ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 7 & 8, 2005


# Forum Original Research Communication

# Identification of Plant Glutaredoxin Targets

NICOLAS ROUHIER,[1,3] ARSENIO VILLAREJO,[2] MANOJ SRIVASTAVA,[3] ERIC GELHAYE,[1] OLIVIER KEECH,[2] MICHEL DROUX,[4] IRIS FINKEMEIER,[5] GÖRAN SAMUELSSON,[2] KARL JOSEF DIETZ,[5] JEAN-PIERRE JACQUOT,[1] and GUNNAR WINGSLE[3]

## ABSTRACT

**Glutaredoxins (Grxs) are small ubiquitous proteins of the thioredoxin (Trx) family, which catalyze dithiol–disulfide exchange reactions or reduce protein-mixed glutathione disulfides. In plants, several Trx-interacting proteins have been isolated from different compartments, whereas very few Grx-interacting proteins are known. We describe here the determination of Grx target proteins using a mutated poplar Grx, various tissular and subcellular plant extracts, and liquid chromatography coupled to tandem mass spectrometry detection. We have identified 94 putative targets, involved in many processes, including oxidative stress response [peroxiredoxins (Prxs), ascorbate peroxidase, catalase], nitrogen, sulfur, and carbon metabolisms (methionine synthase, alanine aminotransferase, phosphoglycerate kinase), translation (elongation factors E and Tu), or protein folding (heat shock protein 70). Some of these proteins were previously found to interact with Trx or to be glutathiolated in other organisms, but others could be more specific partners of Grx. To substantiate further these data, Grx was shown to support catalysis of the stroma β-type carbonic anhydrase and Prx IIF of *Arabidopsis thaliana,* but not of poplar 2-Cys Prx. Overall, these data suggest that the interaction could occur randomly either with exposed cysteinyl disulfide bonds formed within or between target proteins or with mixed disulfides between a protein thiol and glutathione.** *Antioxid. Redox Signal.* 7, 919–929.

## INTRODUCTION

In all organisms, the two major disulfide reducing enzymatic systems, thioredoxin (Trx)- and glutaredoxin (Grx)-dependent, are able to regulate several cellular processes through dithiol–disulfide exchanges with many target proteins. Trxs and Grxs are small ubiquitous proteins, with conserved CxxC/S active sites, where x represents various amino acids. Their midpoint redox potentials differ, being close to −300 mV for Trx and −200 mV for Grx. In the cytosol and mitochondria, Trxs are reduced via NADPH and an NADPH-dependent thioredoxin reductase (TR) and Grxs via NADPH-dependent glutathione reductase (GR) and glutathione (GSH). A characteristic of Grxs is their efficiency in reducing protein mixed disulfides between proteins and GSH, using only the first catalytic cysteine of the active site (43).

In *Arabidopsis thaliana* and presumably in all higher plants, there are at least 26 Trx isoforms and 31 Grx isoforms predicted to be located in various cellular compartments, not taking into account the significant set of Trx- or Grx-like proteins (28, 37). Up to now, very few plant Grxs have been characterized in terms of expression, localization, or biochemical and structural data (37), whereas the function of most Trx isoforms has been characterized in various plant subcellular compartments (40).

Several recent articles have described the identification of Trx-interacting proteins in plants and in other organisms using proteomics, but Grx-interacting partners have not been studied.

[1]Unité Mixte de Recherches 1136 INRA UHP (Interaction Arbres Microorganismes), IFR 110 Génomique Ecophysiologie et Ecologie Fonctionnelles, Université Henri Poincaré, Vandoeuvre, France.
[2]Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå, Sweden.
[3]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå, Sweden.
[4]Laboratoire Mixte Centre National de la Recherche Scientifique/Bayer CropScience, Lyon, France.
[5]University of Bielefeld, Department of Plant Physiology and Biochemistry/W5, Bielefeld, Germany.

These approaches used either thiol probes (monobromobimane or a cyanide dye 5 maleimide) (25, 26, 47, 49) or, more generally, a protein mutated on the second active-site cysteine as a bait to covalently trap some Trx-linked proteins, by arresting the heterodimer intermittently formed during the catalytic reaction (4, 5, 22, 24, 29, 48). These methods are especially efficient in the case of dithiol–disulfide exchanges. However, in some cases, the interaction between Trxs and their targets does not seem to be linked to the redox state of Trxs. For example, the reduced form of *E. coli* or human Trxs binds to T7 DNA polymerase or to the apoptosis signaling kinase 1, respectively, but not through a covalent interaction involving a disulfide bond (2, 39). Recently, an approach to identify non–redox-regulated Trx partners in *E. coli* was developed (19). A TAP (tandem affinity purification)-tagged Trx fusion was expressed in an *E. coli trxA*$^-$ strain and used to bind *in vivo* some partners. Eighty cytosolic or periplasmic-bound proteins, susceptible to interact with Trx and, for some of them, independently of regulatory cysteines, have been identified this way.

Some well-characterized proteins, such as *E. coli* ribonucleotide reductase and phosphoadenylyl sulfate reductase, poplar type II peroxiredoxin (Prx), or human glutathione peroxidase, are regulated both by Trxs and Grxs (7, 14, 23, 35). Nevertheless, the very electronegative redox potential of the disulfide bridges of some target proteins, or the need to deglutathiolate some others, should in principle allow us to identify some specific partners of Trxs or Grxs, respectively.

This study presents the isolation of Grx-interacting proteins from poplar leaves and stems, *A. thaliana* leaves, *A. thaliana* or potato mitochondria, and pea chloroplast stroma. The five protein extracts were passed through an affinity column constituted by a poplar Grx mutated on the second active-site cysteine (CPYS), followed by tryptic digestion of the targets coupled to liquid chromatography–mass spectrometry (MS)–MS analysis and database search. This poplar Grx was previously characterized and renamed here Grx CxxC4, or GrxC4 for short, according to the classification described previously (37). Many of the identified proteins were already found either to contain a disulfide bridge, to interact with Trx, or to be glutathiolated, but some could be specific Grx target proteins. Moreover, the interaction of poplar GrxC4 with three identified targets, stroma β-type carbonic anhydrase (CA), Prx IIF from *A. thaliana,* and poplar 2-Cys Prx, was investigated in more detail.

## MATERIALS AND METHODS

### *Chemicals and reagents*

NADPH was obtained from Boehringer Mannheim, dithiothreitol (DTT) from Euromedex, Trypsin Gold from Promega, iodoacetamide, yeast GR, and GSH from Sigma, and CNBr Sepharose 4B from Amersham Biosciences. The recombinant poplar GrxC4 C30S was purified as described previously (36).

### *Preparation of the Grx affinity chromatography column*

Ten milligrams of recombinant poplar GrxC4 C30S were extensively dialyzed against a bicarbonate buffer (50 m*M* $NaHCO_3$, pH 8.3). The proteins were mixed together with 1.5 g of washed CNBr Sepharose with gentle shaking overnight at 4°C. The gel was then poured into a 10-ml syringe and washed with bicarbonate buffer. The unreacted CNBr sites were blocked by incubating for 1 h with 1 *M* Tris-HCl, pH 8.0. Finally, the columns were equilibrated in TE buffer (30 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA).

### *Preparation of protein extracts*

The starting material was 100 g of leaves and stems from a 2-month-old poplar, Beaupré clone (*Populus trichocarpa x deltoides*) or 40 g of leaves from 1-month-old *A. thaliana*, ecotype *Columbia*. They were ground with a Waring blender or in a mortar, respectively, in the presence of TE with 1% PVP40 and 25 m*M* ascorbate. The crude extract was filtered through three layers of cloth and centrifuged to remove insoluble materials (16,000 rpm, 1 h). The preparations of potato mitochondria matrix and of pea chloroplast stroma were described previously (5). Mitochondria from 7–8-week-old *A. thaliana* leaves, grown under a photoperiod of 8 h, were obtained by differential centrifugation and phase partitioning. The mitochondrial fraction was slightly contaminated by chloroplasts (5–7% based on chlorophyll content) and by <1% peroxisomes (based on catalase assays). An osmotic shock was further performed by resuspending the mitochondria-enriched extract into 20 m*M* TES (TE containing 200 m*M* NaCl) and freezed-thawed three times in liquid nitrogen. After centrifugation (20,000 *g*, 20 min, 4°C), mitochondrial proteins were precipitated with 80% ammonium sulfate.

### *Isolation of covalently Grx-bound proteins*

The soluble extracts were loaded onto the GrxC4 C30S affinity column overnight at 4°C with a peristaltic pump. The column was washed with TE, TES, and finally TE again until no protein was detected. The proteins, bound to Grx probably through a disulfide bridge, were then eluted with TE supplemented with 10 m*M* DTT, dialyzed against TE, and concentrated. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (15%) was performed in order to assess the complexity of the sample.

### *Tryptic digestion and electrospray MS*

Two alternative methods were used to digest the samples containing the Grx-linked proteins, leading to similar results. The extract was first precipitated on ice for 30 min by addition of an equal volume of 20% trichloroacetic acid, and the pellet washed with 2% trichloroacetic acid. The pellet was then resuspended and incubated in TE containing 8 *M* urea for 30 min at room temperature and the proteins precipitated again following the same procedure. The second method did not use any precipitation to prevent protein losses. Proteins (20 μg) were concentrated using Microcon YM-3 spin filters, denatured, and reduced with a solution containing 6 *M* guanidine, 0.1 *M* Tris-HCl, pH 8.0, 5 m*M* EDTA, and 20 m*M* DTT for 60 min at 56°C. The sample was then alkylated for 30 min at 37°C in the dark, in the same solution, except that DTT was replaced by 55 m*M* iodoacetamide. Finally, in both procedures, the proteins were resuspended in 0.1 *M* ammonium bicarbonate containing 1 μg of trypsin for 20 μg of proteins,

and digested overnight at 37°C. The peptides obtained after digestion were concentrated using a vacuum concentrator, and 0.1% formic acid was added before analysis. If needed, the peptides were purified using Zip TipsC18 pipettes (Millipore). Tandem MS experiments were carried out on a Micromass Q-Tof Ultima (Waters Micromass MS Technologies) hybrid tandem mass spectrometer (8) equipped with a nanoflow electrospray source. MS-MS spectra were analyzed using the Protein Lynx Browser program (MassLynx software, Micromass). The peptides obtained were analyzed against the genomic sequence of *P. trichocarpa* in the case of poplar extract or against *S. tuberosum*, *P. sativum,* and *A. thaliana* nonredundant databases downloaded from the PIR (Protein Information Resources) Website (ftp://ftp.pir.georgetown.edu/pir_databases/). Only the peptides that exactly matched a given protein sequence were considered.

### *Partial purification of* β*-CA from* Arabidopsis *chloroplast*

Chloroplasts from *A. thaliana* were purified following the method described by Kunst (20) with some modifications in order to increase the purity of the preparations (12). The chloroplasts were further purified on a 50% (vol/vol) Percoll gradient (Amersham Biosciences). Intact chloroplasts in the resuspension buffer were sonicated 3 × 30 s and centrifuged at 15,000 *g* for 30 min. The supernatant, containing most of the stromal proteins, was applied to a 1-ml MonoQ anion-exchange column (HiTrap Q FF; Amersham Biosciences) equilibrated with 20 m*M* Tris-HCl buffer, pH 7.8. Bound proteins were eluted with a 30-ml linear gradient from 0 to 800 m*M* NaCl. Each fraction was desalted using PD-10 columns (Amersham Biosciences). The purification process was monitored by subjecting aliquots of each fraction to western blot analysis with antibodies raised against β-CA from spinach and α-CA from *A. thaliana*. The fractions containing β-CA, but devoid of α-CA, were used.

### *CA activity measurements*

CA activity was determined as described previously (46). Basically, the measurements were done in 100-µl reaction mixtures in 20 m*M* HEPES-KOH buffer, pH 8.0, containing 150 µ*M* NADPH, 0.5 units of GR, 500 µ*M* GSH, and 10 µ*M* GrxC4 wild type. The reaction was started by adding a stroma fraction enriched in β-CA. After 20 min of incubation, the activity was measured electrometrically by monitoring the rate of pH change immediately following the addition of $CO_2$-saturated water to the extract at 2°C. The accuracy and rapid response required for this analysis were assured by using a fast-response microelectrode (MI-710 "blue glass," Microelectrodes Inc., U.S.A.) and an 18-bit A/D converter (IOtech Inc., U.S.A.). Differences in the rate of $CO_2$ hydration after the addition of $CO_2$-saturated water to sample and control (buffer) solutions were used to calculate CA activity, which was expressed as Wilbur–Anderson units/mg of protein.

### *Cloning, expression, and purification of poplar 2-Cys Prx and Prx IIF*

The 2-Cys Prx is a chloroplastic enzyme possessing a transit sequence at the N-terminus. The open reading frame, without the sequence encoding the transit peptide, was cloned by PCR from an imbibed seed cDNA library of *P. tremula* (Genbank accession number BU861364) into the plasmid pET-3d using the pfu polymerase (Promega) and the two following oligonucleotides (*Nco*I and *Bam*HI restriction sites underlined):

2-Cys Prx forward:
5′ CCCCCCATGGCTGTCAAAGCCACGAGCGAACTTCCA 3′

2-Cys Prx reverse:
5′ CCCCGGATCCCTATATGGCAGCAAAGTAATC 3′

The recombinant protein was thus produced in the mature form with addition of a methionine and an alanine to improve the translation initiation, leading to a protein with MAKVTSEL and SKDYFAAI at the N-, and C-termini, respectively. The recombinant plasmid was used to transform an *E. coli* BL21(DE3) strain containing the helper plasmid pSBET (36). A 2.4-L culture of the recombinant strain in LB medium at 37°C was induced during 4 h with 100 µ*M* isopropyl-β-D-thiogalactopyranoside. The bacterial cells were collected by centrifugation (5,000 *g*, 15 min) and resuspended in TE. After sonication of the bacterial extract, the proteins were precipitated between 0 and 40% of the saturation in ammonium sulfate, collected by centrifugation, and redissolved in TE. This fraction was extensively dialyzed against TE, subsequently loaded onto a DEAE Sepharose, and then eluted with a 0–400 m*M* NaCl gradient in TE. The fractions containing the recombinant 2-Cys Prx were pooled, concentrated, and dialyzed against TE and stored in aliquots at −20°C at a concentration of 9 mg/ml.

AtPrxIIF cDNA was amplified with the primers prxIIF-F (5′ TCAAAGCTCGCGGAAGGCACT 3′) and prxIIF-R (5′ TTAGATCTGTCCTAAGA 3′) to obtain a product that excluded the coding region for the 28-amino acid signal peptide. The cDNA was cloned in frame into the pCR® T7/NT-TOPO vector (Invitrogen). Expression and purification of the N-terminally 6x His-tagged recombinant protein was performed as described (15).

### *Peroxide-reducing activity of Prxs*

The hydroperoxide-reducing Prx activity was measured at room temperature by following the oxidation of NADPH at 340 nm in the presence of either the TR/Trx system or the GR/GSH/Grx system using a Cary 50 Spectrophotometer (Varian). In the case of 2-Cys Prx, a 500-µl reaction mixture in TE, pH 8.0, contained 150 µ*M* NADPH, 5 µ*M* 2-Cys Prx, 250-µ*M* hydrogen peroxide ($H_2O_2$), and the Trx-reducing system (2 µ*M A. thaliana* TRB and 10 µ*M* poplar Trx h1) or the Grx-reducing system (0.5 unit of GR, 500 µ*M* GSH, and 10 µ*M* poplar GrxC4).

In the case of AtPrx IIF, the 500-µl reaction mixture contained 150 µ*M* NADPH, 250 µ*M* $H_2O_2$, 2 µ*M* Prx IIF, in the presence of the Grx system (0.5 unit of GR, 500 µ*M* GSH, and 6.5 µ*M* poplar GrxC4) or the Trx system (2 µ*M A. thaliana* TRB with 20 µ*M* poplar Trx h1, h2, h3, or *Chamydomonas reinhardtii* Trx Ch1, or 1.3 µ*M E. coli* TR with 20 µ*M E. coli* Trx1). The reactions were started after 1 min of incubation by adding Prxs.

## RESULTS AND DISCUSSION

We have used an affinity chromatography column made with poplar GrxC4 mutated on the second active-site cysteine in combination with various plant extracts to trap and identify Grx target proteins and thereby detect processes potentially regulated by this protein. The mutated GrxC4 C30S used contained only the catalytic cysteine, preventing artifactual interaction with additional thiol groups. Previous studies using Trx as a bait showed a lack of specificity, *i.e.,* a cytosolic Trx is able to interact with chloroplastic or mitochondrial target enzymes (5, 48). Up to now, the localizations of the various plant Grxs are not known, but analysis of *A. thaliana* genome indicates that Grxs are predicted to be present in most plant cell compartments, including chloroplasts and mitochondria (37). Thus, all the proteins retained, whatever their subcellular compartment, are putative Grx-interacting proteins *in vivo*. By using this approach, 94 target proteins have been identified. We have listed all those potential targets in Table 1. The list regroups sequences from poplar, arabidopsis, and potato, either from whole extracts or from purified mitochondrial or chloroplastic preparations. In this list, we have included the closest *Arabidopsis* homologues that are very often orthologues, given the similarity of the sequences. Also indicated is the putative subcellular localization, although one must keep in mind that some of these are just predictions. One surprising finding is the ability of this Grx to bind some well-known Calvin cycle enzymes, such as fructose 1,6-bisphosphatase (FBPase) and phosphoribulokinase (PRK), which present regulatory disulfides possessing a very electronegative redox potential (approximately −300 mV to −360 mV) compared with the one of Grxs (approximately −200 mV for *E. coli* Grxs). This could be explained in several ways: (a) this attack may occur despite the differences in redox potential, perhaps because Grx is different from Trx, being able to act as a monocysteinic enzyme; (b) it may interact with glutathiolated cysteines; (c) it may interact with some proteins independently of their redox state, the addition of DTT during the elution simply modifying the interaction and liberating the protein target; and (d) the redox potential of the target's disulfide could be modified upon interaction with the Grx. The cysteine residues involved in the redox regulation of enzymes are generally conserved, but conversely, the conservation of a cysteine does not necessarily imply that it is involved in redox processes. Nevertheless, screening for conserved cysteines is certainly an interesting indication that it is a potential candidate for redox regulation. Nearly all proteins identified here possess at least one conserved cysteine, except for an inorganic pyrophosphatase and an oxygen-evolving enhancer complex protein. It might be that these proteins interact strongly with one of the Grx targets or directly with Grx, but independently of reactive cysteine. In the latter case, the addition of DTT, but not of NaCl, had altered the interaction. In the case where there is only one conserved cysteine, the regulation could result from the formation of a dimer via an intermolecular disulfide bridge or from a glutathiolation/deglutathiolation process.

The 94 different target proteins found are involved in various metabolic processes (Table 1). All proteins identified will not be described here, but emphasis will be made about new targets and their consequences in terms of regulation.

### *Stress response*

Many peroxidases have been identified, especially some well-known Trx or Grx targets called Prxs. Three of the four subgroups of Prxs have been identified: 2-Cys Prxs, Prx Q, and Prx II. Among Prxs II, three different isoforms have been found: the cytosolic enzyme Prx IIB, which uses preferentially Grxs as electron donors (9, 35); the chloroplastic Prx IIE, which uses only Trxs (9); and the mitochondrial Prx IIF, whose donor is unknown. Two additional $H_2O_2$-degrading enzymes previously identified on Trx columns have also been identified as Grx targets: a catalase and a cytosolic ascorbate peroxidase (22, 48). Nevertheless, Lemaire and co-workers have suggested, based on activity measurements, that only algal, but not plant, catalases are redox-regulated (22).

Many new putative Grx-linked proteins related to stress have been identified: three poplar storage-specific proteins (wound-responsive protein, vegetative and bark storage proteins) involved in wound response (11), two chitinases, and a lipase/hydrolase, which are secreted enzymes containing disulfides as described previously (21). Another putative Grx target is protein phosphatase 2C, which participates in stress signaling. Finally, GR and a predicted chloroplastic Grx isoform have also been trapped on the Grx column.

### *Photorespiration*

Two (H and P) of the four subunits (H, P, T, and L) of the glycine cleavage system have been identified here, confirming that this multienzymatic complex could be redox-regulated in plants, but maybe not in all organisms. Indeed, the bacterial or mammalian sequences of the T and H subunits do not possess the conserved cysteines present in plant sequences. A new putative redox-regulated target is the peroxisomal glycolate oxidase. This enzyme, which converts glycolate into glyoxylate and forms $H_2O_2$ during catalysis, contains only one conserved cysteine. Nevertheless, up to now, no Trx or Grx has been characterized in peroxisomes.

### *Translation*

Some components of the translational machinery seem to be redox-regulated. Two 60S ribosomal proteins and two transcription factors have been isolated as Grx targets. In addition to its role in binding and transport of the aminoacyl-tRNA to the aminoacyl site of the ribosome during the elongation step, the *E. coli* transcription factor elongation factor Tu possesses disulfide reductase activity as it is able to catalyze the formation, reduction, or isomerization of disulfide bridges (32).

The glycyl tRNA synthetase, isolated in this study, is the second tRNA synthetase putatively redox-regulated, because a valyl tRNA synthetase from *Synechocystis* was retained on Trx-based affinity column (24).

### *Calvin cycle and associated reactions*

Trxs were originally discovered as activators of some Calvin cycle enzymes, and also of NADP-malate dehydrogenase, during dark to light transition. Since that time, many other enzymes of the Calvin cycle were found to be regulated by Trx, but none of them by Grx. Here, eight Calvin cycle enzymes have been retained on the Grx column, seven [PRK, ribulose 1,5-diphosphate carboxylase (Rubisco; small and

TABLE 1. GRX-LINKED PROCESSES AND TARGETS IN PLANT

| *Name and functional class* | *Localization*[a] | *Peptides*[b] | *Homologue At*[c] | *Cys*[d] | *Trx targets*[e] |
|---|---|---|---|---|---|
| *Stress-related reactions* | | | | | |
| Wound-responsive protein[1] | SP | 2[1] | At3g17210 | 1 | |
| WIN4.5 vegetative storage protein[1] | SP | 1[1] | No | 4 | |
| Bark storage protein[1] | ? | 3[1] | No | 4 | |
| 2-Cys peroxiredoxin*[1,2,4] | P | 3[1], 1[2,4] | At3g11630 | 2 | 4, 22, 25, 29 |
| Peroxiredoxin Q[1] | P | 3[1] | At3g26060 | 2 | 29 |
| Peroxiredoxin IIB*[2] | C | 1[2] | At1g65980 | 2 | 36 |
| Peroxiredoxin IIE*[1,2] | P | 1[1], 2[2] | At3g52960 | 2 | 9, 22, 24 |
| Peroxiredoxin IIF*[5] | M | 4[5] | At3g06050 | 2 | 5 |
| Ascorbate peroxidase[2] | C | 1[2] | At1g07890 | 1 | 48 |
| Catalase[1,5] | Px | 8[1], 4[5] | At4g35090 | 4 | 5, 22 |
| Glutathione reductase[1] | P, M | 1[1] | At3g54660 | 4 | |
| Glutaredoxin*[1,5] | P | 3[1], 1[5] | At2g20270 | 2 | |
| Formate dehydrogenase[3] | M | 2[3] | At5g14780 | 3 | 5 |
| Aldehyde dehydrogenase[1,2,3] | M | 1[1], 2[2], 3[3] | At3g48000 | 5 | 5 |
| GDSL-motif lipase/hydrolase[1] | SP | 2[1] | At5g45670 | 8 | |
| Endochitinase[1] | SP | 1[1] | At2g43580 | 15 | 25 |
| Class I chitinase[1] | SP | 1[1] | At3g12500 | 14 | |
| Protein phosphatase 2C[1] | C | 1[1] | At3g11410 | 7 | |
| Kunitz trypsin inhibitor[1] | C | 3[1] | At1g73330 | 6 | 25 |
| Germin-like protein[1] | SP | 1[1] | At5g20630 | 2 | 24, 47, 48 |
| *Hormone metabolism* | | | | | |
| Allene oxide cyclase[1] | P | 2[1] | At3g25780 | 1 | 5 |
| *Photorespiration* | | | | | |
| Glycine decarboxylase protein H[1] | M | 1[1] | At1g32470 | 1 | 5 |
| Glycine decarboxylase protein P[1,2,5] | M | 3[1,2], 2[5] | At4g33010 | 11 | 5 |
| Glycolate oxidase[1,2] | Px | 2[1], 2[2] | At3g14420 | 1 | |
| *Translation* | | | | | |
| 60S ribosomal protein L30[1] | C | 1[1] | At3g18740 | 3 | |
| 60S ribosomal protein L13[1] | C | 1[1] | At3g24830 | 2 | |
| Elongation factor Tu[1,4] | P | 1[1], 3[4] | At4g20360 | 1 | 4 |
| Elongation factor 2[1] | C | 4[1] | At1g56070 | 10 | 22, 48 |
| Nucleoside diphosphate kinase III*[1] | P | 1[1] | At4g11010 | 1 | 5 |
| Glycyl tRNA synthetase[1] | M | 1[1] | At1g29880 | 10 | |
| *Calvin cycle and associated reactions* | | | | | |
| Phosphoribulokinase[1,2] | P | 3[1], 2[2] | At1g32060 | 4 | 4 |
| NADP glyceraldehyde-3-phosphate dehydrogenase[1,2,4] | P | 5[2], 4[1,4] | At1g12900 | 5 | 4, 42 |
| Transketolase[1,2,4] | P | 3[1], 1[2,4] | At3g60750 | 5 | 4 |
| Fructose 1,6-bisphosphatase[1,2,4] | P | 2[1]4, 12 | At3g54050 | 7 | 38 |
| Triose phosphate isomerase*[1] | P | 3[1], 1[2] | At2g21170 | 4 | 4, 47 |
| Fructose-bisphosphate aldolase*[1,2,4] | P | 3[1], 1[2], 3[4] | At4g38970 | 2 | 22 |
| Phosphoglycerate kinase*[1,2] | P | 2[1], 3[2] | At1g56190 | 2 | |
| Rubisco small chain[1,2,4] | P | 1[1], 3[2], 4[4] | At1g67090 | 4 | 4, 22, 29 |
| Rubisco large chain[1,2,4] | P | 3[1], 7[2,4] | rbcL | 8 | 22, 24 |
| Rubisco activase[4] | P | 1[4] | At1g73110 | 2 | 4, 29 |
| Carbonic anhydrase β*[1,2,4] | P | 5[1], 3[2], 2[4] | At3g01500 | 5 | 4 |
| *Glycolysis* | | | | | |
| Enolase*[1] | C | 3[1] | At2g36530 | 4 | 22, 25, 47 |
| NAD glyceraldehyde-3-phosphate dehydrogenase*[1] | C | 1[1] | At3g04120 | 2 | 25, 47, 48 |
| *Chaperonins* | | | | | |
| 14-3-3 protein*[1] | C | 1[1] | At5g65430 | 2 | |
| Cyclophilin*[1] | C | 1[1] | At4g01310 | 4 | 25, 29, 47 |
| Protein disulfide isomerase*[5] | SP | 2[5] | At1g21750 | 4 | 47 |
| Heat shock protein 70 kDa[1] | P | 2[1] | At5g49910 | 2 | 4, 22, 48 |
| Heat shock protein 70 kDa*[1] | C | 1[1] | At1g56410 | 7 | 22 |
| Heat shock protein 70 kDa[3] | M | 4[3] | At5g09590 | 3 | 5 |
| Chaperonin HSP 60 α[1,2,4] | P | 1[1,2,4] | At2g28000 | 1 | 4, 22, 48 |
| Chaperonin HSP 60 β[1,4] | P | 1[1,4] | At5g56500 | 4 | 48 |
| Chaperonin HSP 60*[3] | M | 4[3] | At2g33210 | 4 | 5 |

TABLE 1. GRX-LINKED PROCESSES AND TARGETS IN PLANT (*continued*)

| *Name and functional class* | *Localization*[a] | *Peptides*[b] | *Homologue At*[c] | *Cys*[d] | *Trx targets*[e] |
|---|---|---|---|---|---|
| *Citric acid cycle and associated reactions* | | | | | |
| NAD-dependent malate dehydrogenase*[1,5] | M | 2[1], 2[5] | At1g53240 | 3 | 5, 25 |
| Isocitrate dehydrogenase NADP[1] | M | 1[1] | At5g14590 | 6 | 5, 47 |
| NAD-dependent malate dehydrogenase*[1] | C | 5[1] | At5g43330 | 5 | 25, 47, 48 |
| Malic enzyme[1,5] | M | 1[1], 3[5] | At2g13560 | 2 | 5 |
| Succinyl CoA ligase β subunit[3] | M | 2[3] | At2g20420 | 5 | 5 |
| Aconitate hydratase[3] | C | 1[3] | At2g05710 | 8 | 5, 22, 25 |
| Succinate dehydrogenase[3] | M | 2[3] | At5g66760 | 8 | 5 |
| Dihydrolipoamide dehydrogenase[1] | M | 1[1] | At1g48030 | 3 | 5 |
| *Sulfur metabolism* | | | | | |
| Methionine synthase[1] | C | 2[1] | At5g20980 | 3 | 48 |
| Cysteine synthase[1] | P | 1[1] | At3g59760 | 3 | 4 |
| *S*-Adenosyl-L-homocysteine hydrolase[1] | C | 1[1] | At4g13940 | 9 | |
| *Nitrogen metabolism* | | | | | |
| Alanine aminotransferase[1,2] | C | 2[1], 2[2] | At1g23310 | 3 | 5, 25, 47 |
| Ferredoxin-dependent glutamate synthase[1,4] | P | 1[1], 2[4] | At2g41220 | 13 | 24 |
| Glutamine synthetase[1,4] | P | 1[1], 2[4] | At5g35630 | 4 | 4, 22, 29, 48 |
| Glutamate dehydrogenase[3] | M | 1[3] | At5g18170 | 4 | 5 |
| *Electron transfer/photosynthesis/respiration* | | | | | |
| NADH ubiquinone oxidoreductase 75 kDa[2] | M | 2[2] | At4g37510 | 12 | 5 |
| NADH ubiquinone oxidoreductase 22.5 kDa[3] | M | 1[3] | At5g52840 | 3 | |
| NADH dehydrogenase subunit 9[2] | M | 1[2] | nad9 | 2 | |
| Ferredoxin 2Fe 2S[4] | P | 1[4] | At1g10960 | 5 | 22 |
| Ferredoxin NADP reductase[1] | P | 1[1] | At5g66190 | 4 | |
| 16-kDa polypeptide oxygen evolving complex (PsbQ)[1] | P | 2[1] | At4g21280 | 0 | |
| *Starch synthesis* | | | | | |
| ADP glucose pyrophosphorylase[2,4] | P | 1[2], 1[4] | At5g48300 | 4 | 22, 47 |
| Reversibly glycosylated polypeptide/ UDP glucose transglycosylase[1] | C | 1[1] | At5g15650 | 8 | 22, 47 |
| *Protein degradation* | | | | | |
| Subtilase[2] | P | 2[2] | At4g20850 | ? | |
| Peptidase α subunit[3] | M | 2[3] | At3g16480 | 1 | |
| ATP-dependent Clp protease[1] | P | 2[1] | At1g02560 | 1 | 4 |
| *ATP metabolism/energy* | | | | | |
| ATP synthase β chain[3] | M | 9[3] | At5g08670 | 2 | 5 |
| ATP synthase α chain[3] | M | 3[3] | At2g07698 | 5 | 5 |
| Adenosine kinase[1,2] | C | 1[1], 4[2] | At5g03300 | 3 | |
| Inorganic pyrophosphatase*[1,2] | P | 2[1], 2[2] | At5g09650 | 0 | 22 |
| *Others* | | | | | |
| Unknown protein[3] | M | 3[3] | At5g10860 | 1 | 5 |
| Plastid developmental protein[5] | P | 2[5] | At3g15000 | 1 | |
| Glycerol-3-phosphate dehydrogenase*[2] | P | 1[2] | At3g10370 | 9 | |
| Formamidase[1] | C | 1[1] | At4g37560 | 9 | |
| Tyrosine transaminase[1,2] | C | 3[1], 1[2] | At4g23600 | 3 | |
| Chalcone flavonone isomerase[1] | C | 1[1] | At3g55120 | 1 | |
| Lectin/myrosinase binding protein[2] | C | 2[2] | At3g16390 | ? | |
| Cell division cycle protein 23[2] | M | 2[2] | At3g48150 | 7 | |
| Uridyltransferase[1] | C | 1[1] | At2g03730 | 5 | |
| Phenylalanine ammonia lyase[1] | C | 1[1] | At5g04230 | 4 | |
| Red chlorophyll catabolite reductase[1] | P | 1[1] | At4g37000 | 2 | |

[a]Putative subcellular localization of the various targets were determined using TargetP and Psort programs. C, cytosol; M, mitochondria; P, plastid; Px, peroxisomes; SP, secretory pathway.

[b]Number of peptides found with exact match to the sequences of the databases.

[c]Accession number of the closest homologue in *A. thaliana*.

[d]Number of strictly conserved cysteines in photosynthetic organisms.

[e]Identified as a probable Trx target in photosynthetic organisms.

*Identified as a glutathiolated protein in some organisms.

[1]Identified from a total protein extract of *Populus trichocarpa* × *deltoides*.

[2]Identified from a total protein extract of *Arabidopsis thaliana*.

[3]Identified from a mitochondrial protein extract of *Solanum tuberosum* or *A. thaliana*.

[4]Identified from a chloroplastic protein extract of *Pisum sativum*.

[5]Identified from a mitochondrial protein extract of *A. thaliana*.

large subunits), NADP glyceraldehyde phosphate dehydrogenase, triose phosphate isomerase, fructose bisphosphate aldolase, fructose 1,6-bisphosphatase, transketolase] are putative or confirmed Trx targets, whereas phosphoglycerate kinase was identified for the first time as putatively redox-regulated. Actually, with the identification of phosphoglycerate kinase, 10 of the 11 enzymes of the Calvin cycle, except phosphopentose epimerase, can be considered as potential Trx or Grx targets. Two Calvin cycle-related enzymes, Rubisco activase, which is also regulated by the chloroplastic Trx f (50) and retained on a Trx m column (29), and β-CA, previously retained on a Trx column, were also identified in this study (4, 29, 50).

### *Chaperones*

Three of the chaperones identified in this study represent various isoforms of the 70-kDa heat shock protein (HSP70) predicted to be cytosolic, plastidic, or mitochondrial. Two HSP70 from yeast or *A. thaliana* were shown to be redox-regulated, presumably via a conserved cysteine, by an *A. thaliana* Trx-like protein called TDX (tetratricopeptide domain containing Trx), which contains an HSP70-interacting protein in the N-terminal part, and a Trx domain in the C-terminus (45). When aligning isoforms of the three subcellular compartments, the critical cysteine discussed below is only conserved in cytosolic and mitochondrial isoforms, but not in chloroplastic ones. This suggests that the chloroplastic isoform, which was found many times in the proteomic analysis of Trx targets (4, 48), might not be regulated in the same way as the cytosolic and mitochondrial isoforms. Nevertheless, it contains two conserved cysteines in different positions.

Another protein involved in protein folding or assembly is the chaperonin HSP60. In the chloroplast, two subunits, α and β, can be distinguished. These two proteins display, respectively, one and four conserved cysteines with one cysteine in a common conserved position. Interestingly, a mitochondrial isoform, also targeted by Grx and Trx, does not contain this cysteine, but four others are conserved. Similar to HSP70, the various HSP60 isoforms might not be regulated by identical mechanisms.

An isoform of cyclophilin and protein disulfide isomerase have also been identified. Recently, Trx was shown to regulate cyclophilin activity in plants (30). A 14-3-3 protein is proposed for the first time as redox-regulated in plants. On the other hand, a proteomic study has identified a Trx and a Prx as binding partners of 14–3-3 (27).

### *Citric acid cycle and associated reactions*

Of the eight enzymes of the citric acid cycle, six were shown to be putative Trx-interacting proteins, but none of these interactions was firmly established biochemically. Here, we have identified four enzymes involved directly in this cycle (acetyl-CoA ligase, succinate dehydrogenase, NAD-malate dehydrogenase, isocitrate dehydrogenase) and three in related reactions (malic enzyme, cytosolic aconitase, and cytosolic NAD-malate dehydrogenase) (see Table 1). Finally, a dihydrolipoamide dehydrogenase, one of the components of the α-ketoglutarate dehydrogenase, glycine cleavage system, or pyruvate dehydrogenase complexes, was also trapped on the Grx column.

### *Sulfur metabolism*

Three enzymes involved in sulfur metabolism, cysteine synthase, *S*-adenosyl-L-homocysteine (SAH) hydrolase, and methionine synthase, have been identified in this study.

The cysteine synthase isolated here is a putative chloroplastic isoform. Eight isoforms of cysteine synthase are potentially present in the *A. thaliana* genome and predicted to be localized in the cytosol, chloroplasts, and mitochondria. The chloroplastic and mitochondrial isoforms were proposed to be Trx-linked (4, 5). An argument to support this idea is that DTT is necessary for cysteine synthase activation (34). Surprisingly, mitochondrial isoforms do not possess conserved cysteines and cytosolic isoforms were not detected here, although of the two cysteines they possess, one is common with plastidic isoforms, which contain three overall.

A new putative Grx target protein, SAH hydrolase, catalyzes the hydrolysis of SAH, produced from *S*-adenosyl-L-methionine-dependent methylation reactions, into L-homocysteine and adenosine. The adenosine formed was shown, for example, to be used for the synthesis of the purine ring of caffeine (18). One enzyme able to use adenosine is adenosine kinase, which catalyzes the salvage synthesis of adenine monophosphate from adenosine and ATP. This enzyme was also identified for the first time as potentially redox-regulated.

On the other hand, homocysteine can be used to regenerate methionine by methylation via vitamin $B_{12}$-independent methionine synthase. *A. thaliana* contains three functional isoforms of this enzyme: one is chloroplastic and two are cytosolic (31). A cytosolic isoform was identified here, but all these isoforms could be Trx- or Grx-dependent as they possess three conserved cysteines, but two of them bind zinc. Whether Trx and Grx regulate this enzyme by dithiol–disulfide exchange, by glutathiolation, or just by reducing cysteines to allow zinc incorporation is not known. Finally, the methyl cycle seems to be mainly controlled by redox regulation, because in addition to methionine synthase and SAH hydrolase, *S*-adenosyl-L-methionine synthetase was identified as a Trx target in *C. reinhardtii* (22).

### *Electron transfer*

Some subunits of the four mitochondrial electron transfer chain complexes were suspected in a previous study of being Trx-linked proteins. Here, we have identified three subunits of the NADH ubiquinone oxidoreductase. In addition to the previously identified 75-kDa subunit, a subunit of 9 kDa and another of 22.5 kDa could be Grx-linked proteins. One iron-sulfur-containing protein, ferredoxin, has been retained. In *S. cerevisiae* and *E. coli*, Grxs are involved in iron-sulfur assembly (1, 33). The situation could be similar in plants, and Grxs could participate in the assembly of Fe/S cluster of ferredoxin. Finally, the psbQ-encoded subunit of oxygen-evolving enhancer (OEE) complex could be an additional polypeptide of photosystem II redox-regulated. It has been shown earlier that the psbO-encoded OEE1 is a putative Trx target and possesses Trx-like activity in algae (13, 22).

### *Polysaccharide synthesis*

ADP-glucose pyrophosphorylase, a central enzyme in starch synthesis that catalyzes the formation of ADP glucose

from glucose-1-phosphate, was identified. This enzyme was already shown to be regulated by chloroplastic m and f Trxs (3). UDP-glucose transglycosylase or reversibly glycosylated protein is an enzyme that undergoes self-glycosylation and is involved in cell-wall polysaccharide synthesis. The latter enzyme is also a putative Trx target (22, 47).

### *ATP metabolism/energy*

In this study, we have identified the α and β subunits of the mitochondrial ATPase, which were also described as Trx-linked proteins, in addition to the γ subunit. Surprisingly, only the vacuolar, but not chloroplastic, $H^+$-ATPase was retained on Trx column, whereas both were shown to be redox-regulated (41, 44). Concerning nucleoside diphosphate kinase, three different isoforms, found in cytosol, chloroplast, and mitochondria, contain zero, one, or two conserved cysteines, respectively. Only the mitochondrial isoform was identified as a Trx or Grx target. The situation may be similar to that of some carbon fixation enzymes (FBPase, PRK, NADP malate dehydrogenase), where insertions or additions of segments with conserved cysteines were shown to be responsible for their redox regulation (38).

### *Grx activation of 2-Cys Prx, Prx IIF, and CA*

To estimate the accuracy of the method used, we measured the activity of three of the target enzymes, *i.e.,* recombinant poplar 2-Cys Prx (Pt2-Cys Prx), *A. thaliana* Prx IIF (At-PrxIIF), and a partially purified chloroplastic β-CA, in the presence of the Grx system and sometimes the Trx system. So far, only the activity of 2-Cys Prx has been shown to be coupled to reductive regeneration by Trx, whereas the Grx dependency of these enzymes has not been tested to our knowledge. On the other hand, Grxs were shown to be donors to cytosolic Prxs II, but not to the chloroplastic isoform Prx IIE (9, 35). To minimize the side reactions due to GSH alone, we have deliberately kept it at a suboptimal concentration of 0.5 m*M*. It is thus expected that the effects recorded here could be more pronounced in *in vivo* conditions.

Figure 1 displays Pt2-Cys Prx or AtPrxIIF hydroperoxide reducing activity and β-CA activity in the presence of the Grx-reducing system. The two Prxs clearly behave differently with respect to the nature of the electron donor. Even at the highest concentration used, Grx was unable to serve as a donor to Pt2-Cys Prx (Fig. 1A and data not shown), but it is able to reduce AtPrxIIF (Fig. 1B). On the other hand, 2-Cys Prx is reduced by PtTrxh1 and 3 (data not shown), whereas AtPrxIIF was not reduced whatever the Trx used (Fig. 1B).

The activity of β-CA is enhanced 2–2.5 fold by adding GrxC4 compared with the control without Grx-reducing system. The second control without Grx indicates that GSH is not able by itself to enhance CA activity. Previously, it was shown that two exposed cysteinyl residues were critical for activity and structural folding, especially at the tetramer–tetramer interface (6). In addition, it has been shown that the activity of this CA isoform, which is inhibited under oxidative conditions, is partially restored by DTT (17) Altogether, these results suggest that CA activity could be regulated by GrxC4. Whether Grx acts as a reductant or as a structural factor in the activation process of β-CA is still unknown. Nevertheless, this is the first report of a positive effect of Grx on this type of protein.

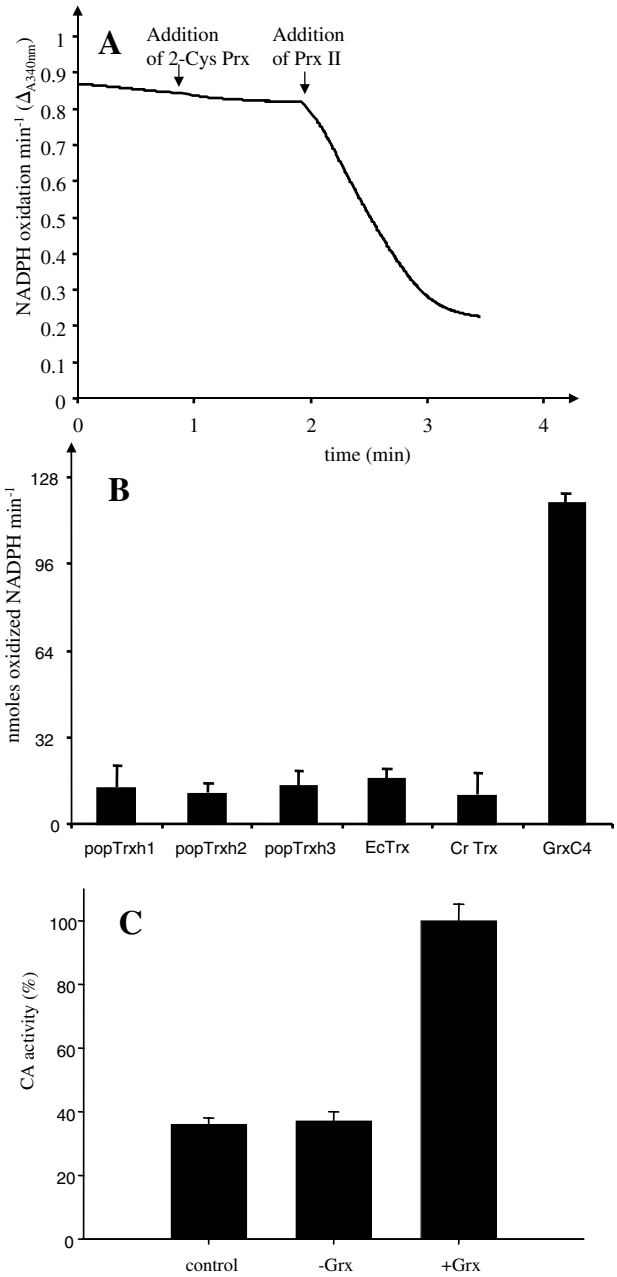


**FIG. 1. Grx dependency of the activity of three target enzymes.** (**A**) Grx-dependent activity of 2-Cys Prx. The activity of Pt2-Cys Prx was tested by measuring NADPH oxidation at 340 nm in the presence of the Grx system (GR, GSH, and poplar GrxC4). As a control, the previously identified Grx-dependent Prx II of poplar was added. (**B**) Mitochondrial Prx IIF activity. The activity of AtPrxIIF was tested spectrophotometrically in the presence of Trx systems (At TRB with poplar Trx h1, 2, 3 or *C. reinhardtii* CH1 or *E. coli* thioredoxin reductase and Trx1) or the Grx system (GR, GSH, and poplar GrxC4), by following the oxidation of NADPH at 340 nm. (**C**) Grx-dependent activity of stroma β-type CA. Control, stroma sample without Grx-reducing system; −Grx, stroma sample incubated without Grx but with GSH; +Grx, stroma sample incubated with 10 μ*M* GrxC4 and GSH. Measurements were done in stroma fractions from three different *A. thaliana* chloroplast preparations. The CA activity in the stroma fraction used for this study was 1,089 ± 29 Wilbur–Anderson units/mg of protein.

## CONCLUSION

Up to now, only four proteins were putative or confirmed Grx targets in plants, whereas more than 30 different Grxs are present in higher plant genomes (37). Three subgroups were distinguished among the Grx family based essentially on the sequence of their active site, either CxxC/S, CGFS, or CCxC/S amino acid sequences (x represent various amino acids). The only characterized Grxs belong to the subgroup of proteins with CxxC/S active sites.

The cytosolic poplar Prx II recently characterized as the first Grx target was also found in this study (35). Another target is a $H^+/Ca^{2+}$ exchanger from *A. thaliana* (10). This protein was not identified here, presumably because of its membrane localization and thus its hydrophobic character. Moreover, this protein was shown to interact with a specific subgroup of Grx with CGFS active site, whereas the study reported here was realized with a CxxC/S active-site Grx. It would thus be interesting to test if this exchanger could also interact with Grx from the two other groups. Two other proteins, a triose phosphate isomerase and an aldolase, found to be glutahiolated in plant extracts (16), could be putative interacting partners of Grxs because they are involved in deglutathiolation processes. These two enzymes were also identified in this study, but whether they interact with Grxs by dithiol–disulfide exchanges or through deglutathiolation needs yet to be investigated.

In conclusion, this study allowed the identification of 94 putative Grx targets, involved in several cellular processes and located in different subcellular compartments. The activity of two of the three targets tested is indeed regulated by Pt GrxC4. Whether the activity of 2-Cys Prx is regulated by glutathiolation, for example, needs to be investigated. Overall, these results suggest that Grx is able to randomly attack exposed disulfide bridges and has a broad capacity to reduce oxidized disulfides and to provide electrons to many enzymatic reduction reactions. Apparently, like Trx, Grx plays a pivotal role in redox regulation and redox homeostasis in plant cells.

## ACKNOWLEDGMENTS

This research was supported by grants to J.P.J. from MNRT and BQR from Université Henri Poincaré. K.J.D. acknowledges support by the DFG (Di346/6; FOR 387).

## ABBREVIATIONS

CA, carbonic anhydrase; DTT, dithiothreitol; FBPase, fructose 1,6-bisphosphatase; GR, glutathione reductase; Grx, glutaredoxin; GSH, reduced glutathione; $H_2O_2$, hydrogen peroxide; HSP, heat shock protein; MS, mass spectrometry; PRK, phosphoribulokinase; Prx, peroxiredoxin; Rubisco, ribulose-1,5-bisphosphate carboxylase oxygenase; SAH, *S*-adenosyl-L-homocysteine; TE, 30 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA; TES, TE containing 200 m*M* NaCl; TR, thioredoxin reductase; Trx, thioredoxin.

## REFERENCES

1. Achebach S, Tran QH, Vlamis-Gardikas A, Mullner M, Holmgren A, and Unden G. Stimulation of Fe-S cluster insertion into apoFNR by *Escherichia coli* glutaredoxins 1, 2 and 3 *in vitro. FEBS Lett* 565: 203–206, 2004.
2. Adler S and Modrich P. T7-induced DNA polymerase. Requirement for thioredoxin sulfhydryl groups. *J Biol Chem* 258: 6956–6962, 1983.
3. Ballicora MA, Frueauf JB, Fu Y, Schürmann P, and Preiss J. Activation of the potato tuber ADP-glucose pyrophosphorylase by thioredoxin. *J Biol Chem* 275: 1315–1320, 2000.
4. Balmer Y, Koller A, del Val G, Manieri W, Schürmann P, and Buchanan BB. Proteomics gives insight into the regulatory function of chloroplast thioredoxins. *Proc Natl Acad Sci U S A* 100: 370–375, 2003.
5. Balmer Y, Vensel WH, Tanaka CK, Hurkman WJ, Gelhaye E, Rouhier N, Jacquot JP, Manieri W, Schürmann P, Droux M, and Buchanan BB. Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. *Proc Natl Acad Sci U S A* 101: 2642–2647, 2004.
6. Bjorkbacka H, Johansson IM, Skarfstad E, and Forsman C. The sulfhydryl groups of Cys 269 and Cys 272 are critical for the oligomeric state of chloroplast carbonic anhydrase from *Pisum sativum*. *Biochemistry* 36: 4287–4294, 1997.
7. Bjornstedt M, Xue J, Huang W, Akesson B, and Holmgren A. The thioredoxin and glutaredoxin systems are efficient electron donors to human plasma glutathione peroxidase. *J Biol Chem* 269: 29382–29384, 1994.
8. Borchers C, Parker CE, Deterding LJ, and Tomer KB. Preliminary comparison of precursor scans and liquid chromatography–tandem mass spectrometry on a hybrid quadrupole time-of-flight mass spectrometer. *J Chromatogr A* 854: 119–130, 1999.
9. Brehelin C, Meyer EH, de Souris JP, Bonnard G, and Meyer Y. Resemblance and dissemblance of *Arabidopsis* type II peroxiredoxins: similar sequences for divergent gene expression, protein localization, and activity. *Plant Physiol* 132: 2045–2057, 2003.
10. Cheng NH and Hirschi KD. Cloning and characterization of CXIP1, a novel PICOT domain-containing *Arabidopsis* protein that associates with CAX1. *J Biol Chem* 278: 6503–6509, 2003.
11. Davis JM, Egelkrout EE, Coleman GD, Chen TH, Haissig BE, Riemenschneider DE, and Gordon MP. A family of wound-induced genes in *Populus* shares common features with genes encoding vegetative storage proteins. *Plant Mol Biol* 23: 135–143, 1993.
12. Ferro M, Salvi D, Brugiere S, Miras S, Kowalski S, Louwagie M, Garin J, Joyard J, and Rolland N. Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Mol Cell Proteomics* 2: 325–345, 2003.
13. Heide H, Kalisz HM, and Follmann H. The oxygen evolving enhancer protein 1 (OEE) of photosystem II in green algae exhibits thioredoxin activity. *J Plant Physiol* 161: 139–149, 2004.
14. Holmgren A. Glutathione-dependent synthesis of deoxyribonucleotides. Characterization of the enzymatic mechanism of *Escherichia coli* glutaredoxin. *J Biol Chem* 254: 3672–3678, 1979.

15. Horling F, Lamkemeyer P, König J, Finkemeier I, Kandlbinder A, Baier M, and Dietz KJ. Divergent light-, ascorbate-, and oxidative stress-dependent regulation of expression of the peroxiredoxin gene family in *Arabidopsis*. *Plant Physiol* 131: 317–325, 2003.
16. Ito H, Iwabuchi M, and Ogawa K. 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, 2003.
17. Johansson IM and Forsman C. Kinetic studies of pea carbonic anhydrase. *Eur J Biochem* 218: 439–446, 1993.
18. Koshiishi C, Kato A, Yama S, Crozier A, and Ashihara H. A new caffeine biosynthetic pathway in tea leaves: utilisation of adenosine released from the *S*-adenosyl-L-methionine cycle. *FEBS Lett* 499: 50–54, 2001.
19. Kumar JK, Tabor S, and Richardson CC. Proteomic analysis of thioredoxin-targeted proteins in *Escherichia coli*. *Proc Natl Acad Sci U S A* 101: 3759–3764, 2004.
20. Kunst L. Preparation of physiologically active chloroplasts from *Arabidopsis*. In: *Methods in Molecular Biology, Vol. 82.* Arabidopsis *Protocols*, edited by Martinez-Zapater J and Salinas J. Totowa, NJ: Humana Press Inc., 1998, pp. 43–53.
21. Lee K, Lee J, Kim Y, Bae D, Kang KY, Yoon SC, and Lim D. Defining the plant disulfide proteome. *Electrophoresis* 25: 532–541, 2004.
22. Lemaire SD, Guillon B, Le Marechal P, Keryer E, Miginiac-Maslow M, and Decottignies P. New thioredoxin targets in the unicellular photosynthetic eukaryote *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 101: 7475–7480, 2004.
23. Lillig CH, Prior A, Schwenn JD, Aslund F, Ritz D, Vlamis-Gardikas A, and Holmgren A. New thioredoxins and glutaredoxins as electron donors of 3′-phosphoadenylylsulfate reductase. *J Biol Chem* 274: 7695–7698, 1999.
24. Lindahl M and Florencio FJ. Thioredoxin-linked processes in cyanobacteria are as numerous as in chloroplasts, but targets are different. *Proc Natl Acad Sci U S A* 100: 16107–16112, 2003.
25. Maeda K, Finnie C, and Svensson B. Cy5 maleimide labelling for sensitive detection of free thiols in native protein extracts: identification of seed proteins targeted by barley thioredoxin h isoforms. *Biochem J* 378: 497–507, 2004.
26. Marx C, Wong JH, and Buchanan BB. Thioredoxin and germinating barley: targets and protein redox changes. *Planta* 216: 454–460, 2003.
27. Meek SE, Lane WS, and Piwnica-Worms H. Comprehensive proteomic analysis of interphase and mitotic 14–3-3 binding proteins. *J Biol Chem* 279: 32046–32054, 2004.
28. Meyer Y, Vignols F, and Reichheld JP. Classification of plant thioredoxins by sequence similarity and intron position. *Methods Enzymol* 347: 394–402, 2002.
29. Motohashi K, Kondoh A, Stumpp MT, and Hisabori T. Comprehensive survey of proteins targeted by chloroplast thioredoxin. *Proc Natl Acad Sci U S A* 98: 11224–11229, 2001.
30. Motohashi K, Koyama F, Nakanishi Y, Ueoka-Nakanishi H, and Hisabori T. Chloroplast cyclophilin is a target protein of thioredoxin. Thiol modulation of the peptidyl-prolyl *cis-trans* isomerase activity. *J Biol Chem* 278: 31848–31852, 2003.
31. Ravanel S, Block MA, Rippert P, Jabrin S, Curien G, Rebeille F, and Douce R. Methionine metabolism in plants: chloroplasts are autonomous for *de novo* methionine synthesis and can import *S*-adenosylmethionine from the cytosol. *J Biol Chem* 279: 22548–22557, 2004.
32. Richarme G. Protein-disulfide isomerase activity of elongation factor EF-Tu. *Biochem Biophys Res Commun* 252: 156–161, 1998.
33. Rodriguez-Manzaneque MT, Tamarit J, Belli G, Ros J, and Herrero E. Grx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes. *Mol Biol Cell* 13: 1109–1121, 2002.
34. Rolland N, Ruffet ML, Job D, Douce R, and Droux M. Spinach chloroplast *O*-acetylserine (thiol)-lyase exhibits two catalytically non-equivalent pyridoxal-5′-phosphate-containing active sites. *Eur J Biochem* 236: 272–282, 1996.
35. Rouhier N, Gelhaye E, Sautiere PE, Brun A, Laurent P, Tagu D, Gerard J, de Fay E, Meyer Y, and Jacquot JP. Isolation and characterization of a new peroxiredoxin from poplar sieve tubes that uses either glutaredoxin or thioredoxin as a proton donor. *Plant Physiol* 127: 1299–1309, 2001.
36. Rouhier N, Gelhaye E, and Jacquot JP. Exploring the active site of plant glutaredoxin by site-directed mutagenesis. *FEBS Lett* 511: 145–149, 2002.
37. Rouhier N, Gelhaye E, and Jacquot JP. Plant glutaredoxins: still mysterious reducing systems. *Cell Mol Life Sci* 61: 1266–1277, 2004.
38. Ruelland E and Miginiac-Maslow M. Regulation of chloroplast enzyme activities by thioredoxins: activation or relief from inhibition? *Trends Plant Sci* 4: 136–141, 1999.
39. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, and Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17: 2596–2606, 1998.
40. Schürmann P and Jacquot JP. Plant thioredoxin systems revisited. *Annu Rev Plant Physiol Plant Mol Biol* 51: 371–400, 2001.
41. Schwarz O, Schürmann P, and Strotmann H. Kinetics and thioredoxin specificity of thiol modulation of the chloroplast $H^+$-ATPase. *J Biol Chem* 272: 16924–16927, 1997.
42. Sparla F, Pupillo P, and Trost P. The C-terminal extension of glyceraldehyde-3-phosphate dehydrogenase subunit B acts as an autoinhibitory domain regulated by thioredoxins and nicotinamide adenine dinucleotide. *J Biol Chem* 277: 44946–44952, 2002.
43. Starke DW, Chock PB, and Mieyal JJ. Glutathione-thiyl radical scavenging and transferase properties of human glutaredoxin (thioltransferase). Potential role in redox signal transduction. *J Biol Chem* 278: 14607–14613, 2003.
44. Tavakoli N, Kluge C, Golldack D, Mimura T, and Dietz KJ. Reversible redox control of plant vacuolar $H^+$-ATPase activity is related to disulfide bridge formation in subunit E as well as subunit A. *Plant J* 28: 51–59, 2001.

45. Vignols F, Mouaheb N, Thomas D, and Meyer Y. Redox control of Hsp70-Co-chaperone interaction revealed by expression of a thioredoxin-like *Arabidopsis* protein. *J Biol Chem* 278: 4516–4523, 2003.
46. Villarejo A, Shutova T, Moskvin O, Forssen M, Klimov VV, and Samuelsson G. A photosystem II-associated carbonic anhydrase regulates the efficiency of photosynthetic oxygen evolution. *EMBO J* 21: 1930–1938, 2002.
47. Wong JH, Balmer Y, Cai N, Tanaka CK, Vensel WH, Hurkman WJ, and Buchanan BB. Unraveling thioredoxin-linked metabolic processes of cereal starchy endosperm using proteomics. *FEBS Lett* 547: 151–156, 2003.
48. Yamazaki D, Motohashi K, Kasama T, Hara Y, and Hisabori T. Target proteins of the cytosolic thioredoxins in *Arabidopsis thaliana*. *Plant Cell Physiol* 45: 18–27, 2004.
49. Yano H, Wong JH, Lee YM, Cho MJ, and Buchanan BB. A strategy for the identification of proteins targeted by thioredoxin. *Proc Natl Acad Sci U S A* 98: 4794–4799, 2001.
50. Zhang N and Portis AR Jr. Mechanism of light regulation of Rubisco: a specific role for the larger Rubisco activase isoform involving reductive activation by thioredoxin-f. *Proc Natl Acad Sci U S A* 96: 9438–9443, 1999.

Address reprint requests to:
*Nicolas Rouhier, Ph.D.*
*Unité Mixte de Recherches 1136 INRA UHP (Interaction Arbres Microorganismes)*
*IFR 110 Génomique Ecophysiologie et Ecologie Fonctionnelles*
*Université Henri Poincaré BP 239*
*54506 Vandoeuvre Cedex, France*

*E-mail:* nrouhier@scbiol.uhp-nancy.fr

Received for publication October 5, 2004; accepted January 19, 2005.

**This article has been cited by:**


1. Yves Meyer , Christophe Belin , Valérie Delorme-Hinoux , Jean-Philippe Reichheld , Christophe Riondet . 2012. Thioredoxin and Glutaredoxin Systems in Plants: Molecular Mechanisms, Crosstalks, and Functional Significance. *Antioxidants & Redox Signaling* **17**:8, 1124-1160. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Qingyu Wu, Julie Lin, Jian-Zhong Liu, Xiaofei Wang, Wansang Lim, Myungmin Oh, Jungeun Park, C. B. Rajashekar, Steven A. Whitham, Ning-Hui Cheng, Kendal D. Hirschi, Sunghun Park. 2012. Ectopic expression of Arabidopsis glutaredoxin AtGRXS17 enhances thermotolerance in tomato. *Plant Biotechnology Journal* **10**:8, 945-955. [CrossRef]
3. Koen Van Laer, Lieven Buts, Nicolas Foloppe, Didier Vertommen, Karolien Van Belle, Khadija Wahni, Goedele Roos, Lennart Nilsson, Luis M. Mateos, Mamta Rawat, Nico A. J. van Nuland, Joris Messens. 2012. Mycoredoxin-1 is one of the missing links in the oxidative stress defence mechanism of Mycobacteria. *Molecular Microbiology* n/a-n/a. [CrossRef]
4. Erin M.G. Allen , John J. Mieyal . Protein-Thiol Oxidation and Cell Death: Regulatory Role of Glutaredoxins. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. María Belén Bosco, Mabel Cristina Aleanzi, Alberto Álvaro Iglesias. 2012. Plastidic Phosphoglycerate Kinase from Phaeodactylum tricornutum: On the Critical Role of Cysteine Residues for the Enzyme Function. *Protist* **163**:2, 188-203. [CrossRef]
6. Karl-Josef Dietz . 2011. Peroxiredoxins in Plants and Cyanobacteria. *Antioxidants & Redox Signaling* **15**:4, 1129-1159. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
7. Sophie Alvarez, Leslie HicksSurvey in Plant Root Proteomics **20113331**, 215-256. [CrossRef]
8. M. C. Marti, I. Florez-Sarasa, D. Camejo, M. Ribas-Carbo, J. J. Lazaro, F. Sevilla, A. Jimenez. 2011. Response of mitochondrial thioredoxin PsTrxo1, antioxidant enzymes, and respiration to salinity in pea (Pisum sativum L.) leaves. *Journal of Experimental Botany* **62**:11, 3863-3874. [CrossRef]
9. Marika Lindahl , Alejandro Mata-Cabana , Thomas Kieselbach . 2011. The Disulfide Proteome and Other Reactive Cysteine Proteomes: Analysis and Functional Significance. *Antioxidants & Redox Signaling* **14**:12, 2581-2642. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
10. Nicolas Navrot, Christine Finnie, Birte Svensson, Per Hägglund. 2011. Plant redox proteomics. *Journal of Proteomics* . [CrossRef]
11. R. Garg, S. Jhanwar, A. K. Tyagi, M. Jain. 2010. Genome-Wide Survey and Expression Analysis Suggest Diverse Roles of Glutaredoxin Gene Family Members During Development and Response to Various Stimuli in Rice. *DNA Research* **17**:6, 353-367. [CrossRef]
12. Esther Jortzik, Karin Fritz-Wolf, Nicole Sturm, Marieke Hipp, Stefan Rahlfs, Katja Becker. 2010. Redox Regulation of Plasmodium falciparum Ornithine #-Aminotransferase. *Journal of Molecular Biology* **402**:2, 445-459. [CrossRef]
13. Christophe H. Marchand, Hélène Vanacker, Valérie Collin, Emmanuelle Issakidis-Bourguet, Pierre Le Maréchal, Paulette Decottignies. 2010. Thioredoxin targets in Arabidopsis roots. *PROTEOMICS* **10**:13, 2418-2428. [CrossRef]
14. Nicolas Rouhier. 2010. Plant glutaredoxins: pivotal players in redox biology and iron-sulphur centre assembly. *New Phytologist* **186**:2, 365-372. [CrossRef]
15. Kamel Chibani, Jérémy Couturier, Benjamin Selles, Jean-Pierre Jacquot, Nicolas Rouhier. 2010. The chloroplastic thiol reducing systems: dual functions in the regulation of carbohydrate metabolism and regeneration of antioxidant enzymes, emphasis on the poplar redoxin equipment. *Photosynthesis Research* **104**:1, 75-99. [CrossRef]
16. Sabarinath Sundaram, Bala Rathinasabapathi. 2010. Transgenic expression of fern Pteris vittata glutaredoxin PvGrx5 in Arabidopsis thaliana increases plant tolerance to high temperature stress and reduces oxidative damage to proteins. *Planta* **231**:2, 361-369. [CrossRef]
17. Yves Meyer, Bob B. Buchanan, Florence Vignols, Jean-Philippe Reichheld. 2009. Thioredoxins and Glutaredoxins: Unifying Elements in Redox Biology. *Annual Review of Genetics* **43**:1, 335-367. [CrossRef]
18. Samuel W. Fan, Richard A. George, Naomi L. Haworth, Lina L. Feng, Jason Y. Liu, Merridee A. Wouters. 2009. Conformational changes in redox pairs of protein structures. *Protein Science* **18**:8, 1745-1765. [CrossRef]
19. A. Shahpiri, B. Svensson, C. Finnie. 2009. From Proteomics to Structural Studies of Cytosolic/Mitochondrial-Type Thioredoxin Systems in Barley Seeds. *Molecular Plant* **2**:3, 378-389. [CrossRef]
20. S ALVAREZ, G WILSON, S CHEN. 2009. Determination of in vivo disulfide-bonded proteins in Arabidopsis. *Journal of Chromatography B* **877**:1-2, 101-104. [CrossRef]
21. Laure Michelet, Mirko Zaffagnini, D. LemaireThioredoxins and Related Proteins 401-443. [CrossRef]

22. Holger Fey, Dario Piano, Ruth Horn, David Fischer, Matthias Schmidt, Stephanie Ruf, Wolfgang P. Schröder, Ralph Bock, Claudia Büchel. 2008. Isolation of highly active photosystem II core complexes with a His-tagged Cyt b559 subunit from transplastomic tobacco plants. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1777**:12, 1501-1509. [CrossRef]
23. Katharina Dueckershoff, Stefan Mueller, Martin J. Mueller, Joerg Reinders. 2008. Impact of cyclopentenone-oxylipins on the proteome of Arabidopsis thaliana. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1784**:12, 1975-1985. [CrossRef]
24. Abderrakib Zahid, Samia Afoulous, Roland Cazalis. 2008. Thioredoxin h System and Wheat Seed Quality. *Cereal Chemistry* **85**:6, 799-807. [CrossRef]
25. M OELZE, A KANDLBINDER, K DIETZ. 2008. Redox regulation and overreduction control in the photosynthesizing cell: Complexity in redox regulatory networks. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1780**:11, 1261-1272. [CrossRef]
26. S. Rinalducci, L. Murgiano, L. Zolla. 2008. Redox proteomics: basic principles and future perspectives for the detection of protein oxidation in plants. *Journal of Experimental Botany* **59**:14, 3781-3801. [CrossRef]
27. A MEYER. 2008. The integration of glutathione homeostasis and redox signaling. *Journal of Plant Physiology* **165**:13, 1390-1403. [CrossRef]
28. L. Tarrago, E. Laugier, P. Rey. 2008. Protein-Repairing Methionine Sulfoxide Reductases in Photosynthetic Organisms: Gene Organization, Reduction Mechanisms, and Physiological Roles. *Molecular Plant* **2**:2, 202-217. [CrossRef]
29. N. Hosoya-Matsuda, K. Inoue, T. Hisabori. 2008. Roles of Thioredoxins in the Obligate Anaerobic Green Sulfur Photosynthetic Bacterium Chlorobaculum tepidum. *Molecular Plant* **2**:2, 336-343. [CrossRef]
30. S. Barranco-Medina, T. Krell, L. Bernier-Villamor, F. Sevilla, J.-J. Lazaro, K.-J. Dietz. 2008. Hexameric oligomerization of mitochondrial peroxiredoxin PrxIIF and formation of an ultrahigh affinity complex with its electron donor thioredoxin Trx-o. *Journal of Experimental Botany* **59**:12, 3259-3269. [CrossRef]
31. Elke Ströher, Karl-Josef Dietz. 2008. The dynamic thiol–disulphide redox proteome of the Arabidopsis thaliana chloroplast as revealed by differential electrophoretic mobility. *Physiologia Plantarum* **133**:3, 566-583. [CrossRef]
32. Filipe Gama, Claire Bréhélin, Eric Gelhaye, Yves Meyer, Jean-Pierre Jacquot, Pascal Rey, Nicolas Rouhier. 2008. Functional analysis and expression characteristics of chloroplastic Prx IIE. *Physiologia Plantarum* **133**:3, 599-610. [CrossRef]
33. Karl-Josef Dietz. 2008. Redox signal integration: from stimulus to networks and genes. *Physiologia Plantarum* **133**:3, 459-468. [CrossRef]
34. Nicolas Rouhier, Stéphane D. Lemaire, Jean-Pierre Jacquot. 2008. The Role of Glutathione in Photosynthetic Organisms: Emerging Functions for Glutaredoxins and Glutathionylation. *Annual Review of Plant Biology* **59**:1, 143-166. [CrossRef]
35. PRAGYA KANT, MICHAL GORDON, SURYA KANT, GASTON ZOLLA, OLGA DAVYDOV, YAIR M. HEIMER, VERED CHALIFA-CASPI, RUTH SHAKED, SIMON BARAK. 2008. Functional-genomics-based identification of genes that regulate Arabidopsis responses to multiple abiotic stresses. *Plant, Cell & Environment* **31**:6, 697-714. [CrossRef]
36. N CHENG. 2008. AtGRX4, an Arabidopsis chloroplastic monothiol glutaredoxin, is able to suppress yeast grx5 mutant phenotypes and respond to oxidative stress. *FEBS Letters* **582**:6, 848-854. [CrossRef]
37. Sakihito Kitajima, Miyo Kurioka, Tadashi Yoshimoto, Mayumi Shindo, Kenji Kanaori, Kunihiko Tajima, Kenji Oda. 2008. A cysteine residue near the propionate side chain of heme is the radical site in ascorbate peroxidase. *FEBS Journal* **275**:3, 470-480. [CrossRef]
38. Takahiro ISHIKAWA, Shigeru SHIGEOKA. 2008. Recent Advances in Ascorbate Biosynthesis and the Physiological Significance of Ascorbate Peroxidase in Photosynthesizing Organisms. *Bioscience, Biotechnology, and Biochemistry* **72**:5, 1143-1154. [CrossRef]
39. Won-Jin Kang, Hyun-Soon Kim, Youn-Il Park, Hyouk Joung, Jae-Heung Jeon. 2007. Differential Expression in Response to Biotic and Abiotic Stress from Three Potato Glutaredoxins Induced during Suberization. *Journal of Plant Biology* **50**:6, 663-670. [CrossRef]
40. Alison M. Winger, Nicolas L. Taylor, Joshua L. Heazlewood, David A. Day, A. Harvey Millar. 2007. Identification of intra- and intermolecular disulphide bonding in the plant mitochondrial proteome by diagonal gel electrophoresis. *PROTEOMICS* **7**:22, 4158-4170. [CrossRef]
41. Sergio Barranco-Medina, Tino Krell, Iris Finkemeier, Francisca Sevilla, Juan-José Lázaro, Karl-Josef Dietz. 2007. Biochemical and molecular characterization of the mitochondrial peroxiredoxin PsPrxII F from Pisum sativum. *Plant Physiology and Biochemistry* **45**:10-11, 729-739. [CrossRef]

42. Christina Vieira Dos Santos, Edith Laugier, Lionel Tarrago, Vincent Massot, Emmanuelle Issakidis-Bourguet, Nicolas Rouhier, Pascal Rey. 2007. Specificity of thioredoxins and glutaredoxins as electron donors to two distinct classes of Arabidopsis plastidial methionine sulfoxide reductases B. *FEBS Letters* **581**:23, 4371-4376. [CrossRef]
43. T. Rausch, R. Gromes, V. Liedschulte, I. Müller, J. Bogs, V. Galovic, A. Wachter. 2007. Novel Insight into the Regulation of GSH Biosynthesis in Higher Plants. *Plant Biology* **9**:5, 565-572. [CrossRef]
44. Stéphane D. Lemaire, Laure Michelet, Mirko Zaffagnini, Vincent Massot, Emmanuelle Issakidis-Bourguet. 2007. Thioredoxins in chloroplasts. *Current Genetics* **51**:6, 343-365. [CrossRef]
45. Dennis Wormuth, Isabelle Heiber, Jehad Shaikali, Andrea Kandlbinder, Margarete Baier, Karl-Josef Dietz. 2007. Redox regulation and antioxidative defence in Arabidopsis leaves viewed from a systems biology perspective. *Journal of Biotechnology* **129**:2, 229-248. [CrossRef]
46. Min Li, Qing Yang, Lianwen Zhang, Han, Li, Yunluan Cui, Qingyu Wu. 2007. Identification of novel targets of cyanobacterial glutaredoxin. *Archives of Biochemistry and Biophysics* **458**:2, 220-228. [CrossRef]
47. Mirko Zaffagnini, Laure Michelet, Christophe Marchand, Francesca Sparla, Paulette Decottignies, Pierre Le Maréchal, Myroslawa Miginiac-Maslow, Graham Noctor, Paolo Trost, Stéphane D. Lemaire. 2007. The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-phosphate dehydrogenase is selectively regulated by glutathionylation. *FEBS Journal* **274**:1, 212-226. [CrossRef]
48. Filipe Gama, Olivier Keech, Françoise Eymery, Iris Finkemeier, Eric Gelhaye, Per Gardeström, Karl Josef Dietz, Pascal Rey, Jean-Pierre Jacquot, Nicolas Rouhier. 2007. The mitochondrial type II peroxiredoxin from poplar. *Physiologia Plantarum* **129**:1, 196-206. [CrossRef]
49. Nicolas Navrot, Nicolas Rouhier, Eric Gelhaye, Jean-Pierre Jacquot. 2007. Reactive oxygen species generation and antioxidant systems in plant mitochondria. *Physiologia Plantarum* **129**:1, 185-195. [CrossRef]
50. Francisco J. Florencio, María Esther Pérez-Pérez, Luis López-Maury, Alejandro Mata-Cabana, Marika Lindahl. 2006. The diversity and complexity of the cyanobacterial thioredoxin systems. *Photosynthesis Research* **89**:2-3, 157-171. [CrossRef]
51. Yves Meyer, Christophe Riondet, Laure Constans, Mohamed Ragab Abdelgawwad, Jean Philippe Reichheld, Florence Vignols. 2006. Evolution of redoxin genes in the green lineage. *Photosynthesis Research* **89**:2-3, 179-192. [CrossRef]
52. Laure Michelet, Mirko Zaffagnini, Vincent Massot, Eliane Keryer, Hélène Vanacker, Myroslawa Miginiac-Maslow, Emmanuelle Issakidis-Bourguet, Stéphane D. Lemaire. 2006. Thioredoxins, glutaredoxins, and glutathionylation: new crosstalks to explore. *Photosynthesis Research* **89**:2-3, 225-245. [CrossRef]
53. Christophe Marchand, Pierre Le Maréchal, Yves Meyer, Paulette Decottignies. 2006. Comparative proteomic approaches for the isolation of proteins interacting with thioredoxin. *PROTEOMICS* **6**:24, 6528-6537. [CrossRef]
54. Eric Gelhaye, Nicolas Navrot, Isabel K. Macdonald, Nicolas Rouhier, Emma Lloyd Raven, Jean-Pierre Jacquot. 2006. Ascorbate peroxidase–thioredoxin interaction. *Photosynthesis Research* **89**:2-3, 193-200. [CrossRef]
55. Sylvie Gillet, Paulette Decottignies, Solenne Chardonnet, Pierre Le Maréchal. 2006. Cadmium response and redoxin targets in Chlamydomonas reinhardtii: a proteomic approach. *Photosynthesis Research* **89**:2-3, 201-211. [CrossRef]
56. S. Xing, A. Lauri, S. Zachgo. 2006. Redox Regulation and Flower Development: A Novel Function for Glutaredoxins. *Plant Biology* **8**:5, 547-555. [CrossRef]
57. E. Ströher, K.-J. Dietz. 2006. Concepts and Approaches Towards Understanding the Cellular Redox Proteome. *Plant Biology* **8**:4, 407-418. [CrossRef]
58. Christina Vieira Dos Santos, Pascal Rey. 2006. Plant thioredoxins are key actors in the oxidative stress response. *Trends in Plant Science* **11**:7, 329-334. [CrossRef]
59. Philip Eaton , Michael J. Shattock . 2005. Protein S-Thiolation: Emphasis on Cell Signaling and Gene Expression. *Antioxidants & Redox Signaling* **7**:7-8, 839-840. [Citation] [Full Text PDF] [Full Text PDF with Links]