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Review

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Plant cell microcompartments: a redox-signaling perspective

Abstract: This review describes how transient protein-protein interactions can contribute to direct information flow between subsequent steps of metabolic and signaling pathways, focusing on the redox perspective. Posttranslational modifications are often the basis for the dynamic nature of such macromolecular aggregates, named microcompartments. The high cellular protein concentration promotes these interactions that are prone to disappear upon the extraction of proteins from cells. Changes of signaling molecules, such as metabolites, effectors or phytohormones, or the redox state in the cellular microenvironment, can modulate them. The signaling network can, therefore, respond in a very flexible and appropriate manner, such that metabolism, stress responses, and developmental steps are integrated by multiple and changing contacts between functional modules. This allows plants to survive and persist by continuously and flexibly adapting to a challenging or even adverse environment.

Keywords: macromolecular crowding; protein-protein interactions; redox signaling; regulation; signal integration; thiol groups.

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Introduction

In this review, we attempt to describe what is known about how signals originating from imbalances in the energy-converting systems of the chloroplasts and the mitochondria are transduced into the nucleus causing an altered gene transcription as a response to reach adaptation to a new steady state. As plants are sessile organisms, they cannot escape adverse conditions, and signaling processes are required to continuously adapt during abiotic and biotic stress, and during development. As becomes evident from studying many types of stress response, a complex network of signal transduction must integrate information from multiple stimuli, and in most cases, redox imbalance plays a role in the early steps (Neill et al., 2002; Fujita et al., 2006; Baudouin, 2011; Spoel and Loake, 2011; Suzuki et al., 2011). In order to understand the integration of the multiple signals that are processed with different outcome in each case depending on the coincidence of specific cues, the molecular components and dynamic changes during signal transfer must be dissected. Following this, the individual components can be integrated into a scheme, to understand how they impact on each other and within the whole signaling network. The complex and variable protein assemblies resulting from protein-protein interactions often take the form of suborganellar functional units, named microcompartments hereafter. Microcompartments often depend on weak protein-protein interactions in their defined cellular microenvironments. Here, they are suggested to be involved in the formation, transduction and execution in redox-signaling processes, particularly in view of their interaction with other signaling cascades.

About 10 years ago, research on MAP kinase pathways opened up a new, productive field in understanding plant signal transduction. It was shown that posttranslational protein modification by phosphorylation plays a pivotal role in two-component systems and MAPK signaling cascades leading to a range of cellular responses during both plant stress and development. Thiol switches are now emerging as additional posttranslational modifications encountered in signal transduction. There is newly emerging evidence for redox-dependent steps acting on phosphorylation cascades of signal transduction. The biochemical link between these two regulatory mechanisms, as recent data suggest (Fujino et al., 2006; Wang et al., 2010), is a further challenge. To unravel their crosstalk will lead to the next level of understanding of

the modular nature of signal transduction cascades in plants.

The cellular microenvironment as a prerequisite for functional networks

Mature plant cells are composed of up to 95% vacuolar space filled with an aqueous solution of rather acidic pH. The remaining volume comprising the cytosol, all organelles, and the nucleus is packed with a high protein concentration (Goodsell, 1991). The organellar compartments are separated from the cytoplasmic matrix by one or two biomembranes equipped with various transporters for controlled exchange between these reaction spaces. In addition, within the cytosol, various protein complexes such as ribosomes, the cytoskeleton, and permanent multienzyme complexes are relatively defined units with specific cellular functions. Structurally less obvious, but just as important for cellular function, are microcompartments that are formed transiently. In order to understand how such weaker protein-protein interactions might be effective and relevant under *in vivo* conditions, it is important to emphasize the extremely high protein concentrations in the cell. Given the lack of free water in these cellular spaces, this enables/enhances their otherwise weak interactions, yielding high binding constants compared to *in vitro* experiments with purified proteins, made with the dilute solutions generated by cell disruption and protein extraction. As transient microcompartments are excellent candidates as potential stepping stones in signal transduction and metabolism, the specific properties relating to their formation are outlined here in more detail.

In vivo, macromolecules exist in a crowded environment, where the excluded volume occupied by these large molecules is predominant and a minor fraction of the total volume is available with bound or free water that can serve as a solvent for small molecules (Minton, 1981; Ellis and Minton, 2006). In such a limited volume, the interaction of proteins with other molecules can be efficiently modified by changes to their surface caused by the binding of small molecules such as metabolites, effectors or ions, or by covalent modifications including redox modifications at cysteine residues that are in the focus of this review. The large size of the enzymes, compared to their small active sites, provides the large surface area required for controlled interaction with other proteins (Srere, 2000). The capacity for rapid and dynamic changes in affinity between molecules means that transient complexes are highly suited for information transfer, as well as forming 'channels' for metabolic pathways.

Redox imbalances as signals to maintain homeostasis

Redox homeostasis is one of the basic requirements of a cell, and deviation from a balanced redox state is indicative of disturbances. Therefore, it is assumed that any imbalance leading to the formation of reactive oxygen/nitrogen species (ROS/RNS) might serve as a signal (Potters et al., 2010). Indeed, upon stress, e.g., due to sudden exposure to high light, plants can respond at different levels. They are capable to rapidly adjust to changed electron fluxes, as well as to induce gene expression for long-term adaptation at the transcriptional level (Scheibe et al., 2005). In order to achieve the latter, imbalances in the electron transport chains of chloroplasts or mitochondria (as central pathways of energy-converting reactions) must initiate signaling processes that are finally perceived in the nucleus, resulting in altered gene expression of organelle-localized proteins. For these studies, transgenic approaches have been used that helped to discover new parts of the complex signaling system (Hanke et al., 2009). Such a scenario is known as retrograde signaling (Nott et al., 2006; Barajas-López et al., 2012; Leister, 2012). Additional factors also impact on plant cells, such as other abiotic factors, pathogens, or morphogenic gradients over cells for determining the coordinated development of cells within tissues. Stress can result in oxidative bursts through plasma membrane NADPH oxidases and/or signal conversion into internal signal transduction chains involving phytohormones (Kwak et al., 2003; Scheibe and Beck, 2011).

Various molecules have been proposed to serve as a redox signal (among others, ROS, RNS, GSH/GSSG, ascorbate). Signal transduction across the cytosoliccompartment is thought to proceed through posttranslational protein-thiol modifications. Upon oxidation, cysteine residues are subject to S-glutathionylation, S-nitrosylation or disulfide bridge formation, or various other oxidation forms of the thiol group, such as sulfenic, sulfinic, and sulfonic acid, whereby the latter two forms appear to be largely irreversible (Spadaro et al., 2009). An overview of these modifications is shown in Figure 1. Redox-dependent regulation has been previously thought to be unique to chloroplasts of photosynthesizing organisms containing both strong sources of reductant and oxidant (Scheibe, 1991; Dietz et al., 2002). For all other cases in

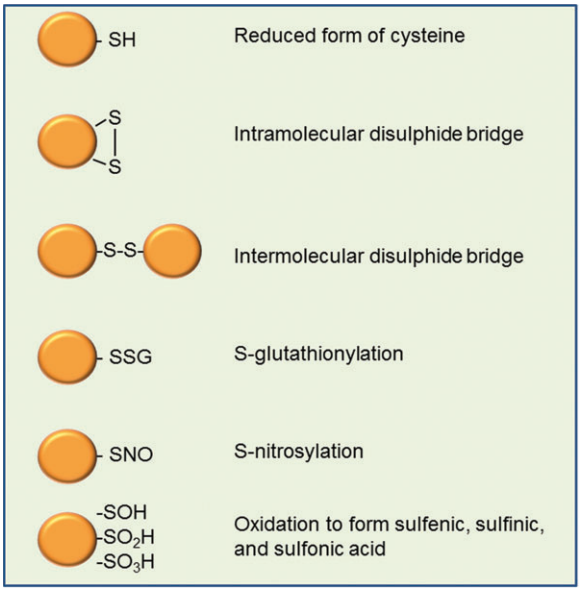

Figure 1 Redox modifications at cysteine residues.
Proteins contain cysteine residues that can occur in the reduced state (-SH), or become oxidized in different ways. Most of these modifications are reversible (catalyzed by thioredoxins, glutaredoxins, etc.). Sulfinic and sulfonic acid formation, as the most severe modes of oxidation, are mainly irreversible. In general, reversible modifications can protect the thiol residues from irreversible damage, and as suggested in this review, provide changed properties and functions.

nonphotosynthesizing cells, any deviation from redox homeostasis has long been considered as stress or damage (Halliwell, 2006). However, since these original discoveries of posttranslational redox regulation of chloroplast enzymes, ample evidence has arisen for redox-dependent regulation and signaling events, taking place also in other cell compartments, namely, non-green plastids, mitochondria, cytosol, and nucleus, of all organisms (see Buchanan et al., 2012).

In order to function in a changing environment, plants must uniquely respond to changes in the quality and availability of photosynthetic light, which are thought to be perceived in the chloroplast. The identity of the retrograde signal generated at the thylakoid membrane, where photo-excitation occurs, remains elusive. ROS generated during photosynthetic electron transport (PET) are frequently suggested to be a source of this signal (Mittler et al., 2004). The predominant species produced are singlet oxygen at photosystem II (PSII) and superoxide at PSI and the plastoquinone (PQ) pool. These species are extremely short lived, but superoxide is rapidly removed by dismutation to the less reactive H_2O_2. H_2O_2 is a more stable molecule, capable of diffusing through membranes, and it has, therefore, been proposed that its leakage out of the chloroplast may be related to the retrograde signal. The possibility that H_2O_2 may regulate cytosolic protein activity after diffusing out of the chloroplast is discussed in detail below. Alternatively, it has also been suggested that oxidative damage to membranes by ROS could result in the production of hormones such as abscisic acid and jasmonic acid, which could then be transported easily out of the chloroplast (Mullineaux and Karpinski, 2002).

H_2O_2 is capable of damaging cellular components by oxidation and can be radicalized in the Fenton reaction to the more toxic hydroxyl radical. To prevent this, chloroplasts contain a powerful scavenging system to remove the majority of H_2O_2 produced. Such protective system relies on reductive conversion to H_2O using ascorbate, which is regenerated by glutathione (GSH) and that is, in turn, regenerated by electrons from PET (Mittler et al., 2004; Foyer and Noctor, 2011). The relative proportions of oxidized and reduced ascorbate and GSH in this buffering system are, therefore, subject to change, depending on the amount of free radicals produced in PET, and it has become clear that the redox state of the GSH/GSSG pool is also involved in retrograde signaling (Foyer and Noctor, 2011). The transmission of chloroplast redox state into the cytosol, either by H_2O_2 or by GSH, would directly connect events at the thylakoid membrane to the redox modifications of cellular components. Once the signal has reached the cytosol, dynamic interactions, possibly at the cytoskeleton or at cellular membranes, transmit the retrograde signals, which finally influence gene transcription in the nucleus (Figure 2).

Microcompartmentation and dynamics in redox-dependent basic cellular processes

Electron-transport chains in chloroplasts and mitochondria

Chloroplasts and mitochondria are broadly divided into two separate subcompartments, the membrane systems containing the protein complexes that catalyze redox chemistry of electron flow, and the soluble stroma/matrix, which contains the enzymes necessary for the assimilation and biosynthesis or substrate oxidation, as well as the genetic material encoding genes for some organellar proteins and their means of transcription and translation. In order to efficiently perform electron transport through bioenergetic membranes, the protein

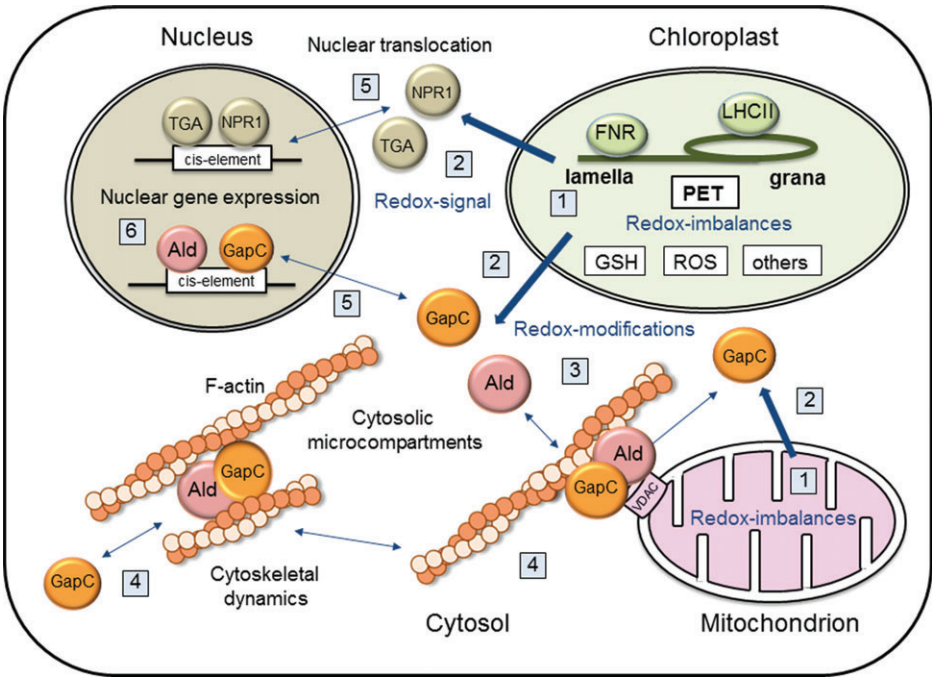

Figure 2 Suggested role of transient microcompartments in retrograde signaling.
The following sequence of events is suggested and shown in the scheme as discussed in this review. Examples are given for their operation in a complex network. 1. Redox-signal resulting from imbalances in chloroplasts or mitochondria (microcompartments in electron transport chains and associated machineries); 2. Transfer of redox signal into cytosol; 3. Redox-modifications of cytosolic proteins (NPR1 and TGA: transcription factors, GapC, Ald: glycolytic enzymes GAPDH, aldolase); 4. Transient formation of cytosolic microcompartments in the cytoskeleton and at outer organellar membranes; 5. Nuclear translocation of cytosolic proteins; 6. Altered nuclear microcompartmentation resulting in changed gene transcription.

complexes involved in reactions transiently assemble and disassemble, forming microcompartments optimized for different forms of electron flow (Dudkina et al., 2006). For more details on these processes, readers are also referred to the review, 'Microcompartments in bioenergetic membranes' in the same issue. The redox state of the PQ pool in the interphotosystem electron transport chain of the thylakoid membrane correlates with many regulatory and signaling processes, providing a mechanism for transducing the redox state of the first electron acceptor in the chain following water oxidation at PSII. One example of redox regulation of microcompartmentation is the control of the 'state transitions', during which the excitation of PSII and PSI is balanced by the transfer of light-harvesting complexes between the two photosystems. There is strong evidence that state transitions are regulated by the redox state of PQ (Mullineaux and Karpinski, 2002; Dietzel et al., 2008). In addition to being regulated, themselves, by redox factors, dynamic changes in PET-component microcompartmentation also impact on the generation of ROS from the PET chain, and the species of ROS produced. Because different free radicals are produced at PSI and PSII (Mittler et al., 2004), any change in the excitation balance between the photosystems, such as that seen in a state transition, will consequently impact on the relative proportion of superoxide and singlet oxygen production. These different radicals have been shown to initiate different signaling cascades (Laloi et al., 2007), and indeed, mutants incapable of state transitions show completely different transcriptional responses to high-light treatment (Bräutigam et al., 2009).

In addition to the redistribution of protein complexes between the separate regions of the thylakoid membrane, activity of the PET chain is also regulated by the recruitment and release of PET chain components that are peripherally bound to the thylakoid. The ferredoxin (Fd):NADP(H) oxidoreductase (FNR) enzyme is present as both soluble and membrane-bound fractions (Hanke et al., 2005), and its recruitment and release from the FNR-binding proteins at the thylakoid is regulated by the pH of the stroma (Alte et al., 2010). The chloroplast pH changes between approximately 6.8 and 8.2 depending on the light intensity, due to the proton pumping associated with PET, providing a rapid response to PET activity.

Redox-dependent regulation of assimilation and dissimilation

Owing to the high protein concentration in the stroma, it has been suggested that some or all of the enzymes of the CO_2-assimilatory Calvin cycle are present in higher heteromeric aggregations and may even be linked to the thylakoids via the CF1-ATPase (Süss et al., 1993). It has been proposed that a complex of five Calvin cycle enzymes, with properties that are distinct from the soluble state, helps increase metabolic flux (Gontero et al., 1988). In order to link the light energy from PET to the endergonic assimilatory processes in the chloroplast stroma, electrons from Fd are diverted to a Fd-thioredoxin reductase (FTR), which reduces the thiol-disulfide exchange protein thioredoxin (Trx) enabling it to modify disulfides on many proteins in a regulatory fashion (Dietz et al., 2002; Schürmann, 2003; Buchanan and Balmers, 2005). This transduces the redox state of PSI to the soluble acceptors in the stroma by reversible posttranslational modification of target enzymes. This electron transfer serves as a light signal, transduced via a soluble electron transport chain consisting of Fd, FTR, and the various Trx that contact and transfer electrons to reduce the disulfide bridges of the target enzymes (Dai, 2000; Schürmann, 2003). While interaction between the electron transport component Fd and the soluble targets via FTR/Trx transmits the basic information that light is available, information about the extent of light activation is contributed by specific metabolites that fine tune this reaction by changing the properties of the interacting partners (Scheibe, 1991).

In most cases of light-dark modulation, posttranslational modifications impact on the redox state, but not the oligomerization state of chloroplast enzymes, changing the properties of soluble enzymes. However, during regulation of the reductive step in the Calvin cycle, catalyzed by the NAD(P)-dependent glyceraldehyde 3-P dehydrogenase (GAPDH, GapA and B), a variable complex formation was found between the two types of subunits. Differences in GAPDH interaction with phosphoribulokinase and CP12, the latter serving as a scaffold in a redox-dependent manner, on the one hand, and with itself to form a hexadecamer of GapA and B, on the other, were also detected. This is connected with altered substrate affinities and, thus, with the actual activation state of the enzyme depending on the metabolic needs (Scheibe et al., 2002). The affinity for effectors and other macromolecules, and thus the propensity for an altered activation state, strongly depends also on the protein concentration, which is extremely high *in situ*, and on the local concentrations of metabolites that serve as activators (Reichert et al., 2000). In general, it seems to be a fundamental property that these redox-dependent short-term changes in activation states are intimately connected with the metabolic state. Consistent with this, local metabolite concentrations appear to affect the redox potentials of the involved cysteine residues (Faske et al., 1995).

Research on metabolons dates back almost 30 years, since then, many examples of metabolic pathways associated with the cytoskeleton or membranes have been analyzed (Ovadi, 1988; Srere, 2000; Winkel, 2004). Here, the formation of such enzyme complexes appears to create microenvironments allowing for efficient catalysis of steps in secondary metabolite biosynthesis, without interfering with basic metabolism. There is also earlier evidence that glycolytic enzymes can be bound to cytoskeletal elements, forming a metabolon with the cytoskeleton serving as a scaffold (Masters, 1996). In similar studies, it has been proposed that the mitochondrial matrix, due to its high protein content, also provides an environment enabling formation of a metabolon containing the Krebs cycle enzymes (Vélot et al., 1997). Such microcompartments might locally provide energy and/or channel metabolites in a directed manner to subcellular sites of specific metabolic demand. Apart from their role in channeling metabolic fluxes, it seems increasing likely that metabolons might also provide the microenvironments for the efficient control of signaling (Zeke et al., 2009).

The role of transient microcompartments in signal transduction

As the input from locally restricted radical production varies depending on the specific situation, it has been suggested that signaling is determined by microenvironments (Terada, 2006). In this section, we will outline the principle behind this concept. Physical organization of the signaling components and their dynamic behavior presumably rely on redox-dependent protein-protein interactions, in a system that is fine-tuned by effector molecules, analogous to that outlined previously for metabolic enzymes, giving rise to highly specific metabolic and signaling pathways.

The formation of transient microcompartments by soluble proteins can be facilitated by scaffold structures. This, indeed, appears to be the case for MAP-kinase cascades in signal transduction chains (Brown and Sacks, 2009). Glycolytic pathway enzymes have also been found associated with cytoskeletal structures (Masters, 1996),

and we have recently shown that GapC and cytosolic aldolase bind to F-actin in a redox-dependent manner, leading to bundling (Wojtera-Kwiczor et al., 2012). As this occurs only under oxidizing conditions, which lead to the inactivation of the enzymes, this alternative function is probably not connected with glycolytic activity, but might, instead, play a role in mediating a redox signal (see Figure 2). The involvement of nitric oxide (NO)-modified cytoskeletal elements in signaling has been suggested, based on the results from various studies (Yemets et al., 2011).

Not only cytoskeletal structures but also membranes of cell organelles can serve as a scaffold for the formation of such transient complexes. Proteomic studies have found that many proteins appear to be associated with the chloroplast outer envelope forming a dynamic interface with the cytosol (Breuers et al., 2011). The chloroplast outer envelope binds actin via CHUP1, and this is essential for blue-light-induced chloroplast movement (Oikawa et al., 2008), while the short chain cp-actin appears to be directly involved in chloroplast movements (Ichikawa et al., 2011). The network of actin microfilaments and binding proteins has been shown to be highly dynamic and is involved in many cellular processes, for example, blue-light-dependent organelle movements [for review, see Higaki et al. (2007)]. Programmed cell death (PCD), both upon biotic stress in the hypersensitive response (HR), and as a developmental step required for certain cells during tissue differentiation, is thought to be actively regulated by cytoskeleton dynamics initiated by specific signals [for review, see Smertenko and Franklin-Tong (2011)]. As another example, ROS/NO signaling involving actin organization appears to affect pollen tube growth in self-incompatibility (Wilkins et al., 2011).

Recently, the transcription factor PHD was found to be associated to the plastid envelope and involved in retrograde signaling from the chloroplast to the nucleus (Sun et al., 2011). Furthermore, the Whirly1 transcription factor translocation from the chloroplast to the nucleus has been suggested to initiate senescence (Isemer et al., 2012). In processes analogous to those at the chloroplast outer envelope, the proteins at the outer mitochondrial membrane have been found to form transient protein complexes. Some of these events involve isoforms of the voltage-dependent anion channel (VDAC) (Duncan et al., 2011). VDAC is a mitochondrial porin and is reported to additionally bind various cytosolic proteins, forming a dynamic interface between these organelles and the cytosol (Colombini, 2004). This is confirmed by the finding that the mitochondria also associate with glycolytic enzymes (Giegé et al., 2003). We suggest that the glycolytic enzymes GapC and aldolase mediate the binding of actin filaments to the mitochondria in a redox-dependent manner (Wojtera-Kwiczor et al., 2012). This hypothesis is confirmed by work with the animal system, where the regulation of apoptosis is dependent on hexokinase binding to VDAC (Azoulay-Zohar et al., 2004), a process that also appears to be redox dependent (Aram et al., 2010). It is tempting to speculate that these structural changes are part of a signal transmission process along the cytosolic substructures that finally lead to an output related to the flow of information to the nucleus as depicted in Figure 2.

Redox-driven responses for adjustment of metabolism, stress response, and development

To understand how the events at the microcompartment level are transduced, we must first examine how the proteins involved can perceive changes in the redox state of the cell. One possible means of perception involves redox-based posttranslational modification of proteins. While Trx m and f are the redox-mediating molecules that regulate metabolic fluxes, the thiol-containing peroxiredoxins serve in radical scavenging systems. These are present in all compartments, and in the chloroplasts during photosynthesis, they are of particular importance for buffering redox imbalances. The central role of 2-Cys-peroxiredoxins in the dynamic response during stress and adaptation is dependent on a variety of protein-protein interactions that interconnect redox imbalances with the required responses (Muthuramalingam et al., 2009). It is suggested that the aggregation state of the chloroplast peroxiredoxins reflecting their state of oxidation enables them to function as sensors for the degree of oxidative stress and as initiators of a signaling cascade (Dietz, 2008).

It is now clear that in oxidative stress situations, not only ROS but also RNS are formed. The origin of NO as a product of arginine metabolism or as a byproduct of nitrate reduction is not completely solved, but its impact during redox signaling is already established. The formation of S-nitrosothiols such as nitrosoglutathione (GSNO) from NO as well as nitrosylation of protein thiols were first identified in human physiology, but have since been established as a general mechanism, which is also of importance in plant stress signaling and immune responses (Wang et al., 2006). In this context, the S-nitrosoglutathione reductase (GSNOR), a plant enzyme originally

classified as GSH-dependent formaldehyde dehydrogenase (FALDH), turns out to be of prime importance in plant stress response and development, as it is required for NO homeostasis (Leterrier et al., 2011).

For long-term acclimation upon all kinds of impacts coming from changes in the environment or as required for developmental needs, changes in plastid gene expression and protein translation as well as in nuclear gene expression are necessary. In order to ensure that chloroplast gene expression and translation are correctly regulated, the redox state of the PET chain must be transduced into a signal within the chloroplast. It has been shown that chloroplast gene expression is dependent on a chloroplast sensor kinase that can be activated *in vitro* by oxidized PQ (Puthiyaveetil et al., 2012), while chloroplast protein translation is regulated in a redox-dependent manner by a protein-disulfide isomerase (Kim and Mayfield, 1997). Recently, it has been found that two fructokinase-like proteins (FLN1 and 2) are activated by Trx z and appear to regulate plastid RNA polymerase, thus, connecting redox-state to plastid gene expression (Arsova et al., 2010).

Because many chloroplast proteins are nuclear encoded, correct acclimation through altered gene transcription depends on a retrograde signal to transmit information to the nucleus. The redox state of the PQ pool is also heavily implicated in the retrograde redox-signaling cascade from the chloroplast to the nucleus (Pfannschmidt et al., 1999; Fey et al., 2005; Tikkanen et al., 2008), a process in which the protein kinase STN7 appears to play a role (Pesaresi et al., 2011). Interestingly, it was shown that under stress, the redox state of the plastids determines nuclear expression of photosynthesis genes, overriding even the metabolic signal from the sugar status (Oswald et al., 2001).

Dynamic microcompartments in redox signaling

In order for the redox-based changes in protein structure – caused by the posttranslational modifications outlined in the previous section – to result in transduction of a signal, they must first cause dynamic changes in the properties and associations of proteins. In this section, we describe how, upon modification, many proteins change their association and localization. In many cases, enzymes with well-described functions are recruited to these dynamic microcompartments, resulting in additional, newly described functions.

As chloroplasts and mitochondria are major sources of redox imbalances, a mode of transmittance across the organelle membranes into the cytosolic compartment is required for signal transduction to the nucleus. Redox regulation can be assumed to occur in most cell compartments, as the required redox-active compounds such as Trx, Grx, GSH/GSSG, Asc/DHA, NAD(P)/H, as well as enzymes for their regeneration are present in chloroplasts, mitochondria, cytosol, endoplasmatic reticulum, and nucleus (Oelze et al., 2008; Foyer and Noctor, 2012). It is very likely that short-lived signaling molecules such as ROS and RNS or changes in the GSH/GSSG or ascorbate/dehydroascorbate ratios are translated into persistent changes that transduce even minor imbalances to achieve transcriptional changes. In this respect, glycolytic GAPDH has been postulated to be a sensor molecule for the detection of H_2O_2 diffusing out of the chloroplast (Hancock et al., 2006). Oxidation of its highly oxidant-sensitive cysteine residues leads to the inactivation and at the same time to changed properties (Holtgrefe et al., 2008). The same phenomenon has been reported for cytosolic aldolase (van der Linde et al., 2011). This has opened the field of 'moonlighting' functions for these glycolytic enzymes (Jeffery, 1999; Moore, 2004), as described below in several examples. Such redox- and metabolite-dependent switching of enzyme functions and localizations have been widely observed, and indeed, both these cytosolic enzymes could be found colocalized with the mitochondria and with actin (Wojtera-Kwiczor et al., 2012). Nuclear localization of cytosolic GAPDH and aldolase is supported by their DNA-binding properties as described for the *NADP-MDH* gene (Hameister et al., 2007). This gene encodes the redox-modified NADP-dependent malate dehydrogenase (MDH) that is part of the malate valve for controlled release of excess electron pressure from chloroplasts under stress (Scheibe, 2004). In addition, *in vivo* bimolecular fluorescence complementation (BiFC) experiments revealed the nuclear localization of both cytosolic enzymes (Holtgrefe et al., 2008; van der Linde et al., 2011).

Finally, the integration of various signals can be achieved by the transduction of redox modifications to influence the components of the protein-phosphorylation cascades (Wang et al., 2010). It is likely that there are points of convergence of the various signal transduction pathways, which allow for a crosstalk to accommodate many different factors and to initiate an appropriate response (Fujita et al., 2006). Many different signaling cascades have already been suggested for the transfer of retrograde chloroplast signals through the cytosol to the nucleus (Nott et al., 2006), and ROS/RNS are assumed

to be a central component of such signal transduction networks between cellular compartments, integrating developmental events and environmental cues (Baudouin, 2011). The central role of H_2O_2 in an 'information flow' between chloroplast and nucleus has been suggested many times, and it is thought to act as an integrator of development and abiotic stress in plant cells (Petrov and Van Breusegem, 2012). As previously discussed, the cytosolic GAPDH is also proposed to act as a H_2O_2 sensor due to the highly reactive cysteine residue in its active site. The various functions and localizations of GAPDH are thought to depend on differential modifications and, subsequently, on complex formation, as shown repeatedly in non-plant systems (Hwang et al., 2009; Sirover, 2011; Tristan et al., 2011).

In many cases, multiple localizations have been shown for transcription factors, metabolic enzymes, redoxins, and GSH. Whether these occur within a signal transduction process in a redox-dependent manner is largely unknown. Currently, an increasing amount of data helps to elucidate the sequence of events that might take place *in vivo* to finally lead to an altered gene expression in the nucleus (Figure 2).

Nuclear microcompartments for coordinated gene transcription

Various types of oxidative cysteine modifications have been demonstrated to occur under oxidative conditions *in vitro*, leading to the inactivation of cytosolic aldolase and GAPDH. It has been shown that these enzymes can also appear in the nuclear compartment, as well as in the cytosol (Holtgrefe et al., 2008; van der Linde et al., 2011), and the transfer of these components into the nucleus may well be dependent on such modifications (Figure 2). In *Medicago sativa*, cytosolic aldolase associates with the MADS box transcription factor NMH7, and it was suggested that this moonlighting function might be involved in nodule development (Páez-Valencia et al., 2008). Redox-based modifications, resulting in the movement of other transcriptional coactivators are well established for non-expressor of PR genes1 (NPR1), a regulator of pathogen defense. NPR1 is localized in the cytoplasm as an oligomer linked through disulfide bridges facilitated by S-nitrosylation, and its reduction by Trx leads to monomerization and transfer to the nucleus (Tada et al., 2008; Yu et al., 2012). There, NPR1 interacts with TGA transcription factors and serves as a coactivator, mediating DNA-binding, and, thus, initiating the transcription of PR genes.

Besides redox-controlled translocation processes into the nucleus, there is also support for the participation of a redox process in shaping transcription factor activity within the nucleus. With the recent findings that ROXYs, land-plant specific glutaredoxins, interact with TGA transcription factors such as PERIANTHIA (PAN) in the nucleus to achieve normal flower development, this implies a novel function for a glutaredoxin-controlled process in the nucleus (Li et al., 2009, 2011; Ziemann et al., 2009; Murmu et al., 2010). ROXY19/GRX480 has been shown to negatively regulate the plant defense processes and interacts with TGA2 and TGA6 (Ndamukong et al., 2007). Moreover, ROXY19/GRX480 exerts a repressive function on the expression of a reporter gene in a transient expression system, which depends on the presence of a functional GSH-binding site (Zander et al., 2011). For TGA1, it was previously shown that Cys-260 and Cys-266 in TGA1 form an intramolecular disulfide bridge under oxidizing conditions, which is reduced in a redox-dependent manner (Després et al., 2000). Most recently, additional complex posttranslational modifications of Cys residues in purified, recombinant TGA1 proteins have been reported, including the formation of an intramolecular disulfide bridge between Cys-172 and Cys-287 as well as S-nitrosylation or S-glutathionylation of Cys-260 and Cys-266 (Lindermayr et al., 2010).

The striking findings that interactions between proteins from the same classes, namely, TGAs, ROXYs, and NPRs, exert crucial functions in two seemingly unrelated pathways, namely, stress response and plant development, could indicate another point of integration of various types of redox signals and emphasizes the importance of the participation of thiol switches in the nucleus (Li and Zachgo, 2009). The overexpression of ROXY1 causes an increased ROS production, which could contribute to the higher pathogen susceptibility in these plants (Wang et al., 2009), providing support for a crosstalk between the two processes. Given the availability of GSH in the nucleus, this enables a transfer of redox poise to the transcription factors and indicates the existence of redox-regulated microcompartments controlling transcription in the nucleus (Diaz Vivancos et al., 2010).

How could these nuclear microenvironments be envisioned? From animal cells, it is known that the individual chromosomes occupy distinct positions in the nucleus, referred to as chromosome territories. As a result of different compaction levels, different chromosome segments adopt a complex organization and topography within their chromosome territory

(Meldi and Brickner, 2011). Gene-rich regions tend to be oriented toward the nuclear interior, whereas gene-poor regions tend to be oriented toward the periphery (Cremer et al., 2006). Indications for a nuclear architecture, with three-dimensional networks of genomic loci in the nuclei of *Drosophila* and mammals imply the existence of a previously unexplored spatial level of gene regulation. The initiation of transcription depends on a coordinated recruitment of the participating multimeric protein complex of the initiation machinery, comprising transcription factors, TATA-binding proteins, and RNA polymerase II. Cytoskeletal proteins such as actin and myosin might have roles in the translocation of nuclear genes, further emphasizing their importance in transcription and shaping a dynamic nuclear architecture (de Lanerolle et al., 2005; Simon and Wilson, 2011). Given the evolutionary conservation between yeast and animal cells, it will be interesting to investigate if chromosomal territories also exist in plant nuclei. Furthermore, this will then allow for unraveling the contribution of redox processes to open DNA-binding sites on different coregulated neighboring chromosomes and to mediate the binding capacity of the transcription factors to *cis*-regulatory DNA elements of target genes – thereby ultimately affecting gene expression in these microcompartments.

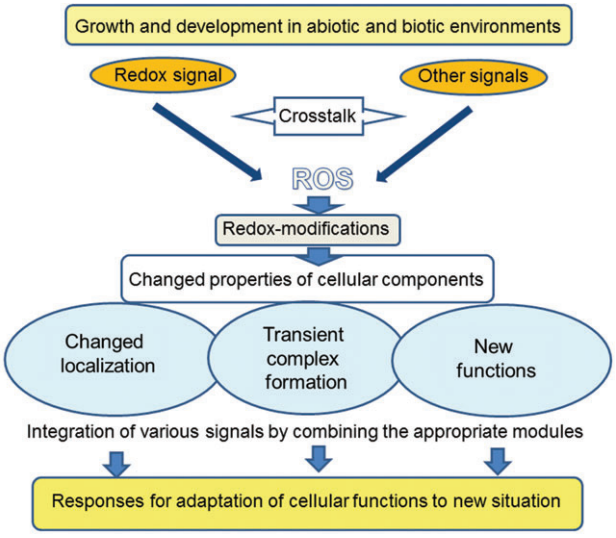

Figure 3 Scheme of the events leading to adaptation of a cell/an organism.
Redox changes and/or other cues initiate modifications and subsequent changes of cellular components that, in turn, lead to an altered nuclear gene expression. The steps during signal transduction include the formation of transient functional modules that can interact and integrate multiple signals to achieve the responses/changes that are required for adapted metabolism under adverse conditions and for developmental processes.

Outlook

In the future, it will be essential to gain insight into the mechanism that transfers redox-dependent signals through various cellular compartments *in vivo*. This demands an investigation of the relevant processes under conditions of high protein concentration, where regulatory redox modification will have maximum impact on weak and transient interactions between proteins. Such information will be essential to understand how short-lived signals from the chloroplasts and mitochondria are stabilized, transduced, and executed in order to reach the site of action in nuclear gene expression. As suggested by Nooren and Thornton (2003), it will be crucial to analyze the interface structures involved in protein-protein interactions against the background of the local environment in the cellular subcompartments, where metabolites, ionic strength, and redox potentials reflect the current situation. These parameters influence such interactions and help to transport this information to the site of its realization. It seems likely that the basic principle is shared with that of redox regulation of chloroplast enzymes (Scheibe, 1991), namely, that each redox-dependent interaction is fine tuned by the local microenvironment, the changing composition of which reflects not only the state of branched metabolic pathways but also the complex network of information transfer (Figure 3).

A similar picture has also arisen for animal cells in the last decades, with the redox modification of proteins implicated in a multitude of signaling processes (Jacob et al., 2006; Go and Jones, 2008; Paulsen and Carroll, 2009; Marozkina and Gaston, 2012). In particular, S-glutathionylation of proteins was found to be involved in many animal diseases (Townsend, 2007). The integration of metabolic processes with signaling networks, based on local and transient redox changes, appears to be a principle that is common to all organisms (Grüning et al., 2010). Learning more about these multiple signaling cascades and their transient interactions will help us to understand this complex network, where many pieces of information that change in space and in time are integrated, such that the plants can flexibly respond to any kind of impact (Leister, 2012). Because plants are sessile photoautotrophic organisms, they are at the mercy of variable light conditions and subject to many different

environmental impacts/cues. In fact, redox-dependent steps have been identified in many of these abiotic and biotic stress situations (Scheibe and Beck, 2011; Scheibe and Dietz, 2012), as well as in many steps during plant development, starting from cell proliferation through all differentiation events and senescence, until cell death (Potters et al., 2010).

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References

Alte, F., Stengel, A., Benz, J.P., Petersen, E., Soll, J., Groll, M., and Bolter, B. (2010). Ferredoxin:NADPH oxidoreductase is recruited to thylakoids by binding to a polyproline type II helix in a pH-dependent manner. Proc. Natl. Acad. Sci. USA *107*, 19260–19265.

Aram, L., Geula, S., Arbel, N., and Shoshan-Barmatz, V. (2010). VDAC1 cysteine residues: topology and function in channel activity and apoptosis. Biochem. J. *427*, 445–454.

Arsova, B., Hoja, U., Wimmelbacher, M., Greiner, E., Üstün, S., Melzer, M., Petersen, K., Lein, W., and Börnke, F. (2010). Plastidial thioredoxin z interacts with two fructokinase-like proteins in a thiol-dependent manner: evidence for an essential role in chloroplast development in *Arabidopsis* and *Nicotiana benthamiana*. Plant Cell *22,* 1498–1515.

Azoulay-Zohar, H., Israelson, A., Abu-Hamad, S., and Shohan-Barmatz, V. (2004). In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. Biochem. J. *377*, 347–355.

Barajas-López, J.D., Blanco, N.E., and Strand, Å. (2012). Plastid-to-nucleus communication, signals controlling the running of the plant cell. Biochim. Biophys. Acta, doi: 10.1016/j.bbamcr.2012.06.020 (in press).

Baudouin, E. (2011). The language of nitric oxide signaling. Plant Biol. *13*, 233–242.

Bräutigam, K., Dietzel, L., Kleine, T., Stroher, E., Wormuth, D., Dietz, K.-J., Radke, D., Wirtz, M., Hell, R., Dormann, P., et al. (2009). Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in Arabidopsis. Plant Cell *21*, 2715–2732.

Breuers, F.K.H., Bräutigam, A., and Weber, A.P.M. (2011). The plastid outer envelope – a highly dynamic interface between plastid and cytoplasm. Front. Plant Sci. *2*, article 97.

Brown, M.D. and Sacks, D.B. (2009). Protein scaffolds in MAP kinase signaling. Cell. Signal. *21*, 462–469.

Buchanan, B.B. and Balmers, Y. (2005). Redox regulation: a broadening horizon. Annu. Rev. Plant Biol. *56*, 187–220.

Buchanan, B.B., Holmgren, A., Jacquot, J.-P., and Scheibe, R. (2012). Fifty years in the thioredoxin field and a bountiful harvest. Biochim. Biophys. Acta *1820*, 1822–1829.

Colombini, M. (2004). VDAC: the channel at the interface between mitochondria and the cytosol. Mol. Cell. Biochem. *256/257*, 107–115.

Cremer, T., Cremer, M., Dietzel, S., Müller. S., Solovei, I., and Fakan, S. (2006). Chromosome territories – a functional nuclear landscape. Curr. Opin. Cell Biol. *18*, 307–316.

Dai, S. (2000). Redox signaling in chloroplasts: cleavage of disulfides by an iron-sulfur cluster. Science *287,* 655–658.

de Lanerolle, P., Johnson, T., and Hofmann, W.A. (2005). Actin and myosin I in the nucleus: what next? Nat. Struct. Mol. Biol. *12*, 742–746.

Després, C., DeLong, C., Glaze, S., Liu, E., and Fobert, P.R. (2000). The Arabidopsis NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell *12*, 279–290.

Diaz Vivancos, P., Wolff, T., Markovic, J., Pallardó, F.V., and Foyer, C.H. (2010). A nuclear glutathione cycle within the cell cycle. Biochem. J. *431*, 169–178.

Dietz, K.-J. (2008). Redox signal integration: from stimulus to networks and genes. Physiol. Plant. *133*, 459–468.

Dietz, K.-J., Link, G., Pistorius, E.K., and Scheibe, R. (2002). Redox regulation in oxigenic photosynthesis. Prog. Bot. *63*, 207–245.

Dietzel, L., Bräutigam, K., and Pfannschmidt, T. (2008). Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry—functional relationships between short-term and long-term light quality acclimation in plants. FEBS J. *275*, 1080–1088.

Dudkina, N.V., Heinemeyer, J., Sunderhaus, S., Boekema, E.J., and Braun, H.-P. (2006). Respiratory chain supercomplexes in the plant mitochondrial membrane. Trends Plant Sci. *11*, 232–240.

Duncan, O., Taylor, N.L., Carrie, C., Eubel, H., Kubiszewski-Jakubiak, S., Zhang, B., Narsai, R., Millar, A.H., and Whelan, J. (2011). Multiple lines of evidence localize signaling, morphology, and lipid biosynthesis machinery to the mitochondrial outer membrane of Arabidopsis. Plant Physiol. *157*, 1093–1113.

Ellis, R.J. and Minton, A.P. (2006). Protein aggregation in crowded environments. Biol. Chem. *387*, 485–497.

Faske, M., Holtgrefe, S., Ocheretina, O., Meister, M., Backhausen, J.E., and Scheibe, R. (1995). Redox equilibria between the regulatory thiols of light/dark-modulated enzymes and dithiothreitol: fine-tuning by metabolites. Biochim. Biophys. Acta *1247*, 135–142.

Fey, V., Wagner, R., Bräutigam, K., Wirtz, M., Hell, R., Dietzmann, A., Leister, D., Oelmüller, R., and Pfannschmidt, T. (2005). Retrograde plastid redox signals in the expression of nuclear genes for chloroplast proteins of *Arabidopsis thaliana*. J. Biol. Chem. *280*, 5318–5328.

Foyer, C.H. and Noctor, G. (2011). Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. *155*, 2–18.

Foyer, C.H. and Noctor, G. (2012). Managing the cellular redox hub in photosynthetic organisms. Plant Cell Environ. *35*, 199–201.

Fujino, G., Noguchi, T., Takeda, K., and Ichijo, H. (2006). Thioredoxin and protein kinases in redox signaling. Sem. Cancer Biol. *16*, 427–435.

Fujita M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., and Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr. Opin. Plant Biol. *9*, 436–442.

Giegé, P., Heazlewood, J.L., Roessner-Tunali, U., Millar, A.H., Fernie, A.R., Leaver, C.J., and Sweetlove, L.J. (2003). Enzymes of glycolysis are functionally associated with the mitochondrion in Arabidopsis cells. Plant Cell *15*, 2140–2151.

Go, Y.-M. and Jones, D.P. (2008). Redox compartmentalization in eukaryotic cells. Biochim. Biophys. Acta *1780*, 1273–1290.

Gontero, B., Cárdenas, M.L., and Ricard, J. (1988). A functional five-enzyme complex of chloroplasts involved in the Calvin cycle. Eur. J. Biochem. *173*, 437–443.

Goodsell, D.S. (1991). Inside a living cell. Trends Biochem. Sci. *16*, 203–206.

Grüning, N.-M., Lehrach, H., and Ralser, M. (2010). Regulatory crosstalk of the metabolic network. Trends Biochem. Sci. *35*, 220–227.

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol. *141*, 312–322.

Hameister, S., Becker, B., Holtgrefe, S., Linke, V., Backhausen, J.E., and Scheibe, R. (2007). Transcriptional regulation of the NADP-dependent malate dehydrogenase: comparative genetics and identification of DNA-binding proteins. J. Mol. Evol. *65*, 437–455.

Hancock, J., Desikan, R., Harrison, J., Bright, J., Hooley, R., and Neill, S. (2006). Doing the unexpected: proteins involved in hydrogen peroxide perception. J. Exp. Bot. *57*, 1711–1718.

Hanke, G.T., Holtgrefe, S., König, N., Strodtkötter, I., Voss, I., and Scheibe, R. (2009). Use of transgenic plants to uncover strategies for maintenance of redox homeostasis during photosynthesis. Adv. Bot. Res. *52*, 209–251.

Hanke, G.T., Okutani, S., Satomi, Y., Takao, T., Suzuki, A., and Hase, T. (2005). Multiple iso-proteins of FNR in Arabidopsis: evidence for different contributions to chloroplast function and nitrogen assimilation. Plant Cell Environ. *28*, 1146–1157.

Higaki, T., Sano, T., and Hasezawa, S. (2007). Actin microfilament dynamics and actin side-binding proteins in plants. Curr. Opin. Plant Biol. *10*, 549–556.

Holtgrefe, S., Gohlke, J., Druce, S., Starmann, J., Klocke, S., Altmann, B., Wojtera, J., Linke, V., Lindermayr, C., and Scheibe, R. (2008). Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modification. Physiol. Plant. *133*, 211–228.

Hwang, N.R., Yim, S.-H., Kim, Y.M., Jeong, J., Song, E.J., Lee, Y., Lee, J.H., Choi, S., and Lee, K.-J. (2009). Oxidative modifications of glyceraldehyde-3-phosphate dehydrogenase play a key role in its multiple cellular functions. Biochem. J. *423*, 253–264.

Ichikawa, S., Yamada, N., Suetsugu, N., Wada, M., and Kadota, A. (2011). Red light, Phot1 and JAC1 modulate Phot2-dependent reorganization of chloroplast actin filaments and chloroplast avoidance movement. Plant Cell Physiol. *52*, 1422–1432.

Isemer, R., Mulisch, M., Schäfer, S., Kirchner, H.U., Koop, K., and Krupinska, K. (2012). Recombinant Whirly1 translocates from transplastomic chloroplasts to the nucleus. FEBS Lett. *583*, 85–88.

Jacob, C., Knight, I., and Winyard, P.G. (2006). Aspects of the biological redox-chemistry of cysteines: from simple redox responses to sophisticated signaling pathways. Biol. Chem. *387*, 1385–1397.

Jeffery, C.J. (1999). Moonlighting proteins. Trends Biochem. Sci. *24*, 8–11.

Kim, J. and Mayfield, S.P. (1997). Protein disulfide isomerase as a regulator of chloroplast translational activation. Science *278*, 1954–1957.

Kwak, J.M., Mori, I.C., Pei, Z.-M., Leonhardt, N., Torres, M.A., Dangl, J.L., Bloom, R.E., Bodde, S., Jones, J.D.G., and Schroeder, J.I. (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO *22*, 2623–2633.

Laloi, C., Stachowiak, M., Pers-Kamczyc, E., Warzych, E., Murgia, I., and Apel, K. (2007). Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA *104*, 672–677.

Leister, D. (2012). Retrograde signaling in plants: from simple to complex scenarios. Front. Plant Sci. *3*, 135.

Leterrier, M., Chaki, M., Airaki, M., Valderrama, R., Palma, J.M., Barroso, J.B., and Corpas, F.J. (2011). Function of S-nitroso-gluthione reductase (GSNOR) in plant development and under biotic/abiotic stress. Plant Signal. Behav. *6*, 789–793.

Li, S. and Zachgo, S. (2009). Glutaredoxins in development and stress responses of plants. Adv. Bot. Res. *52*, 335–403.

Li, S., Lauri, A., Ziemann, M., Busch, A., Bhave, M., and Zachgo, S. (2009). Nuclear activity of ROXY1, a glutaredoxin interacting with TGA factors, is required for petal development in *Arabidopsis thaliana*. Plant Cell *21*, 429–441.

Li, S., Gutsche, N., and Zachgo, S. (2011). The ROXY1 C-terminal L**LL motif is essential for the interaction with TGA transcription factors. Plant Physiol. *157*, 2056–2068.

Lindermayr, C., Sell, S., Müller B., Leister, D., and Durner, J. (2010). Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. Plant Cell *22*, 2894–2907.

Marozkina, N.V. and Gaston, B. (2012). S-Nitrosylation signaling regulates cellular protein interactions. Biochim. Biophys. Acta *1820*, 722–729.

Masters, C. (1996). On the role of the cytoskeleton in metabolic compartmentation. In: The Cytoskeleton. Role in Cell Physiology, Vol. 2, J. Hesketh and I. Pryme, eds. (Greenwich Conn.: JAI Press Inc.), pp. 1–30.

Meldi, L. and Brickner, J.H. (2011). Compartmentation of the nucleus. Trends Cell Biol. *21*, 701–708.

Minton, A.P. (1981). Excluded volume as a determinant of macromolecular structure and reactivity. Biopolymers *20*, 2093–2120.

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends Plant Sci. *9*, 490–498.

Moore, B. (2004). Bifunctional and moonlighting enzymes: lighting the way to regulatory control. Trends Plant Sci. *9*, 221–228.

Mullineaux, P. and Karpinski, S. (2002). Signal transduction in response to excess light: getting out of the chloroplast. Curr. Opin. Plant Biol. *5*, 43–48.

Murmu, J., Bush, M., DeLong, C., Li, S., Xu, M., Khan, M., Fobert, P., Zachgo, S. and Hepworth, S.R. (2010). Arabidopsis bZIP transcription factors TGA9 and TGA10 interact with floral glutaredoxins ROXY1 and ROXY2 and are redundantly required for anther development. Plant Physiol. *154*, 1492–1504.

Muthuramalingam, M., Seidel, T., Laxa, M., Nunes de Miranda, S.M., Gärtner, F., Ströher, F., Kandlbinder, A., and Dietz, K.-J. (2009). Multiple redox and non-redox interactions define 2-Cys peroxiredoxin as a regulatory hub in the chloroplast. Mol. Plant *2*, 1273–1288.

Ndamukong, I., Abdallat, A.A., Thurow, C., Fode, B., Zander, M., Weigel, R., and Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. Plant J. *50*, 128–139.

Neill, S.J., Desikan, R., Clarke, A., Hurst, R.D., and Hancock, J.T. (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. J. Ex. Bot. *53*, 1237–1247.

Nooren, I.M.A. and Thornton, J.M. (2003). Structural characterization and functional significance of transient protein-protein interactions. J. Mol. Biol. *325*, 991–1018.

Nott, A., Jung, H.-S., Koussevitzky, S., and Chory, J. (2006). Plastid-to-nucleus retrograde signaling. Annu. Rev. Plant Biol. *57*, 739–759.

Oelze, M.-L., Kandlbinder, A., and Dietz, K.-J. (2008). Redox regulation and overreduction control in the photosynthesizing cell: complexity in redox regulatory networks. Biochim. Biophys. Acta *1780*, 1261–1272.

Oikawa, K., Yamasato, A., Kong, S.-G., Kasahara, M., Nakai, M., Takahashi, F., Ogura, Y., Kagawa, T., and Wada, M. (2008). Chloroplast outer envelope protein CHUP1 is essential for chloroplast anchorage to the plasma membrane and chloroplast movement. Plant Physiol. *148*, 829–842.

Oswald, O., Martin, T., Dominy, P.J., and Graham, I.A. (2001). Plastid redox state and sugars: interactive regulators of nuclear-encoded photosynthetic gene expression. Proc. Natl. Acad. Sci. USA *98*, 2047–2052.

Ovadi, J. (1988). Old pathways – new concept: control of glycolysis by metabolite-modulated dynamic enzyme associations. Trends Biochem. Sci. *13,* 486–490.

Páez-Valencia, J., Valencia-Mayoral, P., Sánchez-Gómez, C., Contreras-Ramos, A., Hernández-Lucas, I., Martínez-Barajas, E., and Gamboa-deBuen, A. (2008). Identification of fructose-1,6-bisphosphate aldolase cytosolic class I as an NMH7 MADS domain associated protein. Biochem. Biophys. Res. Commun. *376*, 700–705.

Paulsen, C.E. and Carroll, K.S. (2009). Orchestrating redox signaling networks through regulatory cysteine switches. ACS Chem. Biol. *5*, 47–62.

Pesaresi, P., Pribil, M., Wunder, T., and Leister, D. (2011). Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. Biochim. Biophys. Acta *1807*, 887–896.

Petrov, V.D. and Van Breusegem, F. (2012). Hydrogen peroxide – a central hub for information flow in plant cells. AoB Plants, doi: 10.1093/aobpla/pls014.

Pfannschmidt, T., Nilsson, A., and Allen, J.F. (1999). Photosynthetic control of chloroplast gene expression. Nature *397*, 625–628.

Potters, G., Horemans, N., and Jansen, M.A.K. (2010). The cellular redox state in plant stress biology – a charging concept. Plant Physiol. Biochem. *48*, 292–300.

Puthiyaveetil, S., Ibrahim, I.M., and Allen, J.F. (2012). Oxidation-reduction signalling components in regulatory pathways of state transitions and photosystem stoichiometry adjustment in chloroplasts. Plant Cell. Environ. *35*, 347–359.

Reichert, A., Baalmann, E., Vetter, S., Backhausen, J.E., and Scheibe, R. (2000). Activation properties of the redox-modulated chloroplast enzymes glyceraldehyde 3-phosphate dehydrogenase and fructose 1,6-bisphosphatase. Physiol. Plant. *110*, 330–341.

Scheibe, R. (1991). Redox-modulation of chloroplast enzymes. A common principle for individual control. Plant Physiol. *96*, 1–3.

Scheibe, R. (2004). Malate valves to balance cellular energy supply. Physiol. Plant. *120*, 21–26.

Scheibe, R. and Beck, E. (2011). Drought, desiccation and oxidative stress. In: Desiccation Tolerance of Plants. Ecological Sudies, Vol. 215, U. Lüttge, E. Beck and D. Bartels, eds. (Berlin-Heidelberg: Springer Press), pp. 209–231.

Scheibe, R. and Dietz, K.-J. (2012). Reduction-oxidation network for flexible adjustment of cellular metabolism in photoautotrophic cells. Plant Cell Environ. *35*, 202–216.

Scheibe, R., Wedel, N., Vetter, S., Emmerlich, V., and Sauermann, S.-M. (2002). Coexistence of two regulatory NADP-glyceraldehyde 3-P dehydrogenase complexes in higher plant chloroplasts. Eur. J. Biochem. *269*, 5617–5624.

Scheibe, R., Backhausen, J.E., Emmerlich, V., and Holtgrefe, S. (2005). Strategies to maintain redox homeostasis during photosynthesis under changing conditions. J. Exp. Bot. *56*, 1481–1489.

Schürmann, P. (2003). Redox signaling in the chloroplast: the ferredoxin/thioredoxin system. Antioxid. Redox Signal. *5*, 69–78.

Simon, D.N. and Wilson, K.L. (2011). The nucleoskeleton as a genome-associated dynamic 'network of networks'. Nat. Rev. Mol. Cell Biol. *12*, 695–708.

Sirover, M.A. (2011). On the functional diversity of glyceraldehyde-3-phosphate dehydrogenase: biochemical mechanisms and regulatory control. Biochim. Biophys. Acta *1810*, 741–751.

Smertenko, A. and Franklin-Tong, V.E. (2011). Organisation and regulation of the cytoskeleton in plant programmed cell death. Cell Death Diff. *18*, 1263–1270.

Spadaro, D., Yun, B.-W., Spoel, S.H., Chu, C., Wang, Y.-Q., and Loake, G.J. (2009). The redox switch: dynamic regulation of protein function by cysteine modifications. Physiol. Plant. *138*, 360–371.

Spoel, S.H. and Loake, G.J. (2011). Redox-based protein modifications: the missing link in plant immune signalling. Curr. Opin. Plant Biol. *14*, 358–364.

Srere, P.A. (2000). Macromolecular interactions: tracing the roots. Trends Biochem. Sci. *25*, 150–153.

Sun, X., Feng, P., Xu, X., Guo, H., Ma, J., Chi, W., Lin, R., Lu, C., and Zhang, L. (2011). A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. Nat. Commun. *2*, 477.

Suzuki, N., Koussevitzky, S., Mittler, R., and Miller, G. (2011). ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ. *35,* 259–270.

Süss, K.-H., Arkona, C., Manteufel, R., and Adler, K.l. (1993). Calvin cycle multienzyme complexes are bound to chloroplast thylakoid membranes of higher plants i*n situ*. Proc. Natl. Acad. Sci. USA *90*, 5514–5518.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., and Dong, X. (2008). Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. Science *321*, 952–956.

Terada, L.S. (2006). Specificity in reactive oxidant signaling: think globally, act locally. J. Cell Biol. *174*, 615–623.

Tikkanen, M., Nurmi, M., Suorsa, M., Danielsson, R., Mamedov, F., Styring, S., and Aro, E.M. (2008). Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants. Biochim. Biophys. Acta *1777*, 425–432.

Townsend, D.M. (2007). S-Glutathionylation. Mol. Interv. *7*, 313–324.

Tristan, C., Shahani, N., Sedlak, T.W., and Sawa, A. (2011). The diverse functions of GAPDH: views from different subcellular compartments. Cell. Signal. *23*, 317–323.

van der Linde, K., Gutsche, N., Leffers, H.-M., Lindermayr, C., Müller, B., Holtgrefe, S., and Scheibe, R. (2011). Regulation of plant cytosolic aldolase functions by redox-modifications. Plant Physiol. Biochem. *49*, 946–957.

Vélot, C., Mixon, M.B., Teige, M., and Srere, P.A. (1997). Model of a quinary structure between Krebs TCA cycle enzymes: a model for the metabolon. Biochemistry *36*, 14271–14276.

Wang, Y., Yun, B.-W., Kwon, EJ., Hong, J.K., Yoon, J., and Loake, G.J. (2006). S-Nitrosylation: an emerging redox-based post-translational modification in plants. J. Exp. Bot. *57*, 1777–1784.

Wang, Z., Xing, S., Birkenbihl, R.P., and Zachgo, S. (2009). Conserved functions of Arabidopsis and rice CC-type glutaredoxins in flower development and pathogen response. Mol. Plant *2*, 323–335.

Wang, P., Du, Y., Ren, D., and Song, C.-S. (2010). Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis. Plant Cell *22*, 2981–2998.

Wilkins, K.A., Bancroft, J., Bosch, M., Ings, J., Smirnoff, N., and Franklin-Tong, V.E. (2011). Reactive oxygen species and nitric oxide mediate actin reorganization and programmed cell death in the self-incompatibility response of Papaver. Plant Physiol. *156*, 404–416.

Winkel, B.S.J. (2004). Metabolic channeling in plants. Annu. Rev. Plant Biol. *55*, 85–107.

Wojtera-Kwiczor, J., Gross, F., Leffers, H.-M., Kang, M., Schneider, M., and Scheibe, R. (2012). Transfer of a redox-signal through the cytosol by redox-dependent microcompartmentation of glycolytic enzymes at mitochondria and actin cytoskeleton. Front. Plant Sci. doi: 10.3389/fpls.2012.00284.

Yemets, A.I., Krasylenko, Y.A., Lytvyn, D.I., Sheremet, Y.A., and Blume, Y.B. (2011). Nitric oxide signaling via cytoskeleton in plants. Plant Sci. *181*, 545–554.

Yu, M., Yun, B.-W., Spoel, S.H., and Loake, G.J. (2012). A sleigh ride through the SNO: regulation of plant immune function by protein S-nitrosylation. Curr. Opin. Plant Biol. *15*, 424–430.

Zander, M., Chen, S., Imkampe, J., Thurow, C., and Gatz, C. (2011). Repression of the *Arabidopsis thaliana* jasmonic acid/ethylene-induced defense pathway by TGA-interacting glutaredoxins depends on their C-terminal ALWL motif. Mol. Plant *5*, 831–840.

Zeke, A., Lukács, M., Lim, W.A., and Reményi A. (2009). Scaffolds: interaction platforms for cellular signaling circuits. Trends Cell Biol. *19*, 364–374.

Ziemann, M., Bhave, M., and Zachgo, S. (2009). Origin and diversification of land plant CC-type glutaredoxins. Genome Biol. Evol. *1*, 1–12.

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