Ascorbate and Glutathione: The Heart of the Redox Hub[1]

Christine H. Foyer* and Graham Noctor

Centre for Plant Sciences, Faculty of Biology, University of Leeds, Leeds LS2 9JT, United Kingdom (C.H.F.); and Institut de Biologie des Plantes, UMR CNRS 8618, Université de Paris Sud 11, 91405 Orsay cedex, France (G.N.)

The discovery that there is a close relationship between ascorbate and glutathione dates from soon after the characterization of the chemical formulae of the two molecules (Szent-Györgyi, 1931; Hopkins and Morgan, 1936). Similarly, it has long been known that thylakoids can generate hydrogen peroxide (H_2O_2; Mehler, 1951). Following the discovery of superoxide and superoxide dismutase in mammalian cells (McCord and Fridovich, 1969) and their subsequent discovery and study in chloroplasts (Allen and Hall, 1973; Asada et al., 1974), it became obvious that systems must exist to metabolize H_2O_2 produced in this organelle. Once it was accepted that the peroxisomal enzyme, catalase, was not found in the chloroplast, a predominant idea was that chloroplast H_2O_2 must escape to be metabolized by this enzyme or by "guaiacol-type" peroxidases for which no specific substrate had been identified. However, the discovery of thiol-disulfide exchange as a significant mechanism of enzyme regulation led to the finding that H_2O_2 could inactivate photosynthetic metabolism and activate respiratory pathways (Kaiser, 1979; Charles and Halliwell, 1980). Within the context of these exciting developments in oxygen biochemistry, Barry Halliwell, a newly appointed young lecturer at Kings College, London, sought to resolve the issue of how H_2O_2 was metabolized in chloroplasts by assigning his first Ph.D. student, Christine Foyer, to this task. Based on an initial hypothesis that ascorbate and glutathione had the potential to act in detoxification, it was shown that both metabolites and enzymes linking NADPH, glutathione, and ascorbate were found in isolated chloroplast preparations (Foyer and Halliwell, 1976, 1977), and a simple metabolic scheme was proposed (Fig. 1). Even at that time, it was considered that ascorbate oxidase could act as a terminal oxidase and, thus, as a sink for reducing power. However, no specific ascorbate- or glutathione-dependent peroxidase had been identified in plants. The proposed role of ascorbate and glutathione in H_2O_2 metabolism in chloroplasts led to the successful identification of thylakoid-bound and soluble stromal ascorbate peroxidase (APX; Groden and Beck, 1979; Kelly and Latzko, 1979). It was subsequently shown that ascorbate could also be regenerated in the chloroplast by other mechanisms depending on ferredoxin or NADPH (Asada, 1999). Now known as the ascorbate-glutathione or "Foyer-Halliwell-Asada" pathway, the resulting scheme is recognized to be a key player in H_2O_2 metabolism in both animals and plants. Components of this pathway have been shown to be present in animals and in the plant cell, cytosol, mitochondria, and peroxisomes as well as the chloroplast (Edwards et al., 1991; Mittler and Zilinskas, 1991; Jiménez et al., 1997). Here, we explore current concepts on the functions of ascorbate and glutathione in H_2O_2 metabolism and signaling, but also in the wider contexts of plant development and environmental responses. We pay particular attention to studies in which the status of ascorbate and glutathione themselves has been manipulated. Such changes may be linked to or independent of modified activity of dependent enzymes, just as the activity of ascorbate- or glutathione-dependent components may be modified without sustained, marked changes in ascorbate or glutathione status. Among key current questions is the nature of the mechanisms that link changes in ascorbate and glutathione status to downstream signaling, and we discuss these in the light of recent advances, notably information generated from genetically based studies of Arabidopsis (*Arabidopsis thaliana*).

OXYGEN TOXICITY AND CONCEPTS OF OXIDATIVE STRESS

The discovery of superoxide took place within a widespread appreciation of oxygen toxicity. Thus, initial concepts regarding reactive oxygen species (ROS) simply extended this idea to one of heightened reactivity. According to this concept, the formation of ROS merely increased the capacity of oxygen to cause damage to cells. However, the term "oxidative stress" did not become widely used until the 1980s, and the major focus on ROS and photosynthesis during the 1970s was as inevitable, indeed necessary, by-products of the photosynthetic electron transport chain that played an important role in regulation (Foyer

[1] This work was supported by the French Agence Nationale de la Recherche-Genoplante project "Redoxome" and Agence Nationale de la Recherche project "Vulnoz" (Orsay) and by the European Union Marie-Curie Initial Training Network "Chloroplast Signals" project (Leeds, Orsay).

* Corresponding author; e-mail c.foyer@leeds.ac.uk.

www.plantphysiol.org/cgi/doi/10.1104/pp.110.167569

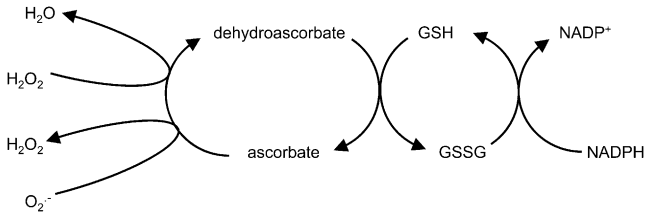

Figure 1. The chloroplast ascorbate-glutathione cycle as proposed in 1976. Scheme adapted from Foyer and Halliwell (1976).

et al., 1990). The term oxidative stress still lacks a precise definition, but the key hallmarks are (1) increased oxidative load (enhanced ROS production); (2) the potential for uncontrolled oxidation due to rates of production exceeding rates of metabolism; (3) oxidative damage to cellular components, which assumes that the rate of oxidation exceeds repair or replacement; causing (4) the accumulation of damaged cellular components that somehow lead to loss of function and eventual death. Despite the vague nature of some of these notions, they have become embedded in the psyche of researchers in this area and have been important both in driving research orientation and strategy and in determining data interpretation. It was within this paradigm that over a decade ago, we reviewed the roles of ascorbate and glutathione and considered that their main functions revolved around "keeping active oxygen under control" (Noctor and Foyer, 1998). In the intervening years, an explosion of research on the signaling functions of ROS in conjunction with ascorbate and glutathione (Fig. 2) has confirmed this notion while at the same time significantly extending it to one of redox signaling (Foyer and Noctor, 2005a, 2005b, 2009). In this review, we propose the concept of ascorbate and glutathione as key players in a redox hub that integrates metabolic information and environmental stimuli to tone responses within the cellular signaling network. We discuss recent data and emerging concepts on the roles of ascorbate and glutathione in important physiological processes such as cell division, cell death, light signaling, and pathogen responses, with a focus on the following issues. What is a redox hub? Within such a hub, how interdependent or independent are ascorbate and glutathione? How important are modifications in their status in transmitting ROS signals, and how crucial are the resulting changes in processes such as cell death? What is their significance as signals in the absence of ROS-driven changes in redox state? How could any of this signaling occur from a mechanistic point of view?

DEFINING THE REDOX HUB

The Collins English dictionary defines the word "hub" as either the central part of a wheel or a focal point. Within this concept, a redox hub can be viewed as an interconnected core of reactions and components able to mediate cross talk between essential bioenergetic processes and signaling pathways. Why should such a redox hub exist? First, all life on earth is driven by reduction-oxidation reactions, in which energy is extracted from electron flow between compounds of differing electrochemical potential. Second, for cells to exist, they have had to evolve mechanisms to control redox potential spans in the soluble phase of the cell, in which biosynthetic processes occur and signals are transduced (Foyer and Noctor, 2005a, 2009). Third, plant growth occurs through developmentally plastic programs within a context of ever-changing environmental conditions that can potentially cause large swings in redox states. Cellular redox homeostasis is an essential buffering mechanism that prevents excessive reduction or oxidation. Redox signaling triggered by perturbations in this buffering system can transmit "current status" information on the balance between external inputs and internal cellular requirements.

Why should ascorbate and glutathione be part of any redox hub? Compounds able to directly oxidize ascorbate and reduced glutathione (GSH) at high rates include ROS such as superoxide or hydroxyl radical, whose probability of production is influenced by the environment. Due to their relatively high cellular concentrations, therefore, ascorbate and GSH act as scavengers or sacrificial nucleophiles. Many primary and secondary metabolites can function similarly. Ascorbate and glutathione are distinguished from most of these compounds by (1) specific enzymes that couple them to peroxide metabolism; (2) the existence of relatively stable oxidized forms; and (3) recycling of these forms to the reduced compounds by high-capacity enzyme-based systems that depend on the key electron carriers, NAD(P)H. Analysis of Arabidopsis mutants has provided convincing demonstrations that ascorbate and glutathione are required for plant development (Vernoux et al., 2000; Cairns et al., 2006; Dowdle et al., 2007). In shoot meristem functions, glutathione also plays overlapping roles with thioredoxin (TRX) systems (Reichheld et al., 2007; Bashandy et al., 2010). Other studies of Arabidopsis mutants have provided unequivocal evidence that both ascorbate and glutathione are multifunctional metabolites that are important in redox homeostasis and signaling as well as in development and defense reactions such as those that occur during plant responses to pathogens (Pastori et al., 2003; Ball et al., 2004; Conklin and Barth, 2004; Parisy et al., 2007; Schlaeppi et al., 2008; Mhamdi et al., 2010a).

Both ascorbate and glutathione are abundant and stable antioxidants with appropriate redox potentials that interact with numerous components and pathways and that are maintained in a generally reduced state. In compartments where the pools are at or close to thermodynamic equilibrium with each other, and assuming an NADP(H) redox potential of −300 mV or lower, glutathione is expected to be almost completely

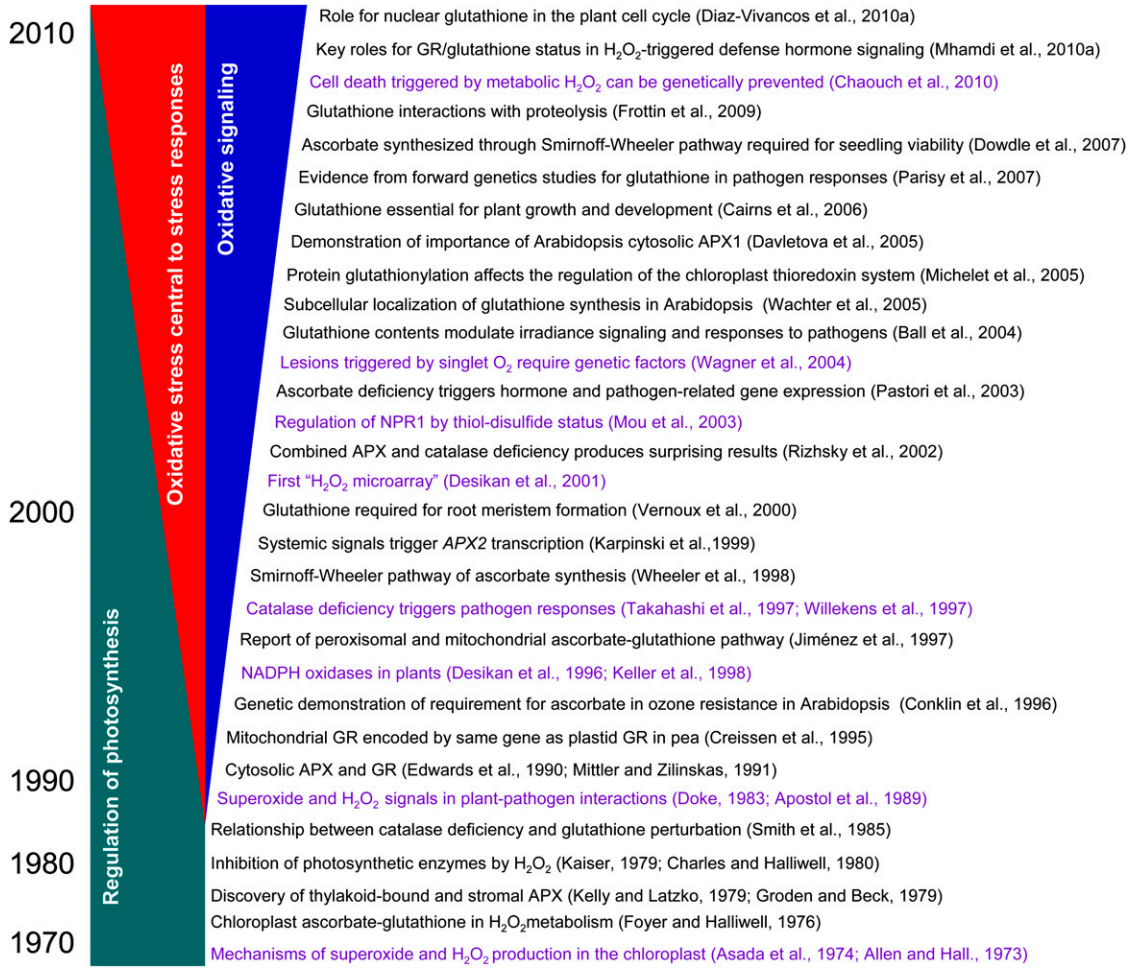

Figure 2. Simple scheme illustrating the timeline of some important advances concerning ascorbate and glutathione during the development of concepts on the roles of ROS in plants since 1970. Purple text shows some of the many related advances in ROS metabolism that may not directly involve ascorbate or glutathione systems. References not cited in the text (in alphabetical order): Apostol et al., 1989; Conklin et al., 1996; Creissen et al., 1995; Davletova et al., 2005; Desikan et al., 1996, 2001; Doke, 1983; Edwards et al., 1990; Keller et al., 1998; Rizhsky et al., 2002; Takahashi et al., 1997; Wachter et al., 2005; Wheeler et al., 1998.

in the reduced form, GSH (Fig. 3). Where they are present together with the enzymes monodehydroascorbate reductase and dehydroascorbate (DHA) reductase (DHAR), the reducing actions of both NAD(P)H and GSH should mean that oxidized ascorbate is present at only trace concentrations under optimal conditions (Fig. 3). Within this highly reducing intracellular context, even the most stable ROS, H_2O_2, has a relatively short lifetime. ROS removal by the ascorbate-glutathione-NADPH system can cause transient or sustained adjustments in all or some of the components of this system. A key theme of this review is that such adjustments have physiological significance, as they can be sensed and transduced to influence multiple signaling pathways, notably those involving phytohormones. Furthermore, even without ROS-induced changes in the system, components such as glutathione and ascorbate can provide current status redox information through proteins such as glutaredoxins (GRX) and enzymes of phytohormone synthesis. Ascorbate is an essential cofactor in several biosynthetic pathways, while glutathione acts as a sulfur source or reductant in metabolism and is also necessary for the formation of GS conjugates involved in biosynthesis, transport, and detoxification.

INTERDEPENDENCE OR INDEPENDENCE OF ASCORBATE AND GLUTATHIONE

Ascorbate and glutathione are part of a highly complex and intricate plant antioxidative system (Mittler et al., 2004). They function alongside catalases in high-capacity redox-homeostatic H_2O_2-processing pathways. While the ascorbate-glutathione pathway has long been considered to function in an integrated fashion, a key question concerns the extent to which the functions and status of the two pools are interdependent (Noctor et al., 2000; Potters et al., 2002; Noctor, 2006).

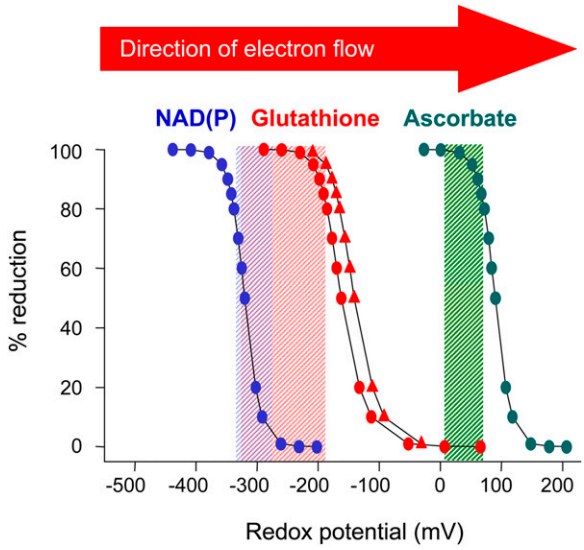

Figure 3. Redox potentials of NAD(P), glutathione, and ascorbate couples. Adapted from Noctor (2006). The shaded backgrounds indicate typical redox states in the absence of stress. For glutathione, the two curves indicate the relationship between reduction state and redox potential at total glutathione concentrations of 1 mM (triangles) and 5 mM (circles).

Although ascorbate and glutathione pools may be configured to respond to perturbation in a compensatory manner (discussed further below), it is now clear from studies of Arabidopsis mutants that the two compounds have specific functions and should not merely be considered as interchangeable antioxidants. Work on GSH-deficient Arabidopsis mutants has shown that glutathione has critical functions in embryo and meristem development (Vernoux et al., 2000; Cairns et al., 2006; Reichheld et al., 2007; Frottin et al., 2009; Bashandy et al., 2010), while complete deficiency in ascorbate synthesized through GDP-L-Gal phosphorylase causes lethality at the seedling stage (Dowdle et al., 2007). Interactions with specific cellular compounds or specific reactions are necessary if the two compounds are to play roles in signaling other than indirect ones in which they influence ROS availability.

Ascorbate and glutathione are differentially influenced by environmental factors. One example is sulfur nutrition, which affects glutathione much more than ascorbate. In contrast, while leaf glutathione contents can be increased by higher irradiances, they are generally not as responsive as ascorbate contents (Grace and Logan, 1996; Willekens et al., 1997). Marked diurnal fluctuations in the leaf ascorbate pool size have been reported with considerable depletion of ascorbate in darkness (Dutilleul et al., 2003; Bartoli et al., 2006) that has been linked to decreased abundance of transcripts encoding the biosynthetic enzymes GDP-D-Man pyrophosphorylase, L-Gal 1-P phosphatase, L-galactono-1,4-lactone dehydrogenase, and GDP-L-Gal phosphorylase in the dark (Yabuta et al., 2007). While the light-dependent stimulation of ascorbate biosynthesis appears to require photosynthetic electron transport activity (Yabuta et al., 2007), ascorbate synthesis and ascorbate regeneration are influenced by light quality as well as quantity (Bartoli et al., 2009). Ascorbate synthesis and accumulation are particularly sensitive to the changing light environment, particularly red/far-red ratios, effects that are in line with the direct interactions between the ascorbate pool and the photosynthetic and respiratory electron transport chains (Bartoli et al., 2006, 2009).

In the ascorbate-glutathione pathway, GSH regenerates ascorbate by reducing DHA, either chemically or via DHARs, enzymes that constitute a class of glutathione *S*-transferases (Dixon et al., 2002). Overexpression of DHAR has highlighted the roles of GSH-dependent ascorbate pools in responses such as stomatal regulation (Chen et al., 2003). However, ascorbate regeneration may be independent of GSH, while DHAR is only one of several routes for GSH oxidation (Fig. 4). Annotated plant GPXs use TRXs more efficiently than GSH (Iqbal et al., 2006), but some glutathione *S*-transferases show GSH-dependent peroxidase activity against H_2O_2 and organic peroxides (Dixon et al., 2009), and some GST-encoding genes are strongly induced by oxidative stress (Vanderauwera et al., 2005; Queval et al., 2007). GSH oxidation that is independent of DHA or of chemical reactions with ROS could also occur through GRX-dependent peroxiredoxin (PRX) or Met sulfoxide reductase (Rouhier et al., 2002; Tarrago et al., 2009). In some cases, however, the physiological reductants of these peroxidases remain to be unequivocally identified. Like GSH, TRX, GRX, and cyclophilins, ascorbate can function as an electron donor in the regeneration of the active form of thiol peroxidases such as PRX. In particular, ascorbate has been shown to reduce the sulfenic acid form of yeast 1-Cys PRX (Monteiro et al., 2007). This nucleus-localized enzyme is expressed in the embryo and aleurone layers of seeds and is encoded by a single gene in Arabidopsis (Dietz, 2003). A recent study in wheat (*Triticum aestivum*) has shown that the oxidized form of the enzyme can be regenerated by ascorbate or by NADPH-dependent TRX reductase (Pulido et al., 2009).

Consistent with the idea that ascorbate pools should remain highly reduced if there are significant amounts of GSH (or other thiols) present, catalase deficiency in Arabidopsis drives glutathione oxidation while ascorbate remains highly reduced (Mhamdi et al., 2010a). This could occur because of the difference in redox potential (Fig. 3) or because reduction of DHA is only one of several routes for GSH oxidation (Fig. 4). In Arabidopsis *cat2* knockouts, specific cytosolic APXs and DHARs are induced in concert, providing evidence that the core ascorbate-glutathione pathway is engaged in the response to increased H_2O_2 availability (Mhamdi et al., 2010a). However, several other genes encoding potential GSH-dependent peroxidases are also induced. Thus, peroxide-triggered glutathione oxidation could be linked to flux through ascorbate pools as well as ascorbate-independent reactions, thereby providing a mechanism by which perturbations

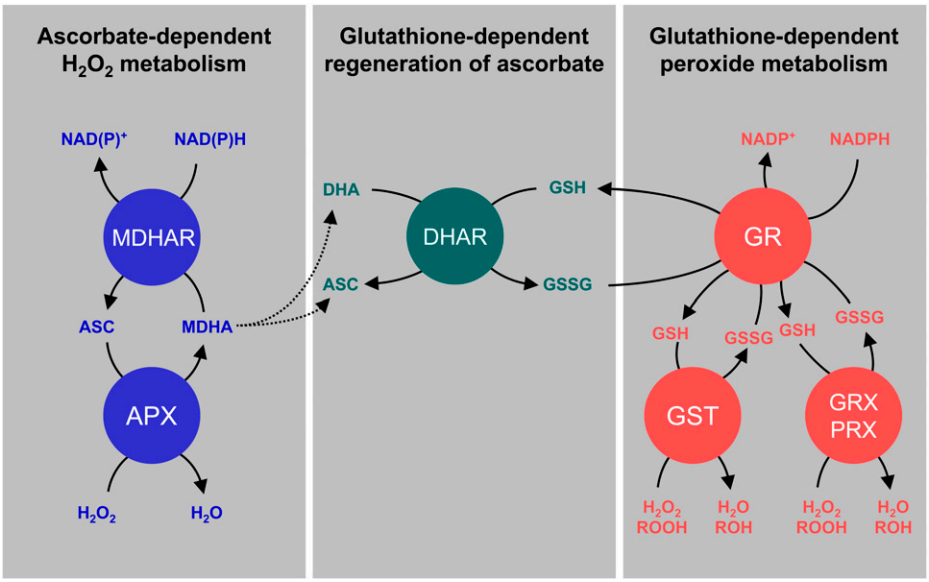

Figure 4. Interdependence and independence of glutathione and ascorbate in peroxide metabolism. The scheme is not meant to show an exhaustive inventory of all mechanisms. For further discussion, see text. ASC, Reduced ascorbate; MDHA(R), monodehydroascorbate (reductase); GST, glutathione *S*-transferase; ROH and ROOH, organic compound with alcohol and peroxide group, respectively.

in glutathione could act to transmit peroxide signals. A key issue here could be changes in NADP(H) redox status and the relatively low capacity of glutathione disulfide (GSSG)-reducing systems such as glutathione reductase (GR) compared with GSH-oxidizing mechanisms (discussed further in Noctor et al., 2010)

THE IMPORTANCE OF MODIFICATIONS IN GLUTATHIONE AND ASCORBATE IN TRANSMITTING ROS SIGNALS

Three properties mark out glutathione as a candidate transmitter of intracellular ROS signals: (1) glutathione is highly reduced under optimal conditions; (2) shifts toward a more oxidized glutathione status are well described in response to increased intracellular ROS availability; and (3) mechanisms exist that are theoretically able to link such shifts to altered redox state, and therefore biological activity, of target proteins. There is quite a good correlation between the expected intracellular H_2O_2 availability and the status of the glutathione pool. Oxidative perturbation of glutathione pools has been well documented in plants with pharmacologically or genetically knocked down catalase activities (Smith et al., 1985; May and Leaver, 1993; Willekens et al., 1997; Queval et al., 2009; Mhamdi et al., 2010a, 2010c). Most data suggest that enhanced ROS availability has less impact on the ascorbate-DHA ratio than on the redox status of the glutathione pool. Moreover, because much of the DHA detected in tissues is probably localized in the apoplast rather than the cytosol, we can assume that plant cells are able to maintain very high cytoplasmic ascorbate-DHA ratios simultaneously with low GSH-GSSG ratios, presumably because of efficient GSH-independent pathways of ascorbate regeneration and/or the difference in redox potential between the GSH/GSSG and ascorbate/DHA couples (Fig. 3).

Direct evidence for a role for glutathione in transmitting H_2O_2 signals is scarce. A recent study compared gene expression patterns in lines in which GSH-GSSG ratios were decreased by increased H_2O_2 availability or by lowered glutathione recycling capacity. Mutants in which one of the two Arabidopsis GR genes (encoding a mainly cytosolic isoform) is knocked out (*gr1*) are aphenotypic and show no evidence of generalized oxidative stress, despite an increase in leaf GSSG (Marty et al., 2009; Mhamdi et al., 2010a). In itself, this shows that plants can tolerate mild constitutive perturbation of the glutathione redox state without adverse consequences on growth and development. However, when compared in identical conditions, *gr1* shows gene expression patterns that partly recapitulate those driven by H_2O_2 in *cat2* (Mhamdi et al., 2010a). Moreover, introduction of the *gr1* mutation into the *cat2* background causes marked modulation of H_2O_2-associated transcript profiles. This observation points to a significant role for glutathione status in transmitting a subset of the signals derived from intracellular H_2O_2. However, because of uncertainty over whether changes in GR-dependent glutathione status are themselves sensed or rather affect gene expression through secondary effects on ROS availability, further work is required to resolve this issue (Mhamdi et al., 2010a).

In vivo studies conducted in wild-type Arabidopsis using redox-sensitive GRX-dependent redox-sensitive GFPs (roGFPs) have reported glutathione redox potential values lower than −300 mV in the cytosol (Meyer et al., 2007; Jubany-Mari et al., 2010). These values may be considered surprising because they imply that GSSG concentrations are in the low nanomolar range. On the other hand, they suggest that the glutathione and NADP(H) redox potentials are close to thermodynamic equilibrium, and it is not clear why this would not be the case in compartments in which

GSSG-reducing mechanisms are active in conditions where GSH turnover is relatively low. However, the growing awareness of interactions between glutathione and TRX systems (Michelet et al., 2005; Reichheld et al., 2007; Marty et al., 2009) and the potential complexity of the reaction mechanisms of the different types of GRX (Gao et al., 2010) mean that it remains to be unequivocally established whether the GFP expressed in vivo really is reporting only on the glutathione redox potential. Typical GSH-GSSG ratios measured in whole tissue extracts (of the order of 10–20) point to redox potentials closer to −200 mV, which would mean that glutathione and NADP(H) potentials are significantly removed from equilibrium (Fig. 3). Differences between analyses of whole tissue, which typically report GSSG contents corresponding to overall concentrations in the micromolar range, and roGFP analyses of cytosolic redox potential, which suggest much lower GSSG concentrations in compartments such as the cytosol, could be reconciled if a significant amount of GSSG is present in compartments such as the endoplasmic reticulum, vacuole, or apoplast, where glutathione reduction capacity is relatively low. Indeed, GSSG can be imported into vacuoles by certain transporters of the multidrug resistance-associated protein family (Tommasini et al., 1993; Lu et al., 1998). Accumulation of GSSG in *cat2* leaves is accompanied by induction at the transcript level of some of these types of transporter (Mhamdi et al., 2010a), and evidence has been presented that a significant proportion of the accumulated GSSG is indeed found in the vacuole (Queval et al., 2010).

Maintenance of low GSSG concentrations under optimal conditions could confer high sensitivity in signal transduction. It would allow relatively small ROS-triggered departures from this highly reduced state to be perceived as significant changes in redox potential by sensitive proteins. A second important factor is glutathione concentration, which in itself affects glutathione redox potential. Even if the GSH-GSSG ratio does not change, decreased concentration causes the redox potential to increase (i.e. become more positive). Indeed, the cytosolic redox potential measured by roGFP was more oxidizing in both glutathione-deficient *cad2* and in GR-deficient *gr1* mutants in which the GSH-GSSG ratio but not the glutathione concentration is decreased (Meyer et al., 2007; Marty et al., 2009). The decreased reduction state of GRXs caused by a more positive glutathione redox potential could explain observations in plants that both lack NADPH-dependent TRX reductase and are partly deficient in glutathione (Reichheld et al., 2007; Bashandy et al., 2010). To date, however, quantification of changes in glutathione redox potential caused by stress or mutations has produced relatively modest values (about 20 mV), and it is still unclear whether such adjustments are a major part of the mechanism of ROS-dependent signaling through the glutathione pool.

In contrast to glutathione, there is no evidence that the ratio of ascorbate to DHA is itself involved in the transmission of ROS signals. Like glutathione, ascorbate scavenges ROS both nonenzymatically and enzymatically and thus limits the lifetime of the ROS signal. Despite the discovery and characterization of several classes of thiol-dependent peroxidases (Rouhier et al., 2002; Dietz, 2003; Iqbal et al., 2006; Dixon et al., 2009), current information suggests that APX is the major H_2O_2-reducing peroxidase in plants (Ishikawa and Shigeoka, 2008). Moreover, like glutathione, the ascorbate pool is highly reduced under optimal conditions, and it can shift toward a more oxidized state as the oxidative load on the cell increases, although such effects are often less marked than those documented for glutathione. While changes in the ascorbate-DHA ratio are frequently considered to be a redox status indicator, there are no mechanisms at present that directly link such shifts in the ascorbate-DHA ratio to altered redox state and the biological activity of target proteins. One complexity in interpreting changes in the ascorbate-DHA ratio in terms of effects on redox state is the possibility that much of the DHA pool is spatially separated from the ascorbate pool. It is now widely accepted that DHA accumulates in the apoplast, which is considered to be the site of ascorbate degradation (Green and Fry, 2005). It is possible, therefore, that the intracellular ascorbate pool is maintained in a largely reduced state even under conditions of enhanced oxidative load, while the DHA pool of the apoplast is enhanced under these conditions because of increased ascorbate export from the cytoplasm coupled to increased oxidation in the apoplast. Furthermore, ascorbate redox state in the apoplast, as reflected by the ascorbate-DHA ratio, is critically important in a number of stress responses such as the control of guard cell signaling and stomatal movement (Chen et al., 2003). Moreover, while ascorbate is a cofactor of endoplasmic reticulum-located prolyl hydroxylase that produces the Hyp-rich glycoproteins required for cell division and expansion, the requirements of cell wall cross-linking are compatible only with a completely oxidized apoplastic ascorbate pool (Kärkönen and Fry, 2006).

In the intracellular environment, the overall abundance of reduced ascorbate may be more important in terms of regulation than the ascorbate-DHA ratio. This is probably because ascorbate is a cofactor for enzymes such as violaxanthin deepoxidase, which is involved in xanthophyll cycle-mediated photoprotection, and for enzymes participating in the biosynthesis of plant hormones such as abscisic acid (ABA), GA, and ethylene (Arrigoni and De Tullio, 2002; Mirica and Klinman, 2008). Ascorbate is also required for the anthocyanin biosynthetic pathway as well as a range of enzymes involved in Hyp, flavonoid, and glucosinolate biosynthesis (Turnbull et al., 2004). It is not surprising, therefore, that ascorbate depletion in the Arabidopsis *vtc2* mutant causes increased susceptibility to high-light-induced stress linked to an inability to accumulate either zeaxanthin or anthocyanin (Müller-Moulé et al., 2004; Giacomelli et al., 2006). In addition

to the direct effects of ascorbate availability on metabolic pathways, the abundance of ascorbate exerts a strong influence on gene expression (Kiddle et al., 2003; Pastori et al., 2003). The mechanisms by which ascorbate controls gene expression remain to be elucidated, but several mechanisms are possible. First, as noted above, the availability of ascorbate may regulate the synthesis and abundance of hormones and thus modulate hormone-signaling pathways. Second, there is a compensation for a decrease in ascorbate by an increase in the abundance of glutathione, potentially enhancing redox signaling through glutathione-dependent pathways in ascorbate-deficient cells. Both types of ascorbate-dependent control over cell signaling and gene expression have been observed in the Arabidopsis *vtc1* and *vtc2* mutants (C.H. Foyer, unpublished data).

OXIDATIVE STRESS, GLUTATHIONE, ASCORBATE, CELL DIVISION, CELL DIFFERENTIATION, AND FATE

Since the end of the 1970s, work on ROS in plants has to some extent been divided between two main research communities. The first has been concerned with photosynthesis and, more recently, respiration (i.e. what might be called "metabolic ROS"). The second has focused on signaling linked to biotic stress, in which ROS are considered to be produced at the cell surface. These two fields established their own paradigms and concepts. While the notion of ROS signaling has traditionally been more readily accepted within the plant-pathogen field, many researchers working on metabolic ROS cling, somewhat tenaciously, perhaps, to notions of indiscriminate "damage" as the main phenomenon through which these compounds produce their physiological effects (Møller et al., 2007). The effects of ROS produced extracellularly and intracellularly may well be distinct. However, the observation that the formation of lesions induced by singlet oxygen produced in the chloroplast requires genetic factors (Wagner et al., 2004) provided key evidence that even ROS-triggered chlorotic lesions are the result of a controlled process rather than the result of accumulated damage. Like lesions induced by chloroplast-generated singlet oxygen, hypersensitive response-like lesions driven by metabolic H_2O_2 produced in the peroxisomes during photorespiration (Foyer et al., 2009) can also be genetically prevented (Chaouch et al., 2010). While plant programmed cell death (PCD) may be defined as the "the genetically-controlled suicide of the cell," the pathways that lead to PCD are complex and often poorly characterized (Cacas, 2010), particularly in relation to ROS. Several studies have shown the involvement of genetic factors in PCD-like processes (Dietrich et al., 1994; Kaminaka et al., 2006), including lesions triggered by ROS (Overmyer et al., 2000, 2003, 2005). However, many genetic studies on PCD and similar processes have assumed rather than demonstrated the involvement of ROS. Given the capacity of several subcellular compartments to generate ROS potentially involved in PCD (Foyer and Noctor, 2003; Mittler et al., 2004), the site of the initial trigger is a key issue. Similarly, the roles of different ROS remain unclear, although it has been shown that different ROS can activate distinct signaling pathways (Gadjev et al., 2006). Thus, it is intriguing that cell death unambiguously triggered by intracellular oxidation can be genetically reverted and that this is the case whether the initial ROS trigger is excess singlet oxygen generated in the chloroplast or H_2O_2 formed in the peroxisomes (Wagner et al., 2004; Chaouch et al., 2010).

Accumulating evidence suggests that modifications in glutathione and/or ascorbate status modulate the signaling cascades that govern genetically controlled suicide programs within the cell. Cytosolic APX has been reported as a key regulator of PCD (de Pinto et al., 2006). The glutathione redox potential has been suggested to act as a key determinant of cell death and dormancy (Kranner et al., 2006). Thus, an increase in glutathione redox potential above a threshold value would cause death and/or growth arrest. Increased glutathione leaf contents resulting from overexpression of glutathione synthesis enzymes in the tobacco (*Nicotiana tabacum*) chloroplast triggered lesion formation and induced pathogenesis-related (*PR*) gene expression (Creissen et al., 1999). No lesions were observed in poplar (*Populus* sp.) with similar increases in tissue glutathione (Noctor and Foyer, 1998), and it has recently been shown that very high glutathione contents can be engineered in tobacco without major detrimental effects on plant development (Liedschulte et al., 2010). Thus, the available data suggest that high levels of glutathione can be tolerated by plants and are not in themselves sufficient to trigger cell death pathways. In catalase-deficient barley (*Hordeum vulgare*) and tobacco, however, oxidative perturbation of glutathione (accumulation of GSSG to many-fold wild-type levels) precedes or accompanies the appearance of lesions (Smith et al., 1985; Willekens et al., 1997). Similar effects occur in Arabidopsis catalase-deficient *cat2* mutants (Queval et al., 2007, 2009). However, lesions in *cat2* are environmentally determined and depend on the conditional accumulation of salicylic acid (SA) through the pathogen-activated isochorismate pathway (Chaouch et al., 2010). Lesions can also be prevented by treating *cat2* plants with myoinositol (Chaouch and Noctor, 2010). Suppression of cell death in *cat2*, whether this is achieved by growth daylength conditions, by blocking SA synthesis, or by myoinositol treatment, is not associated with decreased perturbation of leaf glutathione. Rather, leaf glutathione tends to be more oxidized under conditions in which cell death is prevented. This suggests that death is not caused merely by overoxidation of the glutathione pool. Another relevant finding is that double *cat2 gr1* mutants, in which GSSG can accumulate to extremely high levels, do not engage the necrotic cell death pathways observed in *cat2*, although they do show leaf

bleaching in some conditions (Mhamdi et al., 2010a). The *gr1* mutation also represses SA accumulation and responses in the wild-type background. Although it is impossible to ignore the potential importance of cellular or subcellular compartmentation in such responses, these observations suggest that even quite severe glutathione oxidation is not sufficient to trigger death in mature leaf cells. They raise the intriguing possibility that at least some death pathways may require reductive as well as oxidative events.

The relationship between ROS accumulation at the plasmalemma and that potentially occurring inside the cell during pathogen responses remains unclear. Induction of *PR* gene expression downstream of SA involves cytosolic redox modifications (Mou et al., 2003). Relatively low redox buffering in the apoplast likely means that ROS lifetimes are longer than inside the cell (Foyer and Noctor, 2005b), but plant metabolism can produce ROS at high rates (Foyer and Noctor, 2003). The potentially high capacity of ROS production in the chloroplasts and mitochondria may enable these organelles to make important contributions in certain circumstances (Dutilleul et al., 2003; Joo et al., 2005), and this could involve secondary changes in redox states of cellular redox buffers. Data obtained in *cat2*, in which cell death is among a wide range of SA-dependent responses (Chaouch et al., 2010), highlight the potentially important role of the peroxisomes (discussed further in Mhamdi et al., 2010c). Evidence in favor of a role for photorespiration in lesion formation has been reported by Mateo et al. (2004).

While the above discussion concerns the role of oxidation as a driving force and regulator for genetically programmed cell suicide pathways, recent evidence has demonstrated unequivocally that metabolic oxidation is a crucial regulator of the cell cycle and embryonic stem cell differentiation in animals (Menon et al., 2003; Yanes et al., 2010). These mechanisms are likely generic in eukaryotes, because very similar patterns of recruitment of GSH into the nucleus have been observed in plant and animal cells (Markovic et al., 2007; Diaz-Vivancos et al., 2010a, 2010b). In both cases, GSH is recruited into the nucleus at the G1 phase of the cell cycle and the resultant depletion of the cytosol leads to a readjustment of intracellular redox metabolism and oxidative signaling (Diaz-Vivancos et al., 2010b). GSH recruitment into the nucleus is rapidly followed by a significant accumulation of GSH throughout the cell (Pellny et al., 2009), suggesting that GSH depletion coupled to stromal oxidation causes the activation of glutathione synthesis. We presume that posttranslational activation of γ-glutamylcysteine synthetase (γ-ECS), the first enzyme of the committed path of glutathione synthesis, together with the observed enhanced glutathione synthetase expression (Diaz-Vivancos et al., 2010b), leads to the enhanced GSH production and the larger total GSH pool size that is required for redistribution between the daughter cells following mitosis (Diaz-Vivancos et al., 2010a).

It should be noted that enhanced oxidative load frequently leads to a slow-growth phenotype, as observed in mutants that are deficient in calatase when they are grown in air but not when they are grown in a high-CO_2 atmosphere to limit photorespiratory H_2O_2 production. The slow-growth phenotype that is observed in these circumstances is linked to GSSG accumulation rather than a buildup of H_2O_2 (Mhamdi et al., 2010c). While the development of the shoot apical meristem requires either GSH or reduced TRX (Vernoux et al., 2000; Reichheld et al., 2007), the failure of catalase-deficient plants to grow at optimal rates in air may be caused by an inability to maintain glutathione status at values sufficient to allow the dividing cells to progress rapidly out of G1. However, mild GSSG accumulation is not sufficient to restrict growth, as is evident from the aphenotypic nature of *gr1* knockout mutants (Marty et al., 2009; Mhamdi et al., 2010a). The abundance of GSH in proliferating cells plays a critical role in shoot and root meristem development, exerting control by mechanisms such as the regulation of auxin transport and signaling (Bashandy et al., 2010).

Depletion of the cytosolic GSH pool is accompanied by large changes in the abundance of transcripts encoding proteins that are involved in oxidative defense (Diaz-Vivancos et al., 2010b). GSH recruitment into the nucleus is not impaired in the presence of SA (Diaz-Vivancos et al., 2010b). However, it is likely that plant pathogen response pathways are down-regulated in these circumstances, because depletion of the cytosolic GSH pool leads to a reduced ability to activate SA-dependent *PR* gene expression (Maughan et al., 2010). These observations are consistent with reported links between glutathione status and SA contents or SA-dependent gene expression (Mou et al., 2003; Gomez et al., 2004; Mateo et al., 2006; Koornneef et al., 2008) as well as with the pathogen responses of mutants in glutathione synthesis or reduction (Ball et al., 2004; Parisy et al., 2007; Mhamdi et al., 2010a).

The oxidative regulation of animal embryonic stem cells is considered to be important in allowing them to differentiate in response to in vivo oxidative processes that occur, for example, in conditions such as inflammation (Yanes et al., 2010). Crucially, the GSH-GSSG ratio and ascorbate abundance are differentially regulated during the essential oxidation step that stimulates the embryonic stem cells to begin differentiation (Yanes et al., 2010). While little information is available on comparable processes in plants, it has recently been shown that a transcription factor called UPBEAT1, which regulates the expression of a small set of peroxidases, determines the ROS balance between the different zones and thus controls the transition from cell proliferation to cell elongation within the root (Tsukagoshi et al., 2010). It is also clear that cell identity has a major influence on cell fate, together with the modulation of ROS signaling pathways and responses to abiotic stress (Jiang et al., 2006; Dinneny et al., 2008). In plants, cell identity determines hormone-triggered

gene expression patterns (Dinneny et al., 2008), particularly in relation to the action of defense hormones such as ABA, which use ROS as second messengers. ROS generation is required for polarized cell growth (Foreman et al., 2003). It has long been recognized that the cells of the root quiescent center are in a highly oxidized state as a consequence of the action of auxin (Jiang and Feldman, 2005; Jiang et al., 2006). Crucially, despite the highly oxidizing environment, the quiescent center cells avoid the oxidative initiation of genetically programmed cell suicide pathways.

Taken together, the above studies demonstrate that control of the intracellular redistribution of antioxidants, particularly glutathione, can act as a potent signal in the regulation of the cell cycle (Diaz-Vivancos et al., 2010a, 2010b). Control of redox state could be achieved by the regulation of antioxidant capacity or of ROS production. When the cytoplasmic GSH pool is depleted, the control of redox state in the affected compartments is shifted to ascorbate-dependent processes and related signaling. Similarly, depletion of the ascorbate pool, as observed in the Arabidopsis *vtc1* and *vtc2* mutants, shifts redox control to the glutathione pool, glutathione-dependent processes, and related signaling. Moreover, the response of the cells to ROS-dependent signaling processes is fundamentally dependent on cell identity. Thus, ROS levels that cause cell death in fully expanded cells that are far removed from cell division fail to trigger cell suicide programs in the stem cell niche (Jiang and Feldman, 2005). During the development of the latter, high ROS levels could act as signals for differentiation as they do in animal cells.

GLUTATHIONE, ASCORBATE, AND LIGHT SIGNALING

Genetic studies have identified glutathione content as potentially influential in irradiance signaling and also point to functions in light quality signaling. The Arabidopsis *rax1* mutant was identified through constitutively enhanced expression of the high-light-induced gene, *APX2*, and shown to contain less than 50% of wild-type glutathione contents caused by a mutation in the *GSH1* gene encoding γ-ECS (Ball et al., 2004). Glutathione has also been implicated in systemic electrophysiological signaling pathways leading to acclimation to high light (Szechyńska-Hebda et al., 2010). Studies on the arsenic-tolerant mutants *ars4* and *ars5* have also revealed links between glutathione and photoreceptor signaling (Sung et al., 2007). The *ars5* mutation is affected in a component of the 26S proteasome, while *ars4* is a phytochrome A mutant (Sung et al., 2007, 2009). Mutants for *phyA* showed increased resistance to buthionine sulfoximine as well as to arsenic (Sung et al., 2007), and *ars5* had increased *GSH1* and *GSH2* transcripts and enhanced levels of glutathione when exposed to arsenic (Sung et al., 2009).

Ascorbate fulfills several important roles in the protection of photosynthesis from the adverse effects of high light. In addition, the high-light-inducible cytosolic *APX2* gene has proved to be a useful tool in the analysis of light-associated signaling cascades (Karpinski et al., 1999; Ball et al., 2004; Szechyńska-Hebda et al., 2010). The abundance of ascorbate in leaves is regulated both in response to the amount of light available during the photoperiod and the red/far-red ratio of the incident light (Bartoli et al., 2006, 2009). Because the ascorbate pool is significantly depleted during the dark, it adjusts to prevailing conditions of light quality and quantity within a single photoperiod (Bartoli et al., 2006, 2009). Furthermore, ascorbate deficiency in the *vtc1* and *vtc2* mutants alters the expression of a number of genes encoding chloroplast proteins, effects that are reversed upon the addition of ascorbate (Kiddle et al., 2003). The latter observation suggests that ascorbate might also participate in the repertoire of signals that are transmitted from the chloroplasts to the nucleus in order to coordinate nuclear and chloroplast gene expression. Chloroplast-to-nucleus retrograde signaling, which is considered to be particularly important for the correct assembly of functional chloroplasts, is considered to involve magnesium-protoporphyrin-, ROS-, and ABA-signaling pathways (Koussevitzky et al., 2007). In particular, the nucleus-localized Apetala2-type transcription factor, ABA-INSENSITIVE4 (ABI4), has been shown to have functions in chloroplast-to-nucleus and mitochondria-to-nucleus (retrograde) signaling pathways, and it has been suggested that ABI4 is a "master switch" for the regulation of nuclear genes in response to environmental and developmental cues (Koussevitzky et al., 2007; Giraud et al., 2009). Intriguingly, the transcriptomes of *vtc1* and *vtc2* mutants show very high overlap with those of mutants lacking ABI4 (C.H. Foyer, unpublished data). This observation shows that ascorbate deficiency or failure to sense ABA drives very similar patterns of gene expression.

MECHANISMS OF GLUTATHIONE-DEPENDENT SIGNALING

As well as potential roles in the regulation of the cell cycle, cell death, and light signaling, glutathione status has clearly been implicated in signaling through both SA and jasmonic acid (JA) pathways. Although it remains unclear whether these effects require dynamic changes in glutathione, such changes have been described in response to biotic stress or SA in barley and Arabidopsis (Vanacker et al., 2000; Mou et al., 2003; Mateo et al., 2006; Koornneef et al., 2008). Furthermore, addition of GSH but not GSSG is sufficient to mimic SA in inducing *PR1* (Gomez et al., 2004), an effect presumably occurring through NPR1 reduction and relocation to the nucleus (Mou et al., 2003). NPR1 monomers interact with the reduced form of the TGA1 transcription factor, which targets the activation sequence-1 element in the promoter regions of defense proteins. The NPR1 and TGA1 proteins are *S*-nitrosylated as part of the redox-controlled regulation of defense gene expression in systemic acquired resis-

tance, which employs *S*-nitrosoglutathione (GSNO) as the physiological nitric oxide donor (Lindermayr et al., 2010). Other links between SA and glutathione status have also been reported (Mateo et al., 2006), while glutathione has been implicated in SA-dependent repression of JA signaling through an NPR1-dependent mechanism in the cytosol (Spoel et al., 2003; Koornneef et al., 2008). However, a certain glutathione reduction status appears to be required for both SA and JA signaling (Mhamdi et al., 2010a), while glutathione-deficient mutants have compromised resistance to both microorganisms and insects (Ball et al., 2004; Parisy et al., 2007; Schlaeppi et al., 2008). It is likely that the influence of glutathione status in biotic stress signaling extends beyond that mediated through NPR1 function. It may also impact SA synthesis (Mhamdi et al., 2010a) and effects on JA signaling mediated by certain GRXs (Ndamukong et al., 2007). Redox effects on the F-box protein COI1, which functions in JA perception, may also await discovery (Acosta and Farmer, 2010). Communication is not one way, as JA induces genes encoding glutathione synthesis enzymes as well as GR (Xiang and Oliver, 1998).

Glutathione redox functions are linked to reversible oxidation or conjugation of the Cys sulfur group, resulting in many possible oxidized forms or derivatives. These notably include mixed GS-S-protein disulfides, GS conjugates with electrophilic metabolites, and GSNO (Fig. 5; Foyer and Noctor, 2005b; Lindermayr et al., 2005; Dixon and Edwards, 2010). A potential further layer of possible complexity of redox regulation is added by biochemical interactions between glutathione and TRX systems through *S*-glutathionylation of TRXf or TRX-regulated enzymes (Michelet et al., 2005; Zaffagnini et al., 2007). These studies and others have identified potential effects of modified glutathione status on photosynthetic and respiratory enzymes (Ito et al., 2003; Dixon et al., 2005; Holtgrefe et al., 2008; Zaffagnini et al., 2008; Gao et al., 2009; Palmieri et al., 2010). In many cases, the in vivo mechanisms underlying *S*-glutathionylation remain to be elucidated. They may involve thiol-disulfide exchange between protein thiol groups and GSSG, the conversion of protein thiol groups to thiyl radicals or sulfenic acids, or be mediated by GSNO (Dixon et al., 2005; Holtgrefe et al., 2008; Gao et al., 2009; Palmieri et al., 2010).

GRXs are the outstanding candidates for transmitting changes in glutathione redox state. In plants, they consist of several types defined according to their active-site motif (Lemaire, 2004; Rouhier, 2010). The activities of the different GRXs (over 30 genes in Arabidopsis) remain to be established but include regeneration of PRX, DHAR activity, and assembly of iron-sulfur clusters (Rouhier et al., 2007; Bandyopadhyay et al., 2008; Zaffagnini et al., 2008; Gao et al., 2010). From a signaling perspective, their abilities to regulate the status of protein thiols through reactions leading to disulfides or mixed disulfides [protein (de)glutathionylation] may be key (Fig. 5). Certain GRXs may also interact with ion channels (Cheng and Hirschi, 2003) and play roles in deglutathionylation reactions through monothiol or dithiol mechanisms (Zaffagnini et al., 2008; Couturier et al., 2009; Gao et al., 2009, 2010). Removal of glutathione through a GRX-dependent mechanism is also implicated in the catalytic cycle of Arabidopsis Met sulfoxide reductase B1 (MSRB1),

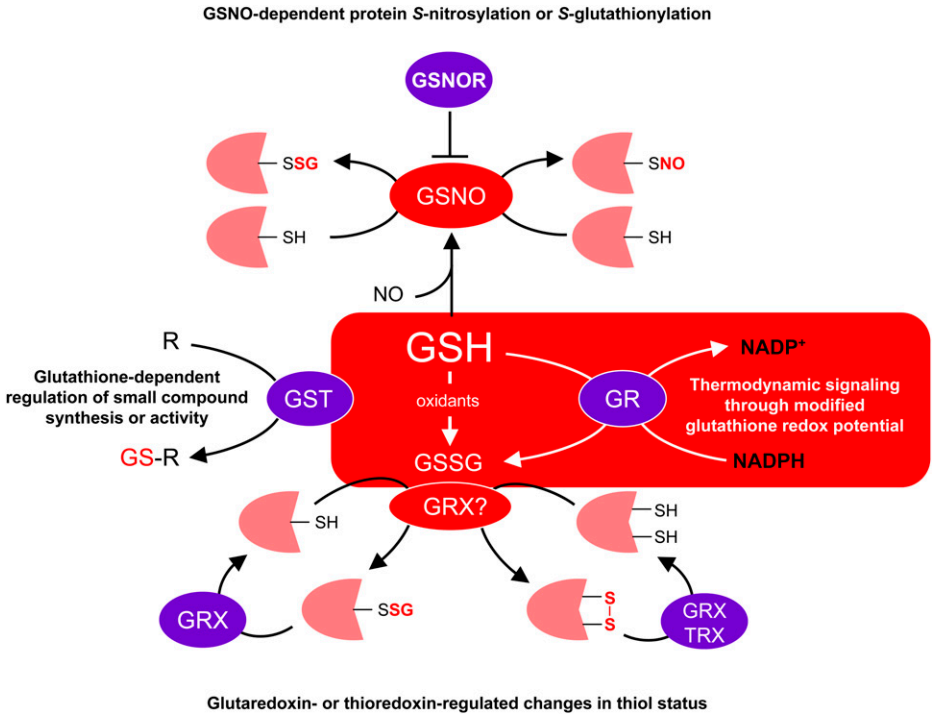

Figure 5. Some possible glutathione-linked signaling mechanisms. GS-R, Glutathione *S*-conjugate; GSNOR, GSNO reductase; R, small organic molecule; SH, sulfhydryl (thiol) group; SSG, glutathionylated protein Cys residue. Other abbreviations as for Figure 4.

whereas MSRB2 is regenerated through a TRX-dependent mechanism (Tarrago et al., 2009).

Both *S*-glutathionylation and *S*-nitrosylation of protein Cys residues may be mediated by GSNO (Holtgrefe et al., 2008; Palmieri et al., 2010). Inhibition of a Met adenosyltransferase by GSNO-triggered *S*-nitrosylation has been proposed to be an important regulatory mechanism in ethylene synthesis (Lindermayr et al., 2006), while modification of PRX by *S*-nitrosylation has been implicated in the regulation of ROS signaling (Romero-Puertas et al., 2007). GSNO may be an important physiological nitric oxide donor in the regulation of NPR1 activity (Tada et al., 2008). It is not yet clear whether changes in glutathione status can affect GSNO availability or the probability of either protein *S*-glutathionylation and *S*-nitrosylation. A key enzyme appears to be GSNO reductase, which is clearly implicated in biotic stress responses (Díaz et al., 2003; Feechan et al., 2005).

An intriguing issue in defining glutathione-dependent signaling concerns the biochemical activities of the numerous CC-type GRX subclass, which appear to be specific to plants. Physiological functions for two of these GRXs have been identified through genetic approaches. *ROXY1* and *ROXY2* are involved in petal and anther development (Li et al., 2009). Overexpression studies have also implicated a third CC-type GRX (GRX480) in JA signaling (Ndamukong et al., 2007). While relatively little is known about their biochemistry, complementation experiments with *ROXY1*, *ROXY2*, and *GRX480* suggest that their functions may be mainly determined by gene expression patterns (Li et al., 2009; Wang et al., 2009; Ziemann et al., 2009). Both this notion and the potential roles of this type of GRX in ROS-triggered signaling are consistent with the gene expression patterns of plants with genetically determined perturbations in glutathione. H_2O_2-triggered alteration of glutathione status in *cat2* and *cat2 gr1* mutants modifies the expression of four CC-type GRXs but not other GRX types. The affected genes included *GRX480* as part of a generalized effect on JA-associated genes but not *ROXY1* or *ROXY2* (Mhamdi et al., 2010a).

ASCORBATE AND GLUTATHIONE AS ROS-INDEPENDENT SIGNALS

While ascorbate and glutathione may play roles in signal regulation and/or transmission during cell death and defense responses, it is less clear how closely their functions in controlling growth interact with ROS. As discussed above, the controlled redistribution of the intracellular GSH pool during the cell cycle has pronounced effects on gene expression and leads to a lowering of the oxidative defenses during cell division (Diaz-Vivancos et al., 2010b). This illustrates the key point that although GSH and ascorbate fulfill similar essential antioxidant roles, they serve different functions in the control of cell division and cell growth. This feature is also evident in the phenotypes produced in mutants that are completely deficient in either ascorbate or glutathione. Both ascorbate and glutathione are irreplaceable in Arabidopsis development. Mutants that are completely deficient in ascorbate synthesized through the "Smirnoff-Wheeler" pathway are seedling lethal (Dowdle et al., 2007), while partial ascorbate deficiency slows the growth of the shoot and the root to a similar extent (Olmos et al., 2006). Knockout mutants for γ-ECS are embryo lethal (Cairns et al., 2006). The Arabidopsis *rml1* mutant, which has very low glutathione, shows a marked inhibition of root development, in which the cells of the primary root meristem arrest at G1, while in comparison shoot development is relatively unaffected (Reichheld et al., 2007). The developmental phenotypes of mutants for Met aminopeptidase 1a can be complemented by supplying GSH but not ascorbate (Frottin et al., 2009). Glutathione is essential for the establishment of root nodules in the legume/rhizobial symbiosis. Depletion of glutathione results not only in a decrease in the number of nodules but also in the expression of early nodulin genes (Frendo et al., 2005).

It is pertinent to address the question of whether changes in NADP(H) redox state drive glutathione-dependent signaling independent of changes in ROS concentration or as a secondary response to ROS-triggered oxidation. Theoretically, such effects could be involved in reductive or oxidative signaling through increases or decreases, respectively, in NADPH-$NADP^+$ ratios. Pharmacological evidence was presented that insufficient NADPH prevents the glutathione reduction state necessary for the activation of *PR1* gene expression (Mou et al., 2003), although it is unclear how NADP(H) pools were affected by this treatment. In general, overall pools of leaf NADP(H) are more stable than glutathione, and a key question concerns how important dynamic changes in NADPH-$NADP^+$ ratios are in response to the environment or during development. While the cytosolic NADPH-$NADP^+$ ratios of photosynthetically active pea (*Pisum sativum*) protoplasts were quite stable in different conditions (light, darkness, CO_2 concentration), short-term alterations in these factors caused marked changes in mitochondrial NADPH-$NADP^+$ ratios (Igamberdiev and Gardeström, 2003). Such changes, if efficiently transmitted to the glutathione pool by GR, could be sufficient to drive significant changes in glutathione redox potential (Noctor et al., 2010). It remains unclear whether such effects actually occur, and ROS-triggered oxidation remains the most obvious candidate for setting in motion dynamic signaling through glutathione. Arabidopsis mutants lacking cytosolic isocitrate dehydrogenase (*icdh*), an NADP-linked enzyme, have very similar NADPH-$NADP^+$ and GSH-GSSG ratios to the wild type (Mhamdi et al., 2010b). At least in steady-state conditions, whole tissue NADPH-$NADP^+$ ratios are not decreased by increased H_2O_2 availability in either *cat2* or double *cat2 icdh* lines. However, GSH-GSSG ratios are decreased

further in double *cat2 icdh* mutants than in *cat2* (Mhamdi et al., 2010b). These observations underscore the potential sensitivity of glutathione status as a signal transmitter while also further emphasizing the close link between glutathione redox status and peroxide availability.

Despite these observations, changes in glutathione concentration could act independently of ROS, either by affecting the glutathione redox potential or the availability of glutathione as a substrate or sulfur donor. For instance, sulfur supply has been implicated in resistance to pathogens (Bloem et al., 2007). Although the underlying causes of this phenomenon remain to be identified, tissue contents of glutathione or precursors could be one of the factors linking sulfur nutrition to the responses of plants to fungal and viral infection (Bloem et al., 2007; Höller et al., 2010). A threshold level of glutathione has been shown to be necessary for production of the phytoalexin camalexin and also to determine pathogen resistance (Parisy et al., 2007).

The ascorbate-deficient *vtc1* and *vtc2* mutants have similar levels of oxidants and do not show symptoms of oxidative stress under optimal growth conditions (Veljovic-Jovanovic et al., 2001; Colville and Smirnoff, 2008). It is thus pertinent to address the question of how ascorbate controls growth and gene expression in the absence of changes in ROS. It has long been recognized that ascorbate and ascorbate oxidase exert a strong influence on plant growth and development, a trait that has largely been attributed to direct effects of ascorbate on cell expansion (Pignocchi et al., 2003; Conklin and Barth, 2004; Pavet et al., 2005). As mentioned above, ascorbate participates in phenoxy radical-mediated cross-linking of cell wall components, leading to cell wall stiffening (Smirnoff, 2000). A strong link between ascorbate and noncellulosic cell wall polysaccharide biosynthesis has also been established (Gilbert et al., 2009).

In addition to direct effects of ascorbate on photosynthesis and cell wall metabolism, the participation of ascorbate in the synthesis of several major hormones such as ABA and GA may also be relevant to ROS-independent signaling pathways. Arabidopsis *vtc1* and *vtc2* have enhanced ABA levels (Pastori et al., 2003) and weaker GA signaling (C.H. Foyer, unpublished data). Evidence to support the view that ABA and ABA signaling participate in ascorbate-dependent control of growth comes from studies on Arabidopsis mutants that lack both ascorbate and ABI4. The *vtc2 abi4* double mutants have low ascorbate levels like the *vtc2* mutant but have the wild-type growth phenotype like the *abi4* mutant (C.H. Foyer, unpublished data). Such observations provide evidence of a strong interaction between ascorbate abundance and ABA signaling pathways in the control of plant growth and development. The ascorbate-ABA interaction could involve both ROS-dependent and ROS-independent pathways, as illustrated in Figure 6.

A further mechanism that might facilitate ascorbate-dependent signaling is related to the large ascorbate gradient that is observed in membranes like the plasma membrane and thylakoid membrane and probably also on the endoplasmic reticulum. While ascorbate is very high in the cytosol, it can be present at much lower levels in the apoplast and thylakoid lumen. Intriguingly, the DHAR family of proteins includes members known to affect membrane conductance. It appears that when some DHARs become oxidized, they can insert directly into membranes, where it is possible that they form "chloride intracellular channels" that may mediate ion movement across the membrane. This behavior has been described for three DHAR-like chloride intracellular channels in mammals and at least one of the Arabidopsis DHAR proteins (Littler et al., 2004; Elter et al., 2007). In this way, the DHARs can act both as an ion channel and as a glutathione-coupled redoxin. Moreover, one of the three Arabidopsis DHARs, DHAR1, is glutathionylated at the conserved catalytic Cys residue, Cys-20 (Dixon et al., 2005), suggesting a further layer of regulation. Many studies have highlighted the influence of ROS on ion channels and their impact on

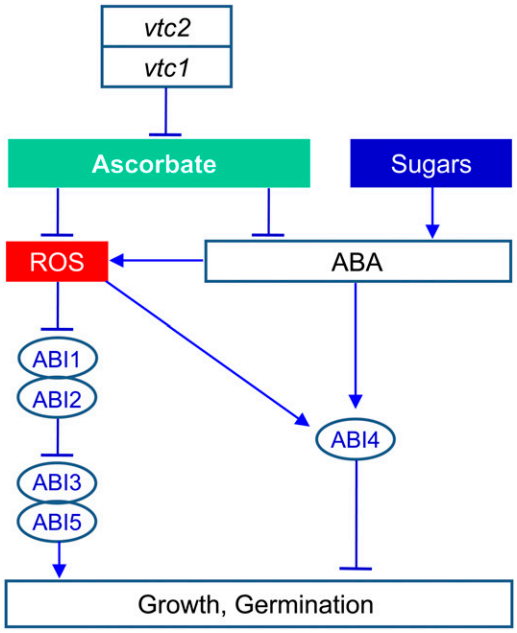

Figure 6. A schematic representation of possible interactions between ascorbate, ROS, and ABA. This scheme depicts the negative effects of the *vtc1* and *vtc2* mutations on the tissue ascorbate pool. A high level of tissue ascorbate will favor lower abundance of both ROS and ABA. However, a low ascorbate pool will favor increases in both ROS and ABA, together leading to an increase in signal transduction through ROS-mediated and ABA-dependent signaling cascades. The ABA signaling components have been placed in this scheme in relation to the control of growth, with ABI4 having a major downstream effect on the signaling cascade leading to the ascorbate-dependent repression of growth. The ABI1 and ABI2 protein phosphatases are involved in the transmission of ROS signals. For simplicity, in this scheme ABI3 and ABI5 have been placed downsteam of ABI1 and ABI2, but other interactions are also possible.

transmembrane ion flux, particularly through coupling to mechanisms that elevate free calcium in the cytosol, in relation to plant stress responses and also stomatal responses to water stress and ABA. The participation of DHARs in the regulation of transmembrane ion flux provides a mechanism that couples ion transport to the redox state of the ascorbate across the membrane, a feature that may be particularly important in stress situations that involve oxidation of the apoplast.

CONCLUDING REMARKS: REQUIREMENT, REGULATION, OR BOTH?

A wheel without a hub will not turn, but it is not the hub itself that determines the rate of rotation. Similarly, in the cellular control network, requirement is not proof of a regulatory role. Reverse genetics approaches are incisive in demonstrating that a compound is indispensable for a given process and thus in providing clues to underlying mechanisms. Such studies have shown unequivocally that ascorbate and glutathione are essential for plant growth and development. It remains less clear how influential changes in the abundance or redox states of these compounds are in determining plant function or responses to the environment. As discussed in this review, however, a number of observations suggest that changes in ascorbate and glutathione status can exert a powerful influence on plant function. These are (1) the effects of modest alterations in ascorbate or glutathione on various physiological processes; (2) the response of both compounds to changes in the environment; (3) dynamic changes, particularly in glutathione, in response to increased intracellular ROS; and (4) the emerging evidence of mechanisms that are potentially able to sense physiologically relevant changes in glutathione and/or ascorbate concentration or redox state.

While a minimal amount of ascorbate may be necessary for phytohormone synthesis, an overwhelming body of evidence derived from the analysis of ascorbate-deficient mutants shows that a 50% to 70% depletion of the total ascorbate pool can exert a profound influence over plant growth, defenses, and responses to environmental triggers. Such observations suggest that although this metabolite is abundant, metabolism and gene expression are highly sensitive to changes in the ascorbate pool size. Moreover, the ability to synthesize GSH rapidly following its sequestration in the nucleus is a critical regulator of cell cycle progression that may influence the ability of auxin to promote root growth. A threshold glutathione concentration may be required to support GRX or glutathione *S*-transferase activity and to allow cells to progress from G1 through the cell cycle. In theory, glutathione has all the attributes required of a sensitive, regulatory redox buffer that is sensed by the cell. The well-known dynamic changes in its concentration and redox state are consistent with this notion, while components such as GRX provide a potential means of coupling such changes to modified protein status and activity. A key issue concerns to what extent any of the signaling mechanisms discussed in this review are impacted by physiologically relevant changes in glutathione status or whether they merely require a threshold concentration or reduction state of the substrate. While the coming years will throw further light on these issues, it is our view that the well-known effects of environmental factors on ascorbate and glutathione, whether mediated via ROS or other factors, mark them out as central compounds that influence signaling intensity through pathways mediated by other factors such as recognized phytohormones.

Received October 15, 2010; accepted November 16, 2010; published January 6, 2011.

LITERATURE CITED

Acosta IF, Farmer EE (2010) Jasmonates. *In* The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi/10.1199:tab.0129, http://www.aspb.org/publications/arabidopsis/

Allen JF, Hall DO (1973) Superoxide reduction as a mechanism of ascorbate-stimulated oxygen uptake by isolated chloroplasts. Biochem Biophys Res Commun **52:** 856–862

Apostol I, Heinstein PF, Low PS (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. Plant Physiol **90:** 109–116

Arrigoni O, De Tullio MC (2002) Ascorbic acid: much more than just an antioxidant. Biochim Biophys Acta **1569:** 1–9

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol **50:** 601–639

Asada K, Kiso K, Yoshikawa K (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J Biol Chem **249:** 2175–2181

Ball L, Accotto GP, Bechtold U, Creissen G, Funck D, Jimenez A, Kular B, Leyland N, Mejia-Carranza J, Reynolds H, et al (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in *Arabidopsis*. Plant Cell **16:** 2448–2462

Bandyopadhyay S, Gama F, Molina-Navarro MM, Gualberto JM, Claxton R, Naik SG, Huynh BH, Herrero E, Jacquot JP, Johnson MK, et al (2008) Chloroplast monothiol glutaredoxins as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters. EMBO J **27:** 1122–1133

Bartoli CG, Tambussi EA, Diego F, Foyer CH (2009) Control of ascorbic acid synthesis and accumulation and glutathione by the incident light red/far red ratio in *Phaseolus vulgaris* leaves. FEBS Lett **583:** 118–122

Bartoli CG, Yu J, Gómez F, Fernández L, McIntosh L, Foyer CH (2006) Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in *Arabidopsis thaliana* leaves. J Exp Bot **57:** 1621–1631

Bashandy T, Guilleminot J, Vernoux T, Caparros-Ruiz D, Ljung K, Meyer Y, Reichheld JP (2010) Interplay between the NADP-linked thioredoxin and glutathione systems in *Arabidopsis* auxin signaling. Plant Cell **22:** 376–391

Bloem E, Haneklaus S, Salac I, Wickenhäuser P, Schnug E (2007) Facts and fiction about sulfur metabolism in relation to plant-pathogen interactions. Plant Biol (Stuttg) **9:** 596–607

Cacas JL (2010) Devil inside: does plant programmed cell death involve the endomembrane system? Plant Cell Environ **33:** 1453–1473

Cairns NG, Pasternak M, Wachter A, Cobbett CS, Meyer AJ (2006) Maturation of Arabidopsis seeds is dependent on glutathione biosynthesis within the embryo. Plant Physiol **141:** 446–455

Chaouch S, Noctor G (2010) *Myo*-inositol abolishes salicylic acid-dependent cell death and pathogen defence responses triggered by peroxisomal hydrogen peroxide. New Phytol **188:** 711–718

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois-Meurinne M, Van Breusegem F, Saindrenan P, Noctor G (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. Plant Physiol **153:** 1692–1705

Charles SA, Halliwell B (1980) Effect of hydrogen peroxide on spinach (*Spinacia oleracea*) chloroplast fructose bisphosphatase. Biochem J **189:** 373–376

Chen Z, Young TE, Ling J, Chang SC, Gallie DR (2003) Increasing vitamin C content of plants through enhanced ascorbate recycling. Proc Natl Acad Sci USA **100:** 3525–3530

Cheng NH, Hirschi KD (2003) Cloning and characterization of CXIP1, a novel PICOT domain-containing *Arabidopsis* protein that associates with CAX1. J Biol Chem **278:** 6503–6509

Colville L, Smirnoff N (2008) Antioxidant status, peroxidase activity, and PR protein transcript levels in ascorbate-deficient *Arabidopsis thaliana vtc* mutants. J Exp Bot **59:** 3857–3868

Conklin PL, Barth C (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant Cell Environ **17:** 959–970

Conklin PL, Williams EH, Last RL (1996) Environmental stress sensitivity of an ascorbic acid-deficient *Arabidopsis* mutant. Proc Natl Acad Sci USA **93:** 9970–9974

Couturier J, Koh CS, Zaffagnini M, Winger AM, Gualberto JM, Corbier C, Decottignies P, Jacquot JP, Lemaire SD, Didierjean C, et al (2009) Structure-function relationship of the chloroplastic glutaredoxin S12 with an atypical WCSYS active site. J Biol Chem **284:** 9299–9310

Creissen G, Firmin J, Fryer M, Kular B, Leyland N, Reynolds H, Pastori G, Wellburn F, Baker N, Wellburn A, et al (1999) Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco plants paradoxically causes increased oxidative stress. Plant Cell **11:** 1277–1292

Creissen G, Reynolds H, Xue YB, Mullineaux P (1995) Simultaneous targeting of pea glutathione reductase and of a bacterial fusion protein to chloroplasts and mitochondria in transgenic tobacco. Plant J **8:** 167–175

Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis*. Plant Cell **17:** 268–281

de Pinto MC, Paradiso A, Leonetti P, De Gara L (2006) Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. Plant J **48:** 784–795

Desikan R, A-H-Mackerness S, Hancock JT, Neill SJ (2001) Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol **127:** 159–172

Desikan R, Hancock JT, Coffey MJ, Neill SJ (1996) Generation of active oxygen in elicited cells of *Arabidopsis thaliana* is mediated by a NADPH oxidase-like enzyme. FEBS Lett **382:** 213–217

Díaz M, Achkor H, Titarenko E, Martínez MC (2003) The gene encoding glutathione-dependent formaldehyde dehydrogenase/GSNO reductase is responsive to wounding, jasmonic acid and salicylic acid. FEBS Lett **543:** 136–139

Diaz-Vivancos P, Dong Y-P, Ziegler K, Markovic J, Pallardó F, Pellny TK, Verrier P, Foyer CH (2010a) Recruitment of glutathione into the nucleus during cell proliferation adjusts whole cell redox homeostasis in *Arabidopsis thaliana* and lowers the oxidative defence shield. Plant J **64:** 825–838

Diaz-Vivancos P, Wolff T, Markovic J, Pallardo FV, Foyer CH (2010b) A nuclear glutathione cycle within the cell cycle. Biochem J **431:** 169–178

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA, Dangl JL (1994) Arabidopsis mutants simulating disease resistance response. Cell **77:** 565–577

Dietz KJ (2003) Plant peroxiredoxins. Annu Rev Plant Biol **54:** 93–107

Dinneny JR, Long TA, Wang JY, Jung JW, Mace D, Pointer S, Barron C, Brady SM, Schiefelbein J, Benfey PN (2008) Cell identity mediates the response of Arabidopsis roots to abiotic stress. Science **320:** 942–945

Dixon DP, Davis BG, Edwards R (2002) Functional divergence in the glutathione transferase superfamily in plants: identification of two classes with putative functions in redox homeostasis in *Arabidopsis thaliana*. J Biol Chem **277:** 30859–30869

Dixon DP, Edwards R (2010) Glutathione *S*-transferases. *In* The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi/10.1199:tab.0131, http://www.aspb.org/publications/arabidopsis/

Dixon DP, Hawkins T, Hussey PJ, Edwards R (2009) Enzyme activities and subcellular localization of members of the Arabidopsis glutathione transferase superfamily. J Exp Bot **60:** 1207–1218

Dixon DP, Skipsey M, Grundy NM, Edwards R (2005) Stress-induced protein *S*-glutathionylation in Arabidopsis. Plant Physiol **138:** 2233–2244

Doke N (1983) Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissues to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. Physiol Plant Pathol **23:** 345–357

Dowdle J, Ishikawa T, Gatzek S, Rolinski S, Smirnoff N (2007) Two genes in *Arabidopsis thaliana* encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J **52:** 673–689

Dutilleul C, Garmier M, Noctor G, Mathieu CD, Chétrit P, Foyer CH, de Paepe R (2003) Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. Plant Cell **15:** 1212–1226

Edwards EA, Rawsthorne S, Mullineaux PM (1990) Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta **180:** 278–284

Edwards R, Blount JW, Dixon RA (1991) Glutathione and elicitation of the phytoalexin response in legume cell cultures. Planta **184:** 403–409

Elter A, Hartel A, Sieben C, Hertel B, Fischer-Schliebs E, Lüttge U, Moroni A, Thiel G (2007) A plant homolog of animal chloride intracellular channels (CLICs) generates an ion conductance in heterologous systems. J Biol Chem **282:** 8786–8792

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for *S*-nitrosothiols in plant disease resistance. Proc Natl Acad Sci USA **102:** 8054–8059

Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, et al (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature **422:** 442–446

Foyer CH, Bloom AJ, Queval G, Noctor G (2009) Photorespiratory metabolism: genes, mutants, energetics, and redox signaling. Annu Rev Plant Biol **60:** 455–484

Foyer CH, Furbank RT, Harbinson J, Horton P (1990) The mechanisms contributing to photosynthetic control of electron transport by carbon assimilation in leaves. Photosynth Res **25:** 83–100

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta **133:** 21–25

Foyer CH, Halliwell B (1977) Purification and properties of dehydroascorbate reductase from spinach leaves. Phytochemistry **16:** 1347–1350

Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant **119:** 355–364

Foyer CH, Noctor G (2005a) Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ **29:** 1056–1071

Foyer CH, Noctor G (2005b) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell **17:** 1866–1875

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Signal **11:** 861–905

Frendo P, Harrison J, Norman C, Hernández Jiménez MJ, Van de Sype G, Gilabert A, Puppo A (2005) Glutathione and homoglutathione play a critical role in the nodulation process of *Medicago truncatula*. Mol Plant Microbe Interact **18:** 254–259

Frottin F, Espagne C, Traverso JA, Mauve C, Valot B, Lelarge-Trouverie C, Zivy M, Noctor G, Meinnel T, Giglione C (2009) Cotranslational proteolysis dominates glutathione homeostasis to support proper growth and development. Plant Cell **21:** 3296–3314

Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inzé D, Mittler R, Van Breusegem F (2006) Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiol **141:** 436–445

Gao XH, Bedhomme M, Veyel D, Zaffagnini M, Lemaire SD (2009) Methods for analysis of protein glutathionylation and their application to photosynthetic organisms. Mol Plant **2:** 218–235

Gao XH, Zaffagnini M, Bedhomme M, Michelet L, Cassier-Chauvat C, Decottignies P, Lemaire SD (2010) Biochemical characterization of

glutaredoxins from *Chlamydomonas reinhardtii*: kinetics and specificity in deglutathionylation reactions. FEBS Lett **584:** 2242–2248

Giacomelli L, Rudella A, van Wijk KJ (2006) High light response of the thylakoid proteome in Arabidopsis wild type and the ascorbate-deficient mutant *vtc2-2*: a comparative proteomics study. Plant Physiol **141:** 685–701

Gilbert L, Alhagdow M, Nunes-Nesi A, Quemener B, Guillon F, Bouchet B, Faurobert M, Gouble B, Page D, Garcia V, et al (2009) GDP-D-mannose 3,5-epimerase (GME) plays a key role at the intersection of ascorbate and non-cellulosic cell-wall biosynthesis in tomato. Plant J **60:** 499–508

Giraud E, Van Aken O, Ho LHM, Whelan J (2009) The transcription factor ABI4 is a regulator of mitochondrial retrograde expression of ALTERNATIVE OXIDASE1a. Plant Physiol **150:** 1286–1296

Gomez LD, Noctor G, Knight MR, Foyer CH (2004) Regulation of calcium signalling and gene expression by glutathione. J Exp Bot **55:** 1851–1859

Grace SC, Logan BA (1996) Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. Plant Physiol **112:** 1631–1640

Green MA, Fry SC (2005) Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-O-oxalyl-L-threonate. Nature **433:** 83–87

Groden D, Beck E (1979) H_2O_2 destruction by ascorbate-dependent systems from chloroplasts. Biochim Biophys Acta **546:** 426–435

Höller K, Király L, Künstler A, Müller M, Gullner G, Fattinger M, Zechmann B (2010) Enhanced glutathione metabolism is correlated with sulfur-induced resistance in tobacco mosaic virus-infected genetically susceptible *Nicotiana tabacum* plants. Mol Plant Microbe Interact **23:** 1448–1459

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

Hopkins FG, Morgan EJ (1936) Some relations between ascorbic acid and glutathione. Biochem J **30:** 1446–1462

Igamberdiev AU, Gardeström P (2003) Regulation of NAD- and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. Biochim Biophys Acta **1606:** 117–125

Iqbal A, Yabuta Y, Takeda T, Nakano Y, Shigeoka S (2006) Hydroperoxide reduction by thioredoxin-specific glutathione peroxidase isoenzymes of *Arabidopsis thaliana*. FEBS J **273:** 5589–5597

Ishikawa T, Shigeoka S (2008) Recent advances in ascorbate biosynthesis and the physiological significance of ascorbate peroxidase in photosynthesizing organisms. Biosci Biotechnol Biochem **72:** 1143–1154

Ito H, Iwabuchi M, Ogawa K (2003) The sugar-metabolic enzymes aldolase and triose-phosphate isomerase are targets of glutathionylation in *Arabidopsis thaliana:* detection using biotinylated glutathione. Plant Cell Physiol **44:** 655–660

Jiang K, Ballinger T, Li D, Zhang S, Feldman L (2006) A role for mitochondria in the establishment and maintenance of the maize root quiescent center. Plant Physiol **140:** 1118–1125

Jiang K, Feldman LJ (2005) Regulation of root apical meristem development. Annu Rev Cell Dev Biol **21:** 485–509

Jiménez A, Hernández JA, Del Rio LA, Sevilla F (1997) Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol **114:** 275–284

Joo JH, Wang S, Chen JG, Jones AM, Fedoroff NV (2005) Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the *Arabidopsis* oxidative stress response to ozone. Plant Cell **17:** 957–970

Jubany-Mari T, Alegre-Batlle L, Jiang K, Feldman LJ (2010) Use of a redox-sensing GFP (c-roGFP1) for real-time monitoring of cytosol redox status in *Arabidopsis thaliana* water-stressed plants. FEBS Lett **584:** 889–897

Kaiser WM (1979) Reversible inhibition of the Calvin cycle and activation of oxidative pentose phosphate cycle in isolated intact chloroplasts by hydrogen peroxide. Planta **145:** 377–382

Kaminaka H, Näke C, Epple P, Dittgen J, Schütze K, Chaban C, Holt BF III, Merkle T, Schäfer E, Harter K, et al (2006) bZIP10-LSD1 antagonism modulates basal defense and cell death in *Arabidopsis* following infection. EMBO J **25:** 4400–4411

Kärkönen A, Fry SC (2006) Effect of ascorbate and its oxidation products on H_2O_2 production in cell-suspension cultures of *Picea abies* and in the absence of cells. J Exp Bot **57:** 1633–1644

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. Science **284:** 654–657

Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase $gp91^{phox}$ subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. Plant Cell **10:** 255–266

Kelly GJ, Latzko E (1979) Soluble ascorbate peroxidase: detection in plants and use in vitamin C estimation. Naturwissenschaften **66:** 617–619

Kiddle G, Pastori GM, Bernard S, Pignocchi C, Antoniw J, Verrier PJ, Foyer CH (2003) Effects of leaf ascorbate content on defense and photosynthesis gene expression in *Arabidopsis thaliana*. Antioxid Redox Signal **5:** 23–32

Koornneef A, Leon-Reyes A, Ritsema T, Verhage A, Den Otter FC, Van Loon LC, Pieterse CMJ (2008) Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol **147:** 1358–1368

Koussevitzky S, Nott A, Mockley TC, Hong F, Sachetto-Martins G, Surpin M, Lim J, Mittler R, Chory J (2007) Signals from chloroplasts converge to regulate nuclear gene expression. Science **316:** 715–719

Kranner I, Birtić S, Anderson KM, Pritchard HW (2006) Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death? Free Radic Biol Med **40:** 2155–2165

Lemaire SD (2004) The glutaredoxin family in oxygenic photosynthetic organisms. Photosynth Res **79:** 305–318

Li S, Lauri A, Ziemann M, Busch A, Bhave M, 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

Liedschulte V, Wachter A, Zhigang A, Rausch T (2010) Exploiting plants for glutathione (GSH) production: uncoupling GSH synthesis from cellular controls results in unprecedented GSH accumulation. J Plant Biotechnol **8:** 807–820

Lindermayr C, Saalbach G, Bahnweg G, Durner J (2006) Differential inhibition of *Arabidopsis* methionine adenosyltransferases by protein *S*-nitrosylation. J Biol Chem **281:** 4285–4291

Lindermayr C, Saalbach G, Dürner J (2005) Proteomic identification of *S*-nitrosylated proteins in Arabidopsis. Plant Physiol **137:** 921–930

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

Littler DR, Harrop SJ, Fairlie WD, Brown LJ, Pankhurst GJ, Pankhurst S, DeMaere MZ, Campbell TJ, Bauskin AR, Tonini R, et al (2004) The intracellular chloride ion channel protein CLIC1 undergoes a redox-controlled structural transition. J Biol Chem **279:** 9298–9305

Lu YP, Li ZS, Drozdowicz YM, Hörtensteiner S, Martinoia E, Rea PA (1998) AtMRP2, an *Arabidopsis* ATP binding cassette transporter able to transport glutathione *S*-conjugates and chlorophyll catabolites: functional comparisons with Atmrp1. Plant Cell **10:** 267–282

Markovic J, Borrás C, Ortega A, Sastre J, Viña J, Pallardó FV (2007) Glutathione is recruited into the nucleus in early phases of cell proliferation. J Biol Chem **282:** 20416–20424

Marty L, Siala W, Schwarzländer M, Fricker MD, Wirtz M, Sweetlove LJ, Meyer Y, Meyer AJ, Reichheld JP, Hell R (2009) The NADPH-dependent thioredoxin system constitutes a functional backup for cytosolic glutathione reductase in Arabidopsis. Proc Natl Acad Sci USA **106:** 9109–9114

Mateo A, Funck D, Mühlenbock P, Kular B, Mullineaux PM, Karpinski S (2006) Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J Exp Bot **57:** 1795–1807

Mateo A, Mühlenbock P, Rustérucci C, Chang CC, Miszalski Z, Karpinska B, Parker JE, Mullineaux PM, Karpinski S (2004) *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. Plant Physiol **136:** 2818–2830

Maughan SC, Pasternak M, Cairns N, Kiddle G, Brach T, Jarvis R, Haas F, Nieuwland J, Lim B, Müller C, et al (2010) Plant homologs of the *Plasmodium falciparum* chloroquine-resistance transporter, *Pf*CRT, are required for glutathione homeostasis and stress responses. Proc Natl Acad Sci USA **107:** 2331–2336

May MJ, Leaver CJ (1993) Oxidative stimulation of glutathione synthesis in *Arabidopsis thaliana* suspension cultures. Plant Physiol **103:** 621–627

McCord JM, Fridovich I (1969) Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). J Biol Chem **244:** 6049–6055

Mehler AH (1951) Studies on reactions of illuminated chloroplasts. I. Mechanisms of the reduction of oxygen and other Hill reagents. Arch Biochem Biophys **33:** 65–77

Menon SG, Sarsour EH, Spitz DR, Higashikubo R, Sturm M, Zhang H, Goswami PC (2003) Redox regulation of the G1 to S phase transition in the mouse embryo fibroblast cell cycle. Cancer Res **63:** 2109–2117

Meyer AJ, Brach T, Marty L, Kreye S, Rouhier N, Jacquot JP, Hell R (2007) Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. Plant J **52:** 973–986

Mhamdi A, Hager J, Chaouch S, Queval G, Han Y, Taconnat L, Saindrenan P, Gouia H, Issakidis-Bourguet E, Renou JP, et al (2010a) Arabidopsis GLUTATHIONE REDUCTASE 1 plays a crucial role in leaf responses to intracellular hydrogen peroxide and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways. Plant Physiol **153:** 1144–1160

Mhamdi A, Mauve C, Gouia H, Saindrenan P, Hodges M, Noctor G (2010b) Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. Plant Cell Environ **33:** 1112–1123

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G (2010c) Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot **61:** 4197–4220

Michelet L, Zaffagnini M, Marchand C, Collin V, Decottignies P, Tsan P, Lancelin JM, Trost P, Miginiac-Maslow M, Noctor G, et al (2005) Glutathionylation of chloroplast thioredoxin *f* is a redox signaling mechanism in plants. Proc Natl Acad Sci USA **102:** 16478–16483

Mirica LM, Klinman JP (2008) The nature of O_2 activation by the ethylene-forming enzyme 1-aminocyclopropane-1-carboxylic acid oxidase. Proc Natl Acad Sci USA **105:** 1814–1819

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

Mittler R, Zilinskas BA (1991) Purification and characterization of pea cytosolic ascorbate peroxidase. Plant Physiol **97:** 962–968

Møller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol **58:** 459–481

Monteiro G, Horta BB, Pimenta DC, Augusto O, Netto LES (2007) Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. Proc Natl Acad Sci USA **104:** 4886–4891

Mou Z, Fan WH, Dong XN (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell **113:** 935–944

Müller-Moulé P, Golan T, Niyogi KK (2004) Ascorbate-deficient mutants of Arabidopsis grow in high light despite chronic photooxidative stress. Plant Physiol **134:** 1163–1172

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

Noctor G (2006) Metabolic signalling in defence and stress: the central roles of soluble redox couples. Plant Cell Environ **29:** 409–425

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol **49:** 249–279

Noctor G, Queval G, Mhamdi A, Chaouch S, Foyer CH (2010) Glutathione. *In* The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD (in press)

Noctor G, Veljovic-Jovanovic S, Foyer CH (2000) Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. Proc Biol Sci **355:** 1465–1475

Olmos E, Kiddle G, Pellny TK, Kumar S, Foyer CH (2006) Modulation of plant morphology, root architecture, and cell structure by low vitamin C in *Arabidopsis thaliana*. J Exp Bot **57:** 1645–1655

Overmyer K, Brosché M, Kangasjärvi J (2003) Reactive oxygen species and hormonal control of cell death. Trends Plant Sci **8:** 335–342

Overmyer K, Brosché M, Pellinen R, Kuittinen T, Tuominen H, Ahlfors R, Keinänen M, Saarma M, Scheel D, Kangasjärvi J (2005) Ozone-induced programmed cell death in the Arabidopsis *radical-induced cell death1* mutant. Plant Physiol **137:** 1092–1104

Overmyer K, Tuominen H, Kettunen R, Betz C, Langebartels C, Sandermann H Jr, Kangasjärvi J (2000) Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell **12:** 1849–1862

Palmieri MC, Lindermayr C, Bauwe H, Steinhauser C, Durner J (2010) Regulation of plant glycine decarboxylase by *S*-nitrosylation and glutathionylation. Plant Physiol **152:** 1514–1528

Parisy V, Poinssot B, Owsianowski L, Buchala A, Glazebrook J, Mauch F (2007) Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J **49:** 159–172

Pastori GM, Kiddle G, Antoniw J, Bernard S, Veljovic-Jovanovic S, Verrier PJ, Noctor G, Foyer CH (2003) Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell **15:** 939–951

Pavet V, Olmos E, Kiddle G, Mowla S, Kumar S, Antoniw J, Alvarez ME, Foyer CH (2005) Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol **139:** 1291–1303

Pellny TK, Locato V, Vivancos PD, Markovic J, De Gara L, Pallardó FV, Foyer CH (2009) Pyridine nucleotide cycling and control of intracellular redox state in relation to poly (ADP-ribose) polymerase activity and nuclear localization of glutathione during exponential growth of Arabidopsis cells in culture. Mol Plant **2:** 442–456

Pignocchi C, Fletcher JM, Barnes J, Foyer CH (2003) The function of ascorbate oxidase in tobacco. Plant Physiol **132:** 1631–1641

Potters G, De Gara L, Asard H, Horemans N (2002) Ascorbate and glutathione: guardians of the cell cycle, partners in crime? Plant Physiol Biochem **40:** 537–548

Pulido P, Cazalis R, Cejudo FJ (2009) An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. Plant J **57:** 132–145

Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gakière B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G (2007) Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death. Plant J **52:** 640–657

Queval G, Jaillard D, Zechmann B, Noctor G (2010) Increased intracellular H_2O_2 availability preferentially drives glutathione accumulation in vacuoles and chloroplasts. Plant Cell Environ (in press)

Queval G, Thominet D, Vanacker H, Miginiac-Maslow M, Gakière B, Noctor G (2009) H_2O_2-activated up-regulation of glutathione in Arabidopsis involves induction of genes encoding enzymes involved in cysteine synthesis in the chloroplast. Mol Plant **2:** 344–356

Reichheld JP, Khafif M, Riondet C, Droux M, Bonnard G, Meyer Y (2007) Inactivation of thioredoxin reductases reveals a complex interplay between thioredoxin and glutathione pathways in *Arabidopsis* development. Plant Cell **19:** 1851–1865

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inzé D, Mittler R (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J **32:** 329–342

Romero-Puertas MC, Laxa M, Mattè A, Zaninotto F, Finkemeier I, Jones AME, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) *S*-Nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell **19:** 4120–4130

Rouhier N (2010) Plant glutaredoxins: pivotal players in redox biology and iron-sulphur centre assembly. New Phytol **186:** 365–372

Rouhier N, Gelhaye E, Jacquot JP (2002) Glutaredoxin-dependent peroxiredoxin from poplar: protein-protein interaction and catalytic mechanism. J Biol Chem **277:** 13609–13614

Rouhier N, Unno H, Bandyopadhyay S, Masip L, Kim SK, Hirasawa M, Gualberto JM, Lattard V, Kusunoki M, Knaff DB, et al (2007) Functional, structural, and spectroscopic characterization of a glutathione-ligated [2Fe-2S] cluster in poplar glutaredoxin C1. Proc Natl Acad Sci USA **104:** 7379–7384

Schlaeppi K, Bodenhausen N, Buchala A, Mauch F, Reymond P (2008) The glutathione-deficient mutant *pad2-1* accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore *Spodoptera littoralis*. Plant J **55:** 774–786

Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, et al (2003) NPR1 modulates cross-talk between salicylate- and jasmonate-depen-

dent defense pathways through a novel function in the cytosol. Plant Cell **15:** 760–770

Smirnoff N (2000) Ascorbic acid: metabolism and functions of a multi-facetted molecule. Curr Opin Plant Biol **3:** 229–235

Smith IK, Kendall AC, Keys AJ, Turner JC, Lea PJ (1985) The regulation of the biosynthesis of glutathione in leaves of barley (*Hordeum vulgare* L). Plant Sci **41:** 11–17

Sung DY, Kim TH, Komives EA, Mendoza-Cózatl DG, Schroeder JI (2009) ARS5 is a component of the 26S proteasome complex, and negatively regulates thiol biosynthesis and arsenic tolerance in Arabidopsis. Plant J **59:** 802–813

Sung DY, Lee D, Harris H, Raab A, Feldmann J, Meharg A, Kumabe B, Komives EA, Schroeder JI (2007) Identification of an arsenic tolerant double mutant with a thiol-mediated component and increased arsenic tolerance in *phyA* mutants. Plant J **49:** 1064–1075

Szechyńska-Hebda M, Kruk J, Górecka M, Karpińska B, Karpiński S (2010) Evidence for light wavelength-specific photoelectrophysiological signaling and memory of excess light episodes in *Arabidopsis*. Plant Cell **22:** 2201–2218

Szent-Györgyi A (1931) On the function of hexuronic acid in the respiration of the cabbage leaf. J Biol Chem **90:** 385–393

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

Takahashi H, Chen Z, Du H, Liu Y, Klessig DF (1997) Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. Plant J **11:** 993–1005

Tarrago L, Laugier E, Zaffagnini M, Marchand C, Le Maréchal P, Rouhier N, Lemaire SD, Rey P (2009) Regeneration mechanisms of *Arabidopsis thaliana* methionine sulfoxide reductases B by glutaredoxins and thioredoxins. J Biol Chem **284:** 18963–18971

Tommasini R, Martinoia E, Grill E, Dietz KJ, Amrhein N (1993) Transport of oxidized glutathione into barley vacuoles: evidence for the involvement of the glutathione-S-conjugate ATPase. Z Naturforsch C **48:** 867–871

Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell **143:** 606–616

Turnbull JJ, Nakajima J, Welford RWD, Yamazaki M, Saito K, Schofield CJ (2004) Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: anthocyanidin synthase, flavonol synthase, and flavanone 3beta-hydroxylase. J Biol Chem **279:** 1206–1216

Vanacker H, Carver TLW, Foyer CH (2000) Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hypersensitive response in the barley-powdery mildew interaction. Plant Physiol **123:** 1289–1300

Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inzé D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol **139:** 806–821

Veljovic-Jovanovic SD, Pignocchi C, Noctor G, Foyer CH (2001) Low ascorbic acid in the *vtc-1* mutant of Arabidopsis is associated with decreased growth and intracellular redistribution of the antioxidant system. Plant Physiol **127:** 426–435

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughan SC, Cobbett CS, Van Montagu M, Inzé D, et al (2000) The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. Plant Cell **12:** 97–110

Wachter A, Wolf S, Steininger H, Bogs J, Rausch T (2005) Differential targeting of *GSH1* and *GSH2* is achieved by multiple transcription initiation: implications for the compartmentation of glutathione biosynthesis in the *Brassicaceae*. Plant J **41:** 15–30

Wagner D, Przybyla D, Op den Camp R, Kim C, Landgraf F, Lee KP, Würsch M, Laloi C, Nater M, Hideg E, et al (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science **306:** 1183–1185

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

Wheeler GL, Jones MA, Smirnoff N (1998) The biosynthetic pathway of vitamin C in higher plants. Nature **393:** 365–369

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, Van Montagu M, Inzé D, Van Camp W (1997) Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J **16:** 4806–4816

Xiang C, Oliver DJ (1998) Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell **10:** 1539–1550

Yabuta Y, Mieda T, Rapolu M, Nakamura A, Motoki T, Maruta T, Yoshimura K, Ishikawa T, Shigeoka S (2007) Light regulation of ascorbate biosynthesis is dependent on the photosynthetic electron transport chain but independent of sugars in *Arabidopsis*. J Exp Bot **58:** 2661–2671

Yanes O, Clark J, Wong DM, Patti GJ, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G (2010) Metabolic oxidation regulates embryonic stem cell differentiation. Nat Chem Biol **6:** 411–417

Zaffagnini M, Michelet L, Marchand C, Sparla F, Decottignies P, Le Maréchal P, Miginiac-Maslow M, Noctor G, Trost P, Lemaire SD (2007) The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-phosphate dehydrogenase is selectively regulated by glutathionylation. FEBS J **274:** 212–226

Zaffagnini M, Michelet L, Massot V, Trost P, Lemaire SD (2008) Biochemical characterization of glutaredoxins from *Chlamydomonas reinhardtii* reveals the unique properties of a chloroplastic CGFS-type glutaredoxin. J Biol Chem **283:** 8868–8876

Ziemann M, Bhave M, Zachgo S (2009) Origin and diversification of land plant CC-type glutaredoxins. Genome Biol Evol **1:** 265–277