Thiol-based redox signaling in the nitrogen-fixing symbiosis

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SYMBIOTIC NITROGEN FIXATION AND ANTIOXIDANT DEFENSES

Legumes are unique among crop plants in their ability to establish symbiotic associations with soil bacteria known collectively as rhizobia. As a result of a molecular dialog between the legume cells and rhizobia, nodules are formed on the roots or, in a few cases, on the stems. Nodules are organs specialized in dinitrogen (N₂) fixation, a biological process in which atmospheric dinitrogen is reduced to ammonia by the nitrogenase enzyme complex of the bacteroids (for a review, see Vance, 2008). The energy required for N₂ fixation derives ultimately from sucrose transported from the leaves to the nodules. In return, the ammonia produced by the bacteroids is assimilated into organic compounds to fulfill the nitrogen demand of both the bacteria and the plant.

Nodules contain abundant metalloproteins, including leghemoglobin and nitrogenase, which are prone to oxidation generating reactive oxygen and nitrogen species (RONS). Some RONS, such as the superoxide radical (O₂•⁻), hydrogen peroxide (H₂O₂) and nitric oxide (NO), perform signaling functions and have been detected in nodules using cytchemical staining, specific fluorescent probes or electron paramagnetic resonance (Santos et al., 2001; Rubio et al., 2004; Sánchez et al., 2010; del Giudice et al., 2011). However, these RONS are potentially cytotoxic, giving rise to highly oxidizing hydroxyl radicals (•OH), nitrogen dioxide (NO₂⁻) and peroxynitrite (ONOO⁻⁻) if their concentrations are not tightly controlled by antioxidant enzymes and metabolites. Nodule antioxidants include ascorbate, thiol tripeptides, superoxide dismutases, catalases, thiol peroxidases and the enzymes of the ascorbate-glutathione pathway (Becana et al., 2010). Here, we will focus on those antioxidants of nodules whose protective and regulatory functions entail thiol groups, paying special attention to the contribution of their redox activities to the lifespan of the symbiosis, from root cell infection to nodule senescence.

BIOSYNTHESIS OF THIOL TRIPETIDES IN LEGUMES

The thiol tripeptide glutathione (GSH; γ-Glu-Cys-Gly) and ascorbate are the major water-soluble antioxidants and redox buffers of plants. In addition, GSH performs multiple and diverse functions, including regulation of cell cycle, sulfur transport and
storage, stress responses and detoxification of heavy metals and xenobiotics (Rausch et al., 2007; Foyer and Noctor, 2011). In legumes, the structural homolog, homoglutathione (hGSH; γGlu-Cys;βAla), may partially or completely replace GSH (Frendo et al., 2001; Matamoros et al., 2003). Both compounds can be found at concentrations of 0.5–1.5 mM in nodules (Matamoros et al., 1999b), similar to the estimated ranges of 1–3 mM GSH and 0.6–0.7 mM hGSH in the chloroplasts (Bergmann and Rennenberg, 1993) or 2–3 mM GSH in the cytosol of root cells (Fricker et al., 2000).

The synthesis of GSH in plants and other organisms is accomplished in two sequential reactions (Figure 1) catalyzed by γ-glutamylcysteine synthetase (γECS) and GSH synthetase (GSHS), both showing a strict requirement for ATP and Mg2+ (Bergmann and Rennenberg, 1993). In legumes, the synthesis of hGSH is also carried out in two steps, involving the same γECS enzyme and a specific hGSH synthetase (hGSHS), which exhibits a much higher affinity for β-alanine than for glycine (Macnicol, 1987; Kläpsch et al., 1988; Frendo et al., 2001; Irurureta-Ormaeta et al., 2002). Detailed work using site-directed mutagenesis of hGSHS has conclusively shown that only two contiguous amino acid residues in the active site (Leu-534 and Pro-535 in M. truncatula) are involved in the catalytic activity (Frendo et al., 2002). Detailed work using site-directed mutagenesis of hGSHS has conclusively shown that only two contiguous amino acid residues in the active site (Leu-534 and Pro-535 in M. truncatula) of hGSHS determine the substrate preference for β-alanine over glycine (Frendo et al., 2001; Galant et al., 2011).

The GSHS and hGSHS genes share high homology (~70% amino acid identity) and are located in tandem on the same chromosome in the model legumes M. truncatula (Frendo et al., 2001) and Lotus japonicus (Matamoros et al., 2003). These findings were consistent with the proposal that the hGSHS gene derived from the GSHS gene by a duplication event occurred after the divergence between the Fabaceae, Solanaceae and Brassicaceae (Frendo et al., 2001). Despite this close relationship, the two genes are differentially regulated in plant organs and in response to stressful conditions or signal compounds such as hormones and BONS. This can be exemplified with studies performed on the two model legumes. Thus, M. truncatula produces exclusively GSH in the leaves and both GSH and hGSH in the roots and nodules (Frendo et al., 1999), whereas L. japonicus produces almost exclusively hGSH in the roots and leaves, but more GSH than hGSH in the nodules (Matamoros et al., 2003). In legumes, the GSH and hGSH contents are positively correlated with the GSHS and hGSHS activities and in general with their mRNA levels (Frendo et al., 1999, Matamoros et al., 2003). In M. truncatula roots, the expression of γECS and GSHS but not of hGSHS is induced by NO (Innocenti et al., 2007). In L. japonicus roots, NO, cytokinins and polyamines up-regulated GSHS whereas hGSHS mRNA and activity were induced by auxins (Clemente et al., 2012). Taken together, these observations suggest the presence of gene-specific cis-acting regulatory elements in the GSHS and hGSHS promoters. However, despite the long time elapsed since the discovery of hGSH in legumes, the reason why this thiol replaces GSH in some legume species and tissues is still a mystery.

Other thiols can be found in plants, but little is known about their roles in the symbiosis and hence they will be only very briefly described here. The GSH and hGSH precursors, cysteine and γ-glutamylcysteine, are found in nodules at concentrations considerably lower (<15%) than GSH or hGSH, in the range of 30–120 μM. As occurs for the tripeptides, both precursors are more abundant in nodules than in roots and leaves, pointing out an active thiol metabolism in N2-fixing nodules (Matamoros et al., 1999b). This conclusion is reinforced by the high transcript levels of the two enzymes involved in cysteine synthesis, serine acetyltransferase and O-acetylserine(thiol)lyase (Figure 1), that can be found in nodules (M. truncatula Gene Expression Atlas1 and L. japonicus Gene Expression Atlas2). Another thiol that can be found in legume nodules and other plant organs are (homo)phytochelatins. These are cysteine-rich polypeptides of general structure (γGlu-Cys)2−11−γGlu or (γGlu-Cys)2−11−βAla, which are synthesized from hGSHS only in the presence of certain metals and metalloids, such as selenium, cadmium, mercury or lead (Figure 1). It has been shown that these polypeptides form complexes with cadmium, which is then sequestrated to the vacuoles, avoiding poisoning of cellular metabolism. Interestingly, three functional phytochelatin synthase genes were found in L. japonicus, which differ in their cadmium response and are all expressed in nodules (Ramos et al., 2007).

![FIGURE 1] Schematics of the (h)GSH biosynthetic pathway. Depicted are also the enzymes forming the cysteine synthase complex, namely, serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase (OASTL), as well as those involved in phytochelatin (PCS) and homophytochelatin (hPCS) synthesis. For the γECS enzyme, the redox switch is drawn as an equilibrium between the more active (oxidized) dimeric form and the less active (reduced) monomeric form. Other abbreviations not used in the text: O-Ac-Ser, O-acetylserine; γGlu-Cys, γ-glutamylcysteine.

1http://mgpa.noble.org/v2/
2http://gpea.noble.org/v2/
REGULATION AND LOCALIZATION OF THIOL SYNTHESIS
The γECS, GSHS and hGSHS genes can be transcriptionally regulated in response to ROS and hormones, as mentioned above. A notable case of this type of regulation is the coordinated induction of the γECS and GSHS genes of Arabidopsis thaliana (Xiang and Oliver, 1998) and of the three genes of L. japonicus (Clemente et al., 2012) exposed to jasmonic acid. However, it has also been demonstrated that the (h)GSH biosynthetic pathway can be controlled at the translational and post-translational levels by modulation of the γECS mRNA stability and enzyme activity, respectively (Rausch et al., 2007; Galant et al., 2011). The post-translational regulation of plant γECS enzymes would occur via a conserved intramolecular disulfide bond that is likely to operate in vivo as a redox switch, in such a way that oxidation shifts the equilibrium toward the more active, dimeric form (Galant et al., 2011; Figure 1).

An additional, but by no means less important, mechanism of regulation may rest on the compartmentation of the thiol biosynthetic pathway. In nodules, subcellular fractionation and immunogold labeling studies have shown that γECS is localized in plastids, whereas GSHS and hGSHS are localized in both the plastids and cytosol (Moran et al., 2005; Clemente et al., 2012). A localization of GSHS in cowpea (Vigna unguiculata) nodule mitochondria needs to be confirmed by electron microscopy and examined in other legume nodules (Moran et al., 2007; Galant et al., 2011). Similar subcellular localizations have been reported for the enzymes of A. thaliana, where γECS is confined to the plastids and GSHS is predominantly located to the cytosol (Rausch et al., 2007; Galant et al., 2011). Because γ-glutamylcysteine needs to be exported from the plastids to the cytosol, where most (h)GSH synthesis takes place, subcellular compartimentation provides a potential conduit for transporting redox signals out of the chloroplast and probably of other plastids (Mullineaux and Rausch, 2005).

ROLES OF THIOLS IN NODULE FORMATION AND FUNCTIONING
A recent electron microscopy study of pea (Pisum sativum) nodules with a GSH-specific antibody revealed that this thiol is present in the bacteroids, mitochondria, cytosol and nuclei of infected cells (Matamoros et al., 2013). Furthermore, as nodules progress from the young to mature stage, total glutathione (reduced + oxidized) decreases in the mitochondria but increases in the bacteroids, cytosol and nuclei, which indicates differential turnover of the thiol or its redistribution between nodule compartments. The finding of GSH in nuclei of infected cells suggests that the thiol performs additional functions to the regulation of the cell cycle, which will be more important in meristematic cells (Diaz Vivancos et al., 2010). These functions may include DNA antioxidative protection or redox regulation of transcription factors (Matamoros et al., 2013).

At the tissue level, careful dissection of nodules has shown that, in general, the (h)GSH content and the γECS, GSHS and hGSHS activities are particularly high in the meristematic and infected zones of legume nodules (Matamoros et al., 1998b). Remarkably, hGSHS is very active in the cortex of bean nodules. The reasons of this specific distribution are unknown, but could be related to the function of this protein in the vascular bundles or in the O2 diffusion barrier, which are localized to the nodule cortex. These observations have been recently corroborated by using promoter-GUS fusions. Thus, El Mehli et al. (2011) have determined the spatio-temporal gene expression of the (h)GSH synthesis pathway in M. truncatula. The expression of γECS appears to be higher in the meristematic and infection zones of nodules, whereas the hGSHS mRNA is more abundant in the cortex and the GSHS mRNA in the cortex and in the N2-fixing zone.

The concentration of (h)GSH and the N2-fixing activity of nodules are positively correlated during nodule development (Dalton et al., 1993). The two parameters decline with advancing age (Evans et al., 1996; Groten et al., 2005) as well as during stress-induced senescence (Escuredo et al., 1996; Gogorcena et al., 1997; Matamoros et al., 1999a; Marino et al., 2007; Naya et al., 2007). These findings suggest that (h)GSH is important for nodule activity, a hypothesis that was tested by modulating the nodule content of (h)GSH by pharmacological and genetic approaches. The application of a specific inhibitor of (h)GSH biosynthesis (buthionine sulfoximine) or the expression of (h)GSHS in anti-sense orientation caused depletion of (h)GSH in M. truncatula roots (Frendo et al., 2005). The deficiency of (h)GSH synthesis in roots decreased substantially the number of nascent nodules and the expression of some early nodulin genes (Frendo et al., 2005). These results, along with the proposed role of GSH in meristem formation in A. thaliana (Vernoux et al., 2000; Reichheld et al., 2007), suggest that (h)GSH is required for the initiation and maintenance of the nodule meristem. The transcriptomic analysis of (h)GSH-depleted plants during early nodulation revealed down-regulation of genes implicated in meristem formation and up-regulation of salicylic acid-related genes after infection with Sinorhizobium meliloti (Pucciarrello et al., 2009). The potentially enhanced expression of defense genes provides a partial explanation for the negative effects of (h)GSH depletion on the symbiosis. Likewise, the reduction of (h)GSH content in transgenic roots led to a significantly lower N2-fixing activity, which was related to a smaller nodule size (El Msehli et al., 2011). Conversely, the overexpression of γECS resulted in an elevated (h)GSH content, which was associated with enhanced N2 fixation. All these data underpin the importance of (h)GSH in nodule development and functioning.

Although the precise roles of (h)GSH in the onset and life-span of symbiosis are still to be defined, a central role of (h)GSH in the regulation of symbiotic activity via hormone transduction pathways can be already anticipated (Bashandy et al., 2010; Clemente et al., 2012). Moreover, GSH and hGSH act as substrates for key antioxidant enzymes, such as glutathione reductases, glutathione-S-transferases and glutaredoxins (Grx), and hence both thiols probably participate in the regulation of symbiosis via modulation of enzyme activities (Dalton et al., 2009).

THIOL PEROXIDASES AND OTHER REDOXINS OF NODULES
Glutathione peroxidases (Gpxs), peroxiredoxins (Prxs) and thioredoxins (Trxs) are protein components of a regulatory network system (Figure 2) that perceives, modulates and transmits information of the cellular redox state via thiol-disulfide exchange (Dietz et al., 2006; Meyer et al., 2009). Although phylogenetically distant, plant Gpxs and Prxs catalyse similar biochemical reactions (Roubier and Jacquot, 2005). During their catalytic mechanism,
FIGURE 2 | A simplified overview of the thiol-based redox network in legume nodules. Representative cellular compartments of an infected cell showing their contents of proteins, such as Gpxs, Prxs and other redoxs, that contain active cysteine residues. The figure includes also the NTRA/B-T rx-Prx redox systems in the mitochondria and cytosol, and the NTRC-Prx and FTR-T rx redox systems in the plastids. Post-translational modifications involving protein cysteine residues, such as sulfenylation (oxidation of the cysteine thiolate to sulfenic acid), glutathionylation (incorporation of a glutathione moiety) and nitrosylation (incorporation of a nitrosyl group), are shown. GSH in the nucleus may protect DNA from oxidative damage by RONS and modulate activity of transcription factors (TF), activating or inactivating defense and stress-related genes. For simplicity, GSH in the mitochondria or plastids is not indicated. Other abbreviations not used in the text: Fd, ferredoxin; FNR, ferredoxin-NADP$^+$ reductase; FTR, ferredoxin-thioredoxin reductase.

Both types of thiol peroxidases reduce H$_2$O$_2$, lipid hydroperoxides or, in some cases, O$_3$O$_2^-$. Both enzymes contain a disulfide bond on the proteins. Cysteine thiol groups are regenerated by Thrxs, which are in turn reduced by NADPH-thioredoxin reductases (NTRs) with the consumption of NADPH.

Thiol peroxidases are widely distributed in all organisms and are encoded by small multigenic families. The A. thaliana and poplar (Populus trichocarpa) genomes contain eight and six Gpxs, respectively, that are differentially regulated at the transcriptional level in plant organs and in response to stress conditions and growth regulators (Rodriguez Milla et al., 2003; Navrot et al., 2006). The L. japonicus genome contains six Gpxs genes encoding cytosolic, plastidial and mitochondrial isoforms (Ramos et al., 2009). Gpxs are induced by GSH and glutathione reductase using the reducing power of NADPH.

In plants, Prxs are grouped into four classes (PrxQ, PrxII, 2-CPrx and 1-CPrx) that differ in their catalytic mechanisms and subcellular locations (Dietz et al., 2006). The L. japonicus genome encodes eight Prxs, which are localized to the chloroplasts (PrxQ), 2C-PrxA, 2C-PrxB and PrxIIE, mitochondria (PrxIIF), cytosol (PrxIIB/C) and nucleus (1C-Prx). These proteins show specific organ distribution. Thus, 1C-Prx localizes mainly to the embryo and PrxQ levels are very high in leaves compared to other organs. Nodules contain PrxIIB/C in the cytosol, PrxIIF in the mitochondria and low levels of 2C-Prx and PrxIIE in the plastids (Tóvar-Méndez et al., 2011). These proteins are part of Prx-Trx-NTR systems that are operative in the cytosol, plastids and mitochondria (Figure 2). Trxs form a complex family of disulfide oxidoreductases involved in the redox regulation of cell metabolism. In plant tissues, several groups of Trxs have been identified. Trx$^f$, Trx$^l$, Trx$^y$ and Trx$^z$ are localized in the plastids, Trx$^h$ in the cytosol and Trx$^o$ in the mitochondria. In addition, some Trx isoforms have been found in the mitochondria, nucleus and endoplasmic reticulum (Meyer et al., 2009). In legumes, the Trx protein family has been analyzed in detail in M. truncatula (Alkhalfioui et al., 2008) and L. japonicus (Tóvar-Méndez et al., 2011). In nodules, the cytosolic Trx mRNAs are very abundant, whereas mitochondrial Trx$^o$ is moderately expressed and plastidial Trx mRNAs are poorly represented. The Trx$h$ and Trx$o$ isoforms of Medicago sativa and Sesbania rostrata. In most cases, labeling was associated to starch grains, which hints to a role of Gpxs in the regulation of starch metabolism (Ramos et al., 2009).

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are maintained in reduced form by cytosolic and mitochondrial NTRs, whereas in the plastids this function is probably performed by the sequential action of ferredoxin-NADP and ferredoxin-Trx reductases (Tiová-Méndez et al., 2011). Nodule plastids also contain low levels of a singular NTR protein, NTRC, characterized by a tight thiol-based control of redox homeostasis for plant function. Furthermore, these proteins probably fulfill redox-independent specific roles due to their differential ability to interact with other proteins. The precise functions of the thiol-based regulatory network in the N2-fixing symbiosis remain largely undefined.

The importance of bacterial glutathione during symbiosis

The first direct evidence that the GSH pathway of the bacterial symbiotic partner is important for nodulation and N2 fixation was obtained by using S. meliloti mutants impaired in GSH synthesis. In S. meliloti, as in other organisms, GSH is synthesized by γECS and GSS, encoded, respectively, by the gshA and gshB genes. An S. meliloti mutant deficient in gshA was unable to grow under non-stress conditions, precluding any nodulation on alfalfa. Conversely, a gshB mutant was able to grow and nodulate alfalfa, indicating that γ-glutamylcysteine can partially compensate for GSH deficiency. The gshB strain showed nevertheless a delayed-nodulation phenotype coupled to abnormal development and early senescence of nodules. Both gshA and gshB mutants exhibited higher catalase activity than the wild-type, suggesting that the two mutants were experiencing oxidative stress (Harrison et al., 2005). Furthermore, gshB mutants of R. tropici and R. etli were affected in their ability to compete during nodulation of common bean, and nodules induced by gshB mutants displayed early senescence (Riccillo et al., 2000; Tate et al., 2012). A deficiency in GSH was also associated with increased levels of O2•− radicals in nodules infected with the gshB mutant of R. tropici, and thus antioxidant mechanisms dependent on bacterial GSH might be impaired (Muglia et al., 2008). In R. etli, GSH deficiency was linked to a reduction of glutamine uptake in growing cultures, suggesting a complex GSH-glutamine metabolic relationship that may be important for symbiotic efficiency (Tate et al., 2012). Finally, the mutation in the gshA gene of Bradyrhizobium sp. 6144-STZ appears to affect the ability of the bacterium to compete during peanut (Arachis hypogaea) nodulation, but not its capacity to form effective nodules (Sobrevals et al., 2006). Taken together, these results show that the bacterial GSH pool plays a critical role in the rhizobia-plant interaction and that different cellular processes are regulated by, or are dependent on, GSH in free-living rhizobia and in N2-fixing bacteroids.

The role of bacterial Grx and Trx pathways

An in silico analysis of the S. meliloti genome led to the identification of three genes that putatively encode Grx from different classes (Figure 2): Grx1, containing the dithiol CGYC active site of class I Grx; Grx2, containing the monothiol CGFS active site of class II Grx; and Grx3, carrying two domains, an N-terminal Grx domain with a CPYG active site and a C-terminal domain with a methylamine utilization protein motif (Benyamina et al., 2013). Inactivation of one gene or the other showed that Grx1 and Grx2 play different roles, Grx1 in protein deglutathionylation and Grx2 in regulation of iron metabolism. Both grx1 and grx2 mutants were impaired in bacterial growth and in nodule functioning. On one hand, grx1 inactivation led to nodule abortion and absence of bacteroid differentiation; on the other, grx2 inactivation decreased nodule development without modifying bacteroid differentiation. Therefore, both Grx1 and Grx2 appear to be critical proteins for optimal development of the N2-fixing symbiosis. The incapacity of the grx1 mutant to differentiate is remarkably similar to the
phenotype of a mutant (katB katC) affected in catalase activity (Jamet et al., 2003). This observation emphasizes the importance of a fine tuning of the RONS balance in bacteroid differentiation and the key role of S-glutathionylation in modulating the function of protein essential for this process.

In Escherichia coli, the Grx and Trx pathways, the two branches of the thiol-redox system, are functionally redundant. Whereas the simultaneous inactivation of the two pathways is non-viable, inactivation of either of them is viable, indicating that each pathway can fully carry out the essential function of reducing disulfides in the absence of the other (Toledano et al., 2007). This does not appear to be the case in S. meliloti, where grx1 and grx2 mutants were affected in both free-living bacteria and plant-hosted bacteroids, which points out that Grx1 and Grx2 have more specific roles than the corresponding E. coli enzymes. Consistent with the notion of poor redundancy, Trx-like proteins are required for optimal N2-fixation efficiency in S. meliloti and Rhizobium leguminosarum (Vargas et al., 1994; Castro-Sowinski et al., 2007).

POST-TRANSLATIONAL MODIFICATIONS AND REDOX SIGNALING IN NODULES

Several lines of evidence indicate that RONS are key signals that regulate the establishment of symbiosis (Rama et al., 2002; del Giudice et al., 2011; Puppo et al., 2013). Differences in the intensity, duration and localization of RONS might be perceived and transmitted by thiol-dependent mechanisms. Transcription factors that respond to redox changes by interaction with Trxs have been described in yeast, plants and animals (Buchanan and Balmer, 2005). In legumes, the expression of several transcription factors that respond to redox changes by interaction with Trxs-like proteins involved in post-translational modifications such as tyrosine nitration, as demonstrated for glutamine synthetase (Melo et al., 2011) and leghemoglobin (Navascués et al., 2012), respectively.

Redox regulation via cysteine residues is also important for the bacterial partner of the symbiosis, and this will be briefly illustrated here with a few examples. In Bradyrhizobium japonicum, the cellular pool of active FixK2, a crucial regulator of genes required for the micro-aerobic lifestyle, is partly controlled at the post-translational level (Mesa et al., 2009). The FixK2 activity is modulated by an oxidative-dependent inactivation involving a critical cysteine residue near the DNA-binding domain. This post-translational modification might be a strategy to prevent the detrimental activation of the FixK2 regulon depending on the cellular status. The expression of the fixK2 gene itself is activated by the FixL1 system in response to a moderate decrease of O2 tension. Particularly, in bacteroids, where ROS are assumed to be generated as a side product of the high respiration turnover, the FixK2 transient inactivation could prevent the generation of more ROS and guarantee an adequate balance between the beneficial and detrimental effects of respiration.

Thiol-dependent redox sensing also modulates the activity of antioxidant enzymes such as Prxs. Interestingly, an atypical 2C-Prx of R. etli is involved in the defense of bacteroids against H2O2 stress and could require the Trx system as a source of reducing power (Dombret et al., 2009). An S. meliloti 1G-Prx gene is predominantly expressed in alfalfa root nodules and the protein was detected by proteomic analysis (Puppo et al., 2013). Among
the twenty sulphydryl enzymes detected in bacteria, proteins related to carbohydrate and nitrogen metabolism are largely represented, suggesting that sulphydryl regulation may regulate the activity of crucial proteins for nodule functioning (Oger et al., 2012).

In indeterminate nodules, bacterial differentiation is mediated by nodule-specific cysteine-rich (NCR) peptides, which are defense-related antinoculural peptides (Margaret et al., 2003). Kereszt et al. (2003) determined that the thiol tripeptides are essential for the functioning of the thiol regulatory network.

The transcriptional regulation of enzymes in redox signaling and in compartmentalized in plant cells, with export of peptides in the redox switch involving conserved cysteine residues. Importantly also in the case of legumes, the use of enzyme inhibitors and transgenic plants has demonstrated that thiol tripeptides are essential for the functioning of the rhizobia-legume symbiosis.

Several important questions need, however, to be solved. Further research will be required to establish if GSH and hGSH signaling during nodule development. Our knowledge on the function of other components of the thiol regulatory network in legume nodules is still at an infancy. This may be due to the amazingly high number of Prx, Gpx, Trx and Grx isoforms, which are present in multiple cellular compartments and differ in biochemical properties. Also, it will be necessary to assess the role of thiold peroxidases and other reductases during rhizobial infection and to identify their target nodule proteins. Redox-dependent post-translational modifications constitute a versatile adaptation mechanism to changing conditions. The recent development of redox proteomics permits the large-scale identification of proteins that are modified in response to specific stimuli. To shed light on the signaling events that take place in response to RONS, it will be important to characterize nodule proteins that undergo oxidation, nitrosylation or glutathionylation of critical cysteines, and to investigate the impact of these modifications on their biological activities. The generation of mutants and/or transgenic lines will be most helpful to establish the function of individual proteins and metabolites in the rhizobia-legume symbiosis. Finally, a comparative study of the thiol-based signaling mechanisms underlying the symbiotic and pathogenic interactions and the plant responses to environmental cues will provide critical information to enhance nitrogen nutrition in crop legumes as well as their tolerance to abiotic and biotic stress. The improvement of the N2 fixation efficiency is expected, in turn, to have direct beneficial consequences for sustainable agriculture and the environment as this biological process will eventually lead to a reduction in the input of costly and contaminating nitrogen fertilizers.

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Thiol functions in nitrogen-fixing symbiosis

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