of the PQ pool.

In higher plants, only weak indication for PQ-dependent regulation of genes for light-harvesting complex proteins (LHCP) is available. For cab genes, encoding LHCP of photosystem II, PQ-dependent regulation has been experimentally excluded for winter rye by Pursiheimo et al. (2001), who describe a correlation of gene expression with the acceptor availability of photosystem I. In addition, PQ-dependent redox regulation was not observed for nuclear encoded psaF and psaD expression in mustard seedlings (Pfannschmidt et al., 2001b). Interestingly, even in the case of petE2, which to date is the model gene for the regulation of nuclear gene expression by the PQ redox state, other
signals such as the sugar status (Oswald et al., 2001), phytochrome-A (Dijkwel et al., 1997) and abscisic acid (Huijser et al., 2000) are dominant regulators in Arabidopsis thaliana. In pea and tobacco, PQ-dependent regulation is overwritten and antagonized post-translationally by a PET-dependent signal (Sullivan and Gray, 2002). It is tempting to assume that the PQ signal, which is central in regulating state transition (Allen, 1992) and chloroplast transcription of photoreaction centre proteins (Pfannschmidt et al., 1999), is of minor importance for chloroplast-to-nucleus signalling in mature leaves and under most environmental circumstances. According to the gradual model of redox signalling proposed by Pfannschmidt et al. (2001a) PQ triggers the signalling pathway with the highest sensitivity and lowest threshold to redox imbalances. Based on the data available, PQ-dependent signalling appears to have an ancillary or insignificant role in controlling the genetic responses to metabolically relevant redox imbalances in green tissues under ambient growth conditions and moderate stress. This conclusion is supported by a recent study in which regulation of gene expression in response to PQ redox state has been studied on a transcriptome level (Fey et al., 2005). Although small sets of genes have been identified as being responsive to the PQ redox state, the experiments were performed at photon flux densities of less than 40 μmol m⁻² s⁻¹, i.e. conditions that barely have major relevance for regulatory adjustment of photosynthesis under natural conditions.

At higher light intensities (a cloudy day corresponds to 100–300 μmol m⁻² s⁻¹ and a sunny day to 1500–2000 μmol m⁻² s⁻¹) the PQ pool is intermediate reduced under most conditions. However, in the vascular tissues and their bundle sheaths, where the redox state of the PQ pool may be controlled more strongly by NADPH-dependent, presumably NDH-mediated reduction (Peltier and Cournac, 2002), a signalling function is more likely. Therefore, expression of cytosolic apx2, which is preferentially expressed along the main veins (Ball et al., 2004), may correlate with the PQ redox state (Yabuta et al., 2004) as well as with the cellular redox status (Chang et al., 2004) and the availability of low molecular weight antioxidants (Ball et al., 2004).

**Thioredoxin-mediated signals:** At the acceptor site of photosystem I, ferredoxin-thioredoxin reductase reduces thioredoxins (Trx) depending on the electron pressure and the redox state of ferredoxin (Fridlyand and Scheibe, 1999). A genome-wide survey showed various chloroplast thioredoxins, namely four Trx-m, two Trx-f, two Trx-y, and one Trx-x (Collin et al., 2004). Together with other small redox proteins like glutaredoxins (Rouhier et al., 2004) and cyclophilins (Romano et al., 2004) they mediate thiol-disulphide redox interchange of various chloroplast proteins like fructose-1,6-bisphosphatase, malate dehydrogenase, peroxiredoxins, and the RB60-protein with partly overlapping specificity. The redox states of the target proteins modulate the chloroplast metabolite fluxes, ATP synthesis, the release of reduction energy into the cytosol, the chloroplast peroxidase activity and chloroplast translation (Schürmann, 2003; König et al., 2002; Barnes and Mayfield, 2003). As indicated by the spectrum of target proteins (Motohashi et al., 2001), redox regulation depending on the redox state of thioredoxins is manifold and far from being understood comprehensively. In this context, the classification of Trx function as signal, sensor or transmitter of redox information depends on definition. The redox state of thioredoxin is an indicator of redox state and thus a transmitter for subsequent signal generation by downstream events rather than the signal or sensor itself. The sink capacity for consumption of reduction energy of the thiol system is determined by the auto-oxidation rate of target proteins (Schürmann, 2003) and, in case of peroxiredoxins, by the rates of peroxide reduction (König et al., 2002). Electron drainage into the thioredoxin pathways withdraws electron from ferredoxin:NADP⁺-reductase and the Mehler reaction (Fridlyand and Scheibe, 1999) and thus competes with the generation of ROS and possible (reductive) signals in metabolic pathways. The allocation of electrons between the different metabolic sinks has to be adjusted for optimal assimilation rate versus dissipation of excess energy. For example, peroxides produced in the Mehler reaction are detoxified by ascorbate peroxidase or peroxiredoxin yielding dehydroascorbic acid and oxidized peroxiredoxin, respectively. Through the regeneration of reduced ascorbate and peroxiredoxin, both reaction sequences consume further reductive power and relieve electron pressure in the PET chain (Fortis and Ellis, 1996; Dietz et al., 2002). Redox information must be central in the regulation of these processes.

**Photosynthates and the plastidic redox state of NADPH/NADP⁺:** The photosynthetic electron transport drives reductive metabolism. Therefore, basically any metabolite synthesized, depending on the availability of reducing energy, could be a redox signal. Examples for putative signalling metabolites are carbohydrates (‘sugar sensing’), which are synthesized in photosynthesis and consumed in mitochondria by respiration depending on the cellular energy status (‘energy sensing’). Channelling reduction energy between chloroplasts and mitochondria can protect photosynthesis against photo-inhibition (Saradadevi and Raghavendra, 1992). In light-enhanced dark respiration, malate is the preferred redox transmitter (Padmasree et al., 2002). NADH generated by dehydrogenase activity is fed into the respiratory electron transport chain and the alternative oxidase branch (Gardeström et al., 2002). It is open for debate if and how sensing of the cytosolic carbohydrate concentration interferes with redox signalling. Sugar signalling involves, for example, hexokinase (Jang et al., 1997), which drives the expression of various nuclear-encoded chloroplast proteins, including suppression of Rubisco, light-harvesting complex proteins, and...
seduheptulose bisphosphatase (Moore et al., 2003), and SNF1-like kinases (Halford et al., 2003). Some of the target genes, for example, lhcb1 (cab3) and petE2, are also model genes in the analysis of redox signalling (see above). In addition to stimulating respiration, carbohydrate fluxes between chloroplast and cytosol mediate the exchange of information on the chloroplast redox state. Two well-studied examples for redox valves controlled by carbohydrate metabolism are the malate-oxaloacetate shuttle and the triose phosphate/3-phosphoglycerate shuttle (Heineke et al., 1991) (Fig. 2). Both transporters exchange redox energy between the chloroplastic NADPH and the cytosolic NADH-system. Since the chloroplastic NADPH/NADP+ ratio is about 0.5 in the light and the cytosolic NADH/NAD+ ratio about 10−3 (Heineke et al., 1991), the transport is essentially unidirectional due to photosynthetic activity and depends on the photosynthetic electron pressure (triose-phosphate/3-phosphoglycerate shuttle) and Trx-dependent enzyme activation (malate valve).

Other putative signalling molecules are amino acids, whose biosynthesis provides a strong electron sink by the high consumption of reduction energy in carbohydrate biosynthesis and nitrate reduction. That the signalling pathways, which are presently under investigation (Palenchar et al., 2004), could have an impact on chloroplast redox signature is, for example, indicated by co-regulation of the ferredoxin gene At2g27510 and the two ferredoxin-NADP+-reductases At1g30510 and At4g05390. Expression of the proteins, which are involved in distributing electrons from the photosynthetic light chain to NADPH+, is controlled by a carbon/nitrogen signalling pathway with dominance of the carbon component (Palenchar et al., 2004).

Analyses of lhcb1 transcription in winter rye and of 2-Cys Prx (2CPA) in Arabidopsis thaliana indicate regulation by the redox state of chloroplast NADPH/ NADP+ (Pursiheimo et al., 2001; Baier et al., 2004). However, transcriptional activity of cab genes and 2CPA is distinctly regulated in response to photosynthates. While transcription of lhcb1 is suppressed by sugars via the hexokinase-dependent pathway (Moore et al., 2003), regulation of 2CPA is independent of sugar signalling (Baier et al., 2004). Low concentrations of externally applied sugars even increases 2CPA promoter activity by 10–20%. The dominance of redox-regulation of 2CPA was demonstrated by low 2CPA-promoter driven reporter gene activity in the presence of strong electron sinks such as CO2 and NO3 (Baier et al., 2004). Since the inhibition of PET also decreased the promoter activity, regulation depends on photosynthetic activity. Apparently, the acceptor availability of photosystem I, possibly mediated by the redox state of the NADPH/NADP+-system, controls the promoter activity (Baier et al., 2004).

External application of millimolar amounts of peroxides only slightly increased 2CPA transcript amount (Baier and Dietz, 1997; Horling et al., 2003; Baier et al., 2004), while a strong up-regulation was seen upon wounding which rapidly and efficiently suppresses photosynthetic activity (Chang et al., 2004). These data support the view that the regulatory redox signal is of chloroplast origin. Pharmacological studies suggest that signal transduction takes place via protein kinases. In the expression dependent inhibition under reducing conditions a staurosporine-sensitive serine/threonine kinase is involved, while under oxidizing conditions a PD98059-sensitive MAPKK transmits the signal (Horling et al., 2001; Baier et al., 2004). The analysis of Arabidopsis mutants with lower induction of 2CPA, the rimb-mutants, suggests that chloroplast monodehydroascorbate reductase, stromal ascorbate peroxidase, chloroplast CuZn superoxide dismutase csd2, and plastidic malate dehydrogenase are modulated by components of the signalling pathway triggering 2CPA expression (I Heiber and M Baier, unpublished results).

Abscisic acid and violaxanthin-cycle activity: In case of 2CPA gene expression, redox regulation of transcription depends on the plant hormone abscisic acid (ABA) (Baier et al., 2004). In addition, ABA-responsive cis-elements are found in promoters of many nuclear-encoded chloroplast proteins (Weatherwax et al., 1996; Milla et al., 2003). The close interrelation between ABA signalling and photosynthesis is indicated by the isolation of alleles for ABA-biosynthetic enzymes and for ABA signal transduction elements in screens for mutants impaired in the expression of the plastocyanin gene petE2 (Huijser et al., 2000) and the gene for a regulatory subunit of ADP-glucose pyrophosphorylase (apL3; Rook et al., 2001). PetE2 (Huijser et al., 2000) is like 2CPA (Baier et al., 2004) suppressed by ABA, while apL3 (Rook et al., 2001) is like apx2 (Chang et al., 2004) ABA-induced (Fig. 3).
ABA biosynthesis starts inside the chloroplast and depends on xanthoxin synthesized from violaxanthin and the violaxanthin-derivative neoxanthin (Finkelstein and Rock, 2002) (Fig. 3). Most of the violaxanthin within the thylakoid membrane takes part in the xanthophyll cycle, which is a redox reaction system of reversible xanthophyll epoxidation and de-epoxidation (Eskling et al., 1997). In excess light, the xanthophyll cycle is activated for the dissipation of excess energy. De-epoxidation of violaxanthin to zeaxanthin via antheraxanthin requires reduced and protonated ascorbic acid in the thylakoid lumen (Bratt et al., 1995), which makes it not only dependent on the ascorbate content, but also on the redox state of the ascorbate pool. Regeneration of ascorbate is covered by the NADPH-dependent Halliwell–Foyer cycle (Foyer and Halliwell, 1977). However, if dehydroascorbate reduction cannot keep pace with ascorbate oxidation, the xanthophyll cycle gets uncoupled. If violaxanthin accumulates, it may promote ABA synthesis. The hypothesis on the regulation of ABA-biosynthesis by ascorbate availability is supported by the characterization of the ascorbate biosynthetic mutant vtc1 (Pastori et al., 2003). In leaves, which accumulate only 30% of wild-type ascorbate (Conklin et al., 1997), the ABA content increased by 60% (Pastori et al., 2003). In parallel, the expression of 9-cis-epoxycarotenoid dioxygenase (NCED) increased (Pastori et al., 2003), which catalyses the irreversible oxidative cleavage of neoxanthin and/ or violaxanthin to xanthoxin (Finkelstein and Rock, 2002), indicating that ABA biosynthesis in a low ascorbate background is also promoted by the adaptation of gene expression.

The thylakoid lumen is especially sensitive to limitations in ascorbate availability since it depends on non-catalysed diffusion of ascorbate through the thylakoid membrane (Foyer and Lelandais, 1996). The supply with reduced ascorbate to the lumen is affected by the local redox poise on the stromal site, where, for example, the Mehler reaction and ascorbate peroxidase activity strain the ascorbate pool. The redox regulation of ABA biosynthesis dependent on PET is further enhanced by redox-regulation of ABA signal transduction, as oxidative inhibition of the ABA antagonistic phosphatases ABI1 and ABI2 (Meinhard and Grill, 2001; Meinhard et al., 2002) increases ABA sensitivity.

The oxidative stimulation of ABA biosynthesis and ABA signal transduction has a different impact on the expression of various antioxidant enzymes. For example, transcript levels of cytosolic ascorbate peroxidase apx2 are up-regulated by ABA (Cheong et al., 2004). As a consequence of ABA-induced H₂O₂ generation, apx2 is induced under photoinhibitory conditions (Chang et al., 2004). High activity of the antioxidant enzyme helps to balance the cytosolic redox poise, as long as the cytosolic ascorbate availability is sufficient to support H₂O₂ reduction. Thus, it is assumed that the chloroplast ABA signal synergistically increases the cytosolic antioxidant capacity before the extraplastidic compartments are flooded with photo-oxidatively produced ROS. In addition to cytosolic apx2 (Chang et al., 2004), chloroplastic gpx1 is induced by ABA (Milla et al., 2003). GPx1 acts independently of ascorbate by using reduced glutathione as a co-factor, which can more efficiently be regenerated inside the chloroplast by glutathione reductase at the expense of NADPH (Baier et al., 2000; Noctor et al., 2000). Under photoinhibitory conditions, increased amounts of Gpx1 may substitute for chloroplast ascorbate peroxidase, which is susceptible to shifts in the redox poise of ascorbate (Miyake and Asada, 1996).
Expression of 2CPA, which is a peroxidase reducing a broad range of peroxides independent of low-molecular-weight antioxidants as co-factors (Baier and Dietz, 1999a, b; König et al., 2003) is suppressed by ABA (Baier et al., 2004). The antagonism of oxidative induction and ABA suppression may keep the transcript and protein on fairly constant, but high, levels under most growth conditions including stress situations (Baier and Dietz, 1996, 1997; Horling et al., 2003; Baier et al., 2004). Exceptions are observed under severe stresses like wounding (Baier et al., 2004) and limitations in thioredoxin regeneration (Keryer et al., 2004), which is needed for driving peroxiredoxin activity (König et al., 2002). Biochemical analysis of 2-Cys Prx in barley (König et al., 2003) demonstrated that under stress conditions increasing portions of the active site of the enzyme get over-oxidized. The oxidation causes conformational changes leading to decamerization and attachment of the inactive enzyme to the thylakoid membranes (König et al., 2003). Ongoing work with transgenic Arabidopsis thaliana lines and in vitro studies with isolated thylakoids and heterologously expressed PRX suggest that the binding of 2-Cys Prx to the thylakoid membrane modulates PET (P Lamkemeyer, WX Li, M Laxa, K-J Dietz, unpublished results). As outlined above, 2CPA expression is antagonistically regulated by negative inputs through ABA and reductive stimuli, and positive input by oxidative stimuli. This mechanism reduces the rates for re-synthesis of active enzyme under stress conditions (Fig. 4). Another explanation for ABA-dependent suppression of 2CPA may be linked to its substrate spectrum. Prx reduces a wide range of alkyl hydroperoxides (König et al., 2002) some of which are precursors for oxolipid biosynthesis (Blé and Joyard, 1996). Consistent with the finding by Andersson and coworkers (2004) that disruption of AtMYC2, which encodes a transcription factor positively regulating ABA-responses, resulted in elevated expression of jasmonate responsive genes, ABA-suppression of 2CPA may lead to stimulated rates of jasmonate synthesis.

Perspectives

In the context of photosynthesis and in the regulation of antioxidant enzymes, chloroplasts act both as source and target of redox regulation. They are tightly integrated in cellular metabolism, a fact that often complicates the experimental dissecting of signal transduction pathways and pinpointing the causes and consequences of regulatory reactions. During evolution, the endosymbiont maintained its photosynthetic capacity with all the associated potential oxidative hazards. However, most structural and functional genes were transferred from the plastome to the nucleus, including all antioxidant and most regulatory genes. A sophisticated network of regulation evolved composed of anthero- and retrograde signalling pathways with significant crosstalk and efficient feedback.

In chloroplasts, antioxidant enzymes came together from different evolutionary origins (Asada, 2000). They have been adapted for their function in protecting against the risks of oxygenic photosynthesis, while the oxygen concentration and light intensities increased. Together with the chloroplast targeting signals, the promoters have evolved. From double targeting of the antioxidant enzymes glutathione reductase, ascorbate peroxidase, and monodehydroascorbate reductase into chloroplasts and mitochondria (Chew et al., 2003) it has to be assumed that this process is still ongoing. 2-Cys peroxiredoxins and glutathione peroxidase already show compartment-specific targeting and regulation (Baier and Dietz, 1997; Mullineaux et al., 1998). For 2-Cys peroxiredoxin-A, which is very likely of endosymbiotic origin (Baier and Dietz, 1997), by taking over an ancient function in the protection of the photosynthetic membrane, promoter adaptation closed a regulatory circuitry of multi-level redox regulation, in which the chloroplast enzyme is the source and the target of redox regulation (Fig. 4). Analysis of the gpx gene structures (Milla et al., 2003) points to the multiplication of a single ancestor gene. Only gpx1 transcripts, which encode the chloroplast isoforms, are induced by ABA and gpx1 is, besides gpx6, the only gpx gene not responding to the oxolipin derivates jasmonic acid and salicylic acid (Milla et al., 2003). The regulation pattern indicates specific responses to second messengers generated by chloroplast metabolism by moderate (ABA) and strong (oxolipins) redox imbalances.
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