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Review

Plant glutaredoxins: still mysterious reducing systems

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Abstract. Glutaredoxins are ubiquitous oxidoreductases which are similar to thioredoxins and possess a typical glutathione-reducible CxxC or CxxS active site. We present here the current knowledge about these proteins in plants. At least 31 glutaredoxin genes are present in *Arabidopsis thaliana*, a value close to the thioredoxin gene number. Based essentially on active site sequences, a classification of these multiple genes is proposed. The specificity of the various apparently redundant forms within the glutaredoxin group or between glutaredoxin and thioredoxin can be analysed in terms of differential spatiotemporal expression of the genes, specificity vs. target proteins and mode of catalysis (glutathiolation/deglutathiolation processes appear to be a specific function of glutaredoxin). Additional putative functions are proposed for plant glutaredoxins based on their targets in other organisms and in the light of the existence of hybrid proteins containing glutaredoxin modules in their N- or C-terminal part.

Key words. Dithiol; glutaredoxin; glutathiolation; glutathione; monothiol; targets; thioredoxin.

Introduction

Glutaredoxins (Grx) are small ubiquitous oxidoreductases of the thioredoxin (Trx) family. The size of these proteins is generally ~10–15 kDa with an active site sequence CxxC or CxxS required for their redox properties [1]. Grx are maintained reduced with the help of NADPH, glutathione reductase (GR) and glutathione (GSH), whereas cytosolic and mitochondrial Trx are reduced by NADPH and NADPH thioredoxin reductase (NTR) [2–4]. In plants, various isoforms of Trx (Trx m, f, x, y and CDSP32 for chloroplast drought-induced protein of 32 kDa) are also present in the chloroplast [3, 5]. In this organelle, they are reduced via the electron transport chain with the help of two stromal proteins containing Fe-S clusters called ferredoxin and ferredoxin thioredoxin reductase [6]. GR and NTR belong to the pyridine nucleotide disulfide oxidoreductase family, which also comprises proteins such as lipoamide dehydrogenase, mercuric ion reductase and a bifunctional enzyme called Trx and GSSG reductase (TGR) [7]. These proteins are generally dimeric flavoproteins which possess a FAD binding domain, a NADPH binding domain and a dithiol/disulfide center of the CxxC or CxxxxC type. In most organisms, the Trx and GSH/Grx systems are the major reducing molecules and are thus involved in many cellular processes. Trx and Grx are multigenic families of proteins, represented by various isoforms. For example, in *Escherichia coli*, two bicysteinic Trx, three bicysteinic Grx have been characterized so far and one monocysteinic Grx (GenBank accession number NP_416171) also exists [8, 9]. In *Saccharomyces cerevisiae*, there are three bicysteinic Trx, including a mitochondrial isoform, two bicysteinic Grx and three monocysteinic Grx harbouring a CGFS active site [8, 9]. In mammals, there are two bicysteinic Trx or Grx isoforms, one cytosolic and one mitochondrial isoform of each protein and various Trx- or

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Grx-like proteins [8, 9]. The sequencing of complete or near complete genomes from *A. thaliana*, *Oryza sativa* or *Populus trichocarpa* and the occurrence of many expressed sequence tag (EST) sequencing projects for *Triticum aestivum, Zea mays*, *Chlamydomonas reinhardtii* and *Synechocystis* sp. indicate that a more complex diversity occurs in photosynthetic organisms. Indeed, up to 20–30 isoforms of Trx and Grx are present in some of these genomes, raising the question of the redundance and of the specificity of these isoforms, as Grx and Trx sometimes possess similar functions. Many review articles have described the various Trx systems and their functions in plant cells [3, 6]. This review will focus on plant Grx, for which information about expression, localization, and biochemical and structural properties are scarce.

The Grx content of the *A. thaliana* and *Populus* genomes has been analysed to propose a classification of plant Grx. Glutathiolation, one specific function of Grx, will be discussed in detail. The existence of many natural fusion proteins in plants or in other organisms between Grx modules and another module and some data about non-plant Grx targets will also be described in order to provide more information about other putative functions or target proteins of Grx.

The multigenic family of *grx* in genomes of photosynthetic organisms

The occurrence of nearly complete sequenced genomes or of EST-sequencing projects in plants provides valuable data about the abundance of Grx. We will describe below in detail the Grx content of two model plants, *A. thaliana*, an annual herbaceous species, and *P. trichocarpa*, a model of woody plants, and compare them briefly with the Grx content of other photosynthetic organisms.

A. thaliana

The analysis of the *Arabidopsis* genome in MATDB [MIPS (Munich information center for protein sequences) *Arabidopsis thaliana* database] (http://mips.gsf.de/proj/thal/db/index.html) indicates that at least 31 *grx* genes are present among the five nuclear chromosomes. Indeed, in higher plants, all the Grx are nuclear encoded but presumably exported in different compartments. Table 1 presents all the Grx found according to the number of conserved cysteines in the active site: there are 14 bicysteinic and 17 monocysteinic Grx. The MATDB protein entry codes, the size of the proteins including transit peptides, the putative localization and the sequence of the active sites, which is the hallmark of each Grx subclass, were also indicated for each Grx. All the localizations remain putative since no AtGrx (standing for *Arabidopsis thaliana* Grx, abbreviation also used later in the mansucript) has been characterized so far. Clearly, these data have to be taken cautiously, because recent studies indicate that many proteins could be exported into a subcellular compartment without any visible N- or C-terminal extension or could be targeted to several subcompartments. Nevertheless, most of the Grx are assumed to be cytosolic proteins. Among the bicysteinic proteins, three could be secreted, two localized in the chloroplasts but none is predicted to be mitochondrial. Among the monocysteinic proteins, four could be localized in chloroplasts and one in mitochondria, but none is predicted to be secreted.

Figures 1 and 2 present, respectively, an amino acid sequence comparison and a phylogenic tree of all AtGrx. Clearly, these analyses enable separation of the Grx into three classes, essentially as a function of the active site sequences. Only four amino acids are absolutely conserved among all AtGrx, the first cysteine of the active site, and proline, glycine and leucine residues located in the C-terminal part of the protein (fig. 1, in white on black). The identity between all AtGrx ranges from 5 to 95%.

The first well-defined class, characterized by a Cxx[C/S] or more precisely [Y/W]C[G/P/S]Y[C/S] active site, includes the four 'classical' dithiol Grx (CxxC1 to CxxC4) with CGYC, CPYC or CPFC active sites and two close isoforms (CxxC5 and CxxS12) with divergent WCSYC/S active sites. Most of the Grx characterized so far in other organisms belong to this group.

The second class includes four Grx with a CGFS active site (CxxS14 to CxxS17). CxxS17 is a fusion protein between a Trx motif (WASWCDAS active site) in the N-terminal part and three Grx motives (CGFS active site) in the C-terminal part. These proteins belong to the PICOT-HD (protein kinase C interacting cousin of Trx-homology domain) containing proteins [10]. The PICOT motif corresponds to a Grx module with a CGFS active site. This family thus includes, for example, the three monocysteinic Grx of *S. cerevisiae* (Grx 3–5) and one Grx of *Plasmodium falciparum* [11, 12].

The third class is the largest one and contains all the other Grx isoforms which possess an active site of the form CCx[C/S/G] or, more precisely, [S/T/G]CC[M/L][C/S/G]. Some of the proteins of the three classes contain additional cysteines likely to participate to the catalytic mechanism (see below).

Populus trichocarpa

The genome of *P. trichocarpa* is entirely sequenced (http://genome.jgi-psf.org/poplar0/poplar0.home.html) but not yet fully annotated. Nevertheless, large-scale EST sequencing provides more than 125,000 sequences. Up to now, 19 different Grx have been identified in the GenBank database by similarity search with AtGrx (table 1). Thus far, one major difference is the lack of many iso-

Table I. Glutaredoxin content of *A. thaliana* and *Populus sp*.

	Protein entry code	Length	Putative localization	Active site sequence	EST number from poplar
CxxC1	**At5g63030**	**125**	**cytosolic**	**YCGYC**	**BU867240**
CxxC2	**At5g40370**	**111**	**secretory pathway (P)**	**YCPYC**	**BU877060**
CxxC3	**At1g77370**	**130**	**secretory pathway**	**YCPYC**	**BU825153**
CxxC4	**At5g20500**	**135**	**secretory pathway**	**YCPYC**	**BU837457**
CxxC5	**At4g28730**	**174**	**plastidial**	**WCSYC**	**BU833604 ?**
CxxC6	At4g33040	144	cytosolic	SCCMC	BU883329
CxxC7	At3g02000	136	cytosolic (PM)	TCCMC	BU830321
CxxC8	At5g14070	140	cytosolic	TCCMC	?
CxxC9	At1g28480	137	cytosolic	GCCMC	BU811342
CxxC10	At5g11930	145	plastidial (C)	SCCMC	?
CxxC11	At3g62950	103	cytosolic (M)	SCCMC	BU811766
CxxC12	At2g47870	103	cytosolic (M)	SCCMC	BU889749
CxxC13	At2g47880	102	cytosolic	SCCLC	BU892497
CxxC14	At3g62960	102	cytosolic	SCCLC	CF230799
CxxS1	At1g03020	102	cytosolic	SCCMS	BU893638
CxxS2	At5g18600	102	cytosolic	SCCMS	?
CxxS3	At4g15700	102	cytosolic	SCCMS	BU895046
CxxS4	At4g15680	102	cytosolic	SCCMS	BU895046
CxxS5	At4g15690	102	cytosolic	SCCMS	BU895046
CxxS6	At3g62930	102	cytosolic	SCCMS	BU819383
CxxS7	At4g15670	102	cytosolic	SCCMS	BU895046
CxxS8	At4g15660	102	cytosolic	SCCMS	BU895046
CxxS9	At2g30540	102	cytosolic	SCCMS	?
CxxS10	At3g21460	102	mitochondrial	TCCMS	?
CxxS11	At1g06830	99	cytosolic	SCCLS	?
CxxS12	**At2g20270**	**179**	**plastidial (ER)**	**WCSYS**	**BU833604 ?**
CxxS13	At1g03850	150	plastidial (C)	GCCLG	?
CxxS14	*At3g54900*	*173*	*plastidial (M, ER)*	*MCGFS*	*BU875409*
CxxS15	*At3g15660*	*169*	*mitochondrial (P)*	*QCGFS*	*BU827149*
CxxS16	*At2g38270*	*293*	*plastidial (M)*	*QCGFS*	*BI132154*
CxxS17	*At4g04950*	*488*	*cytosolic*	*RCGFS, KCGFS (x2)*	*BI126366*

Data concerning *A. thaliana* Grx come from MATDB, and those of poplar Grx are EST sequences present in GenBank. Bold, normal and italic characters represents the three classes of Grx (CxxC/S, CCxC/S/G and CGFS active sites, respectively). Putative localizations are based on TargetP prediction software (http://www.cbs.dtu.dk/services/TargetP/). When other prediction softwares [Predotar (http://genoplante-info.infobiogen.fr/predotar/predotar.html)] and Psort (http://psort.nibb.ac.jp/form.html)) give different results, these are indicated between parentheses. Abbreviations: C, cytosol; ER, endoplasmic reticulum; M, mitochondria; P, plastid; PM, plasma membrane.

forms with a CCMS active site. The four classical Grx of the CxxC group and the four isoforms of the first group (CGFS) are present. There is also one isoform similar to AtCxxC5 or AtCxxS12, but we found only one EST, which is incomplete. Five CCMC and 2 CCLC different isoforms and 4 CCMS isoforms are also present in the database.

Other organisms

In *O. sativa*, *T. aestivum*, *Z. mays*, *Hordeum vulgare* and *Pinus taeda*, all classes are represented, but it seems there are not as many Grx with a CCxC/S active site in these organisms, whereas they are prominent in *A. thaliana*. It is likely that duplication events occurred in the *A. thaliana* genome for these isoforms. At present, it is not known whether these sequences are all expressed. For the organisms detailed below, the Grx isoforms present are constituted only by the expressed sequences, and since the full genome is not annotated, such duplication events are not yet detectable. In the green alga *C. reinhardtii* and in the cyanobacterium *Synechocystis PCC6803*, 6 and 3 Grx, respectively, are present either with a CxxC/S or a CGFS active site, but no isoforms with a CCxC/S active site [S. Lemaire, unpublished]. It is likely that the Grx of the CCxC/S group appeared later in the evolution and are specific for higher plants.

Catalytic and structural properties: is there a role for the second cysteine of the active site and for additional cysteines?

Very few biochemical and structural informations are available about plant Grx. Nevertheless, many structures of dithiol Grx from *Escherichia coli*, *Homo sapiens* or T_4 phage in oxidized and reduced forms have been resolved by nuclear magnetic resonance (NMR) spectroscopy or X-

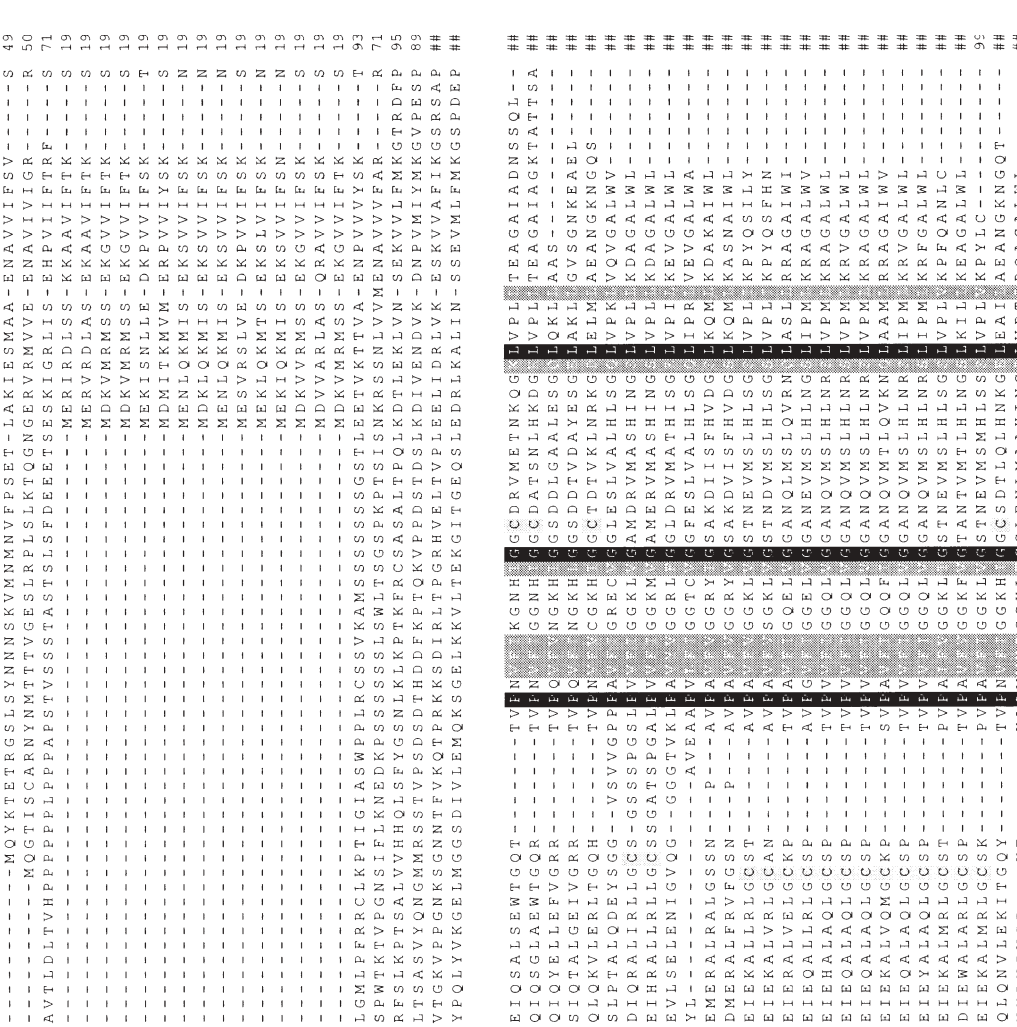

Figure 1. Amino acid sequence comparison of the 31 Grx of *A. thaliana*. The alignment was performed with ClustalW. The protein entry codes are similar to those of table 1. The strictly conserved amino acids are depicted in white on black; the conservative amino acid changes are indicated in white on gray. The second cysteine or serine of the active site and additional conserved cysteines are in black on gray.

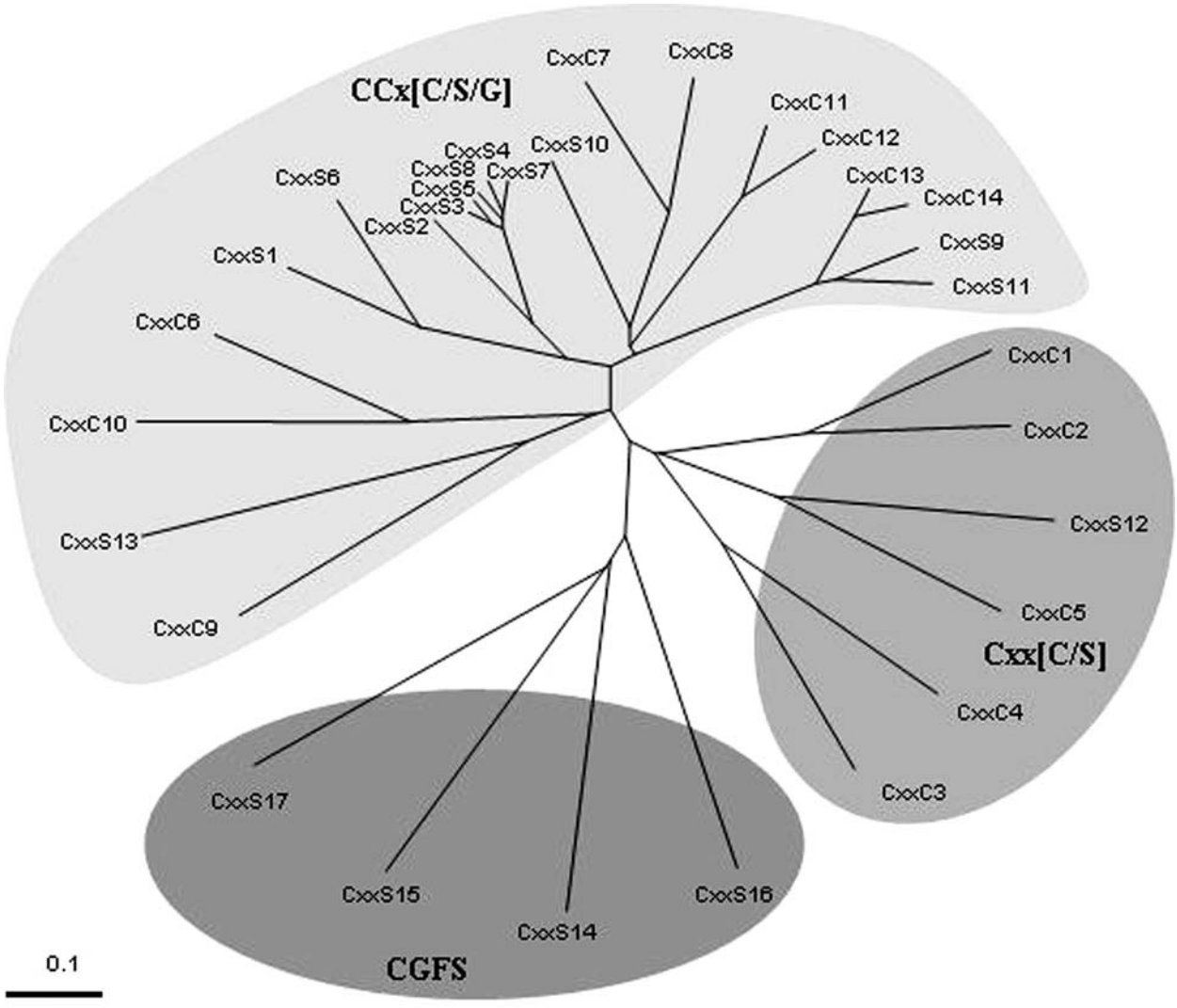

Figure 2. Phylogenetic tree of the various *A. thaliana* Grx isoforms. This tree was drawn using ClustalW. The protein entry codes are identical to those of table 1 and figure 1. Three classes can be distinguished according essentially to the active site sequences: CGFS, Cxx[C/S] and CCx[C/S/G].

ray crystallography (see [13] for a list). All the structures are organized into a Trx fold, consisting of a central β sheet surrounded by α helices. In terms of redox potential, Grx are considered to be weaker reductants than Trx, as their redox potentials are around –190 to –230 mV for *E. coli* Grx isoforms compared with –270 to –330 mV for Trx [14]. Only one poplar isoform with a classical YCPYC active site was characterized by site-directed mutagenesis in term of catalysis and structure [13, 15, 16]. The poplar Grx structure was resolved in complex with glutathione, and the most striking difference compared with the prokaryotic enzymes is the presence of an additional α helix in the N-terminus part [K. D'ambrosio et al., unpublished]. Biochemical studies demonstrate that only the first cysteine of the active site is essential for catalysis with dehydroascorbate (DHA) or type II peroxiredoxin (Prx), a peroxidase involved in the reduction of alkylhydroperoxides [15, 16]. Thus, classification into monocysteinic or bicysteinic Grx should be avoided, since recent data on the *S. cerevisiae* Grx 5 (CGFS active site) indicate that this protein possesses a disulfide bridge involving an extra active site cysteine [17]. This additional cysteine, found 50–54 amino acids after the active site in the consensus motif [I/V/F]G[G/A/S/T]C, is present in seven isoforms of AtGrx (see fig. 1), including CxxC/S Grx (AtGrx CxxC1, CxxC2, CxxC5 and CxxS12) and CGFS Grx (in AtGrx CxxS14, 16 and 17, but surprisingly not in AtGrx CxxS15). The role of this cysteine in the dithiol-containing Grx remains obscure. Its absence in one CGFS isoform and in the CCxC/S isoforms raises the question of the catalytic mechanism used by these isoforms. In the CCxC/S type, another cysteine is partially conserved in many isoforms in the consensus sequence [L/M]GC[S/K/A], located 37–38 amino acids after the active site (see fig. 1).

Expression and localization of glutaredoxins in plants

Very few data are available concerning the distribution of Grx in the different plant organs, and even less is known about their intracellular localizations. Initially, Grx has been identified in spinach leaves and then cloned from complementary DNA (cDNA) libraries of developing seeds of *O. sativa* or of cotyledons from *Ricinus communis* [18–20]. These two Grx, homologous to AtGrx CxxC2, present a distinct expression pattern. Whereas the rice *grx* is expressed exclusively in aleurone layers of seeds [19], the *grx* from *R. communis* is expressed not only in cotyledons but also in hypocotyls, in roots and to a lesser extent in leaves [20]. The gene encoding AtCxxS14 was shown to be expressed in leaves, stems and roots and very weakly in flowers, and it is repressed in seedlings by ion treatment [21]. Moreover, the Grx from *R. communis* is an abundant sieve tube protein of seedlings [20], and the poplar Grx, similar to AtGrx CxxC4, was also localized in the phloem sieve tubes by electronic microscopy and immunofluorescence [unpublished results].

Based on the abundance of each *grx* among the poplar EST in the GenBank database (search was stopped on 10/10/2003), we can estimate in silico the level of expression and the organ localization of each isoform. Out of a total of 114 ESTs encoding poplar Grx, 66 ESTs encode Grx with a CxxC/S active site, 28 encode Grx with a CCxC/S active site and 24 encode Grx with a CGFS active site. PtGrx CxxC2 and CxxC4 are the two most abundantly expressed isoforms (32 and 20 ESTs, respectively). CxxC2 is predominantly expressed in flowers, and CxxC4 in roots.

The functions of Grx in plants and in other organisms: known target proteins

Grx is able to reduce target proteins by dithiol-disulfide exchange using the two active site cysteines in a manner similar to Trx. On the other hand, Grx is a specific and efficient catalyst of protein-glutathione mixed disulfide reduction, a process called deglutathiolation [22]. For this mechanism, only the first cysteine of the active site is required. In animal cells and sometimes in bacteria or yeast, many proteins have been identified as being glutathiolated, especially in response to oxidative conditions and very often by using a proteomics approach. Table 2 presents a complete but non-exhaustive list of the plant and non-plant Grx targets, and indicates whether the target proteins are glutathiolated. If homologues are present in plants, all the targets found in other organisms are also potential interaction partners.

In plants, very little is known about the function of Grx. As their animal counterparts, some plant Grx isoforms are able to reduce dehydroascorbate into ascorbate [15, 20]. More interesting is the capacity of the plant Grx (CxxC type) to reduce the type II Prx [23, 24]. On the other hand, one Grx (CxxC/S type) of *O. sativa* was found to exhibit a GSH-dependent peroxidase activity toward various hydroperoxides as described for the two bicysteinic Grx of *S. cerevisiae* [25, 26]. This is quite surprising since *O. sativa* possesses at least one type II Prx isoform (EST accession number BP184892). The poplar Grx characterized, which belongs to the same group of Grx, does not possess such an activity toward hydroperoxides [27]. Other known targets of Grx in *A. thaliana* are H^+/Ca^{2+} transporters called CAX1 and CAX4 for cation exchanger [21]. Both AtGrx CxxS14 and 16 exhibiting a CGFS active site were found to activate these transporters, probably by a direct interaction which could disrupt the autoinhibition of these transporters. Finally, two proteins of the sugar metabolism from *A. thaliana*, an aldolase and a triose phosphate isomerase, have been found to be glutathiolated and are thus potential targets of Grx for deglutathiolation [28].

In organisms other than plants, Grx are already known to be involved in many processes, such as apoptosis [29], iron sulfur assembly in mitochondria [30, 31] and virion morphogenesis [32]. Moreover, Grx seem to be very important in transduction signaling pathways, as they regulate many transcription factors, kinases and phosphatases; in stress response by regulating various antioxidative enzymes such as Prx and GSH peroxidase; and in cytoskeleton organization, as many proteins are glutathiolated (table 2).

Moreover, many enzymes, listed in table 2, involved in various metabolic pathways are regulated, either by dithiol disulfide exchange or by glutathiolation. One interesting example is the *E. coli* PAPS (3′-phosphoadenylylsulfate) reductase. This enzyme possesses one cysteinyl residue per subunit. In the oxidized form, the enzyme consists of two subunits with an intermolecular disulfide bond, which could be reduced either by Grx or Trx [33]. On the other hand, this cysteine can also be glutathiolated. In this case, only Grx is able to remove GSH [34].

Finally, data on plant target proteins of Grx are scarce compared with other organisms, whereas the number of genes encoding Grx suggests a high representation of this kind of protein. The development of proteomics tools and the emergence of more complete protein databases should allow the identification of new targets. One way, similar to that used for Trx, could be to construct affinity columns with monocysteinic Grx and to retain selectively some covalently interacting proteins, or to use specific probes of thiol groups such as monobromobimane [35, 36]. Another way is to identify glutathiolated proteins by following the methods used for the animal cells, i.e. (i) detection using radiolabeled or biotinylated GSH [28, 37] or (ii) using alkylating agents such as iodoacetamide or N-ethylmaleimide biotin [38, 39].

Table 2. Glutaredoxin target proteins.

	References
Plant Grx targets	
Poplar type II Prx	[23]
Arabidopsis H^+/Ca^{2+} transporter	[21]
Arabidopsis triosephosphate isomerase*	[28]
Arabidopsis aldolase*	[28]
Nonplant Grx targets	
Kinases/phosphatases/transcription factors/signal transduction pathways	
E. coli OxyR	[50]
Human NF1* (nuclear factor 1)	[51]
Rat protein kinase C	[52]
Mouse PEBP2 (polyoma enhancer binding proteins 2)	[53]
Human PTP1B* (protein tyrosine phosphatase 1B)	[54]
Rat H-Ras*	[39]
Human NF-κB (nuclear factor κB) (p50 subunit)	[55, 56]
Human AP1 (activator protein 1)* (c-jun subunit)	[55, 56]
Human CREB (cyclic AMP-response element binding protein)	[55]
Human caspase-3*	[56]
Human Ref1 (redox factor 1)	[57]
Human ASK1 (apoptosis signal-regulating kinase 1)	[58]
Human CRK-like protein*	[38]
Human protein phosphatase 2A	[59]
Mouse cAMP-dependent protein kinase	[60]
Human Akt (Ser/Thr kinase)	[61]
Human Ran-specific GTPase activating protein*	[37]
DNA, RNA, protein synthesis, folding and degradation	
E. coli ribonucleotide reductase	[2]
HIV protease*	[62]
Human cathepsin K*	[63]
Human ubiquitin-conjugating enzymes*	[64, 65]
Human endoplasmic reticulum protein*	[64]
Human SFR1 splicing factor*	[64]
Human 40S ribosomal protein S12*	[64]
Human heat shock cognate 71-kDa protein*	[38]
Human HSP70*	[37,64]
Human HSP60*	[38,64]
Human heat shock protein HSP 90-β*	[38]
Human cyclophilin A*	[37,64]
Human protein disulfide isomerase*	[38,64]
Human translation initiation factor 6*	[38]
Human translation elongation factor*	[64]
Human 40S ribosomal protein SA*	[38]
Human prolyl 4-hydroxylase alpha subunit*	[38]
Human RNA binding protein regulatory subunit*	[64]
Human 14-3-3 protein*	[38]
Human aspartyl-tRNA synthetase*	[38]
Human endoplasmin*	[38]
Human ubiquitin*	[37]
Rat heat shock cognate 70-kDa fragment*	[37]
Saccharomyces cerevisiae 20S proteasome	[66]
Cytoskeleton	
Human actin*	[38, 64, 67]
Human tubulin β1*	[38]
Human vimentin*	[64]
Human laminin*	[38]
Human tropomyosin*	[38, 64]
Human transgelin*	[64]
Human cofilin*	[64]
Human myosin*	[37, 64]
Human profilin*	[37, 64]
Stress response/redox regulation	
Human glutathione peroxidase	[68]
Bovine Cu,Zn superoxide dismutase*	[56]
Human metalloproteinases	[69]

Table 2 (continued)

	References
Human Trx*	[48]
Human peroxiredoxin 1*	[38, 64]
Human peroxiredoxin 4*	[38]
Human peroxiredoxin 6*	[38]
Human stress-induced phosphoprotein 1*	[64]
Rat peroxiredoxin 5*	[37]
Metabolism/energetics	
Rat or human pyruvate kinase*	[38, 70]
Rat ornithine decarboxylase	[71]
Human phosphofructokinase	[72]
Rat S-adenosylmethionine synthetase	[73]
Human aldose reductase*	[38, 74]
Human glyceraldehyde 3-phosphate dehydrogenase*	[75]
E. coli PAPS reductase (*)	[33, 34]
E. coli arsenate reductase*	[76]
Rabbit Ca^{2+} ATPase*	[77]
Rabbit glycogen phosphorylase b*	[39]
Rabbit glycerol phosphate dehydrogenase*	[56]
Bovine haemoglobin*	[56]
Rabbit creatine kinase*	[56]
Yeast alcohol dehydrogenase*	[56]
Rat malate dehydrogenase*	[78]
Human inosine 5′-monophosphate dehydrogenase 2*	[38, 64]
Human enolase*	[38, 64]
Human phosphoglycerate kinase*	[64]
Human, aldolase*	[64]
Human 6-phosphogluconolactonase*	[37, 64]
Human phosphorylase kinase δ*	[64]
Human, triosephosphate isomerase*	[64]
Human dUTP pyrophosphatase*	[64]
Human and rat cytochrome c oxidase*	[37, 64]
Human fructose bisphosphate aldolase A*	[38]
Human nicotinamide N-methyltransferase*	[38]
Human inorganic pyrophosphatase*	[38]
Human fatty acid-binding protein*	[64]
Human β-galactoside soluble protein*	[64]
Human 3-hydroacyl-CoA dehydrogenase type If*	[38]
Human glucose-regulated protein*	[38]
Human histamine release factor*	[38]
Human L-lactate dehydrogenase*	[38]
Human zyrosine hydroxylase	[79]
Human glucosidase II*	[38]
Rat mitochondrial complex I (51- and 75-kDa subunits)	[80]
Rat α-ketoglutarate dehydrogenase*	[81]
Rat enoyl CoA hydratase*	[37]
Other functions	
Human carbonic anhydrase III*	[39, 82]
Human annexin II	[83]
Bovine serum albumin*	[56]
Rat neurogranin/RC3	[84]
Rat neuromodulin/GAP-43	[84]
Human nudix-type motif 6*	[64]
Human T complex protein 1*	[64]
Human lymphocyte-specific protein 1*	[64]
Human nucleosidediphosphate kinase A*	[38]
Human hepatoma-derived growth factor*	[38, 64]
Human ash protein*	[64]
Human My032 protein*	[64]
Human nucleophosmin*	[37, 64]
Human histidine triad nucleotide-binding protein 2*	[37]

Proteins identified as glutathiolated are labelled with an asterisk (*). In other cases, they interact either via dithiol-disulfide exchange, or the mode of catalysis is unknown.

Fusion proteins with a Grx module: physiological significance

In prokaryotes, the genes coding for two proteins which interact with one another are often associated in the same gene cluster or fused together. Computational methods based on phylogenetic profiles [40] and on genome analyses were developed to detect fusion proteins [41] or gene clusters [42]. This organization suggests a possible redox interaction between these proteins in organisms where they are not fused. An example concerning redox-regulated proteins is the fusion between Trx and Trx reductase modules in some bacteria such as *Mycobacterium leprae* [43] or in *A. thaliana* (MATDB protein entry code At2g41680). In all other organisms, these proteins, which also need to interact, are produced as separate proteins.

Using CDART (Conserved Domain Architecture Retrieval Tool, http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi?cmd=rps), various motives can be detected in proteins [44]. These motifs are classified by COG (clusters of orthologous groups of proteins) or pfam (protein families and HMMs) entry codes. In order to identify putative new targets of Grx, this program was used to detect hybrid proteins containing one or many Grx modules (COG0695). Figure 3 presents the schematic organization of these hybrid proteins.

In plants, this type of fusion proteins was already described. The APS (5′-adenylylsulfate) reductase, an enzyme involved in the sulfur metabolism, is coupled to a Trx motif in the C-terminus which possesses a Grx activity [45]. In the red alga *Gracilaria gracilis*, a functional protein is constituted by two Grx modules fused in the N-terminal part to a methionine sulfoxide reductase of type A (MsrA) (COG0225) [N. Rouhier et al., unpublished]. Up to now, only Trx was demonstrated able to reduce MsrA. The existence of this hybrid protein strongly suggests that some MsrA isoforms could be reduced by Grx.

Another example of fusion proteins are enzymes made of a Prx (COG0678) coupled to a Grx domain in the C-terminus. This type of protein, found essentially in bacteria, is functional in hydroperoxide reduction in the presence of GSH as a donor [46]. In all other organisms, the two proteins are not fused. Interestingly, in higher plants the two enzymes, produced as distinct proteins, can interact [23, 24].

An interesting protein, found in eukaryotic organisms such as mammals or platyhelminthes, is constituted by a Grx domain in the N-terminus fused to a Trx reductase (COG1249) in the C-terminus [7]. This protein is 'trifunctional', as it possesses Trx reductase, GR and Grx activities. The previously described PICOT-HD features proteins comprising a Trx-like motif (pfam00085) in the N-terminus associated with one, two and even three Grx modules in the case of AtGrx CxxS17 (fig. 3).

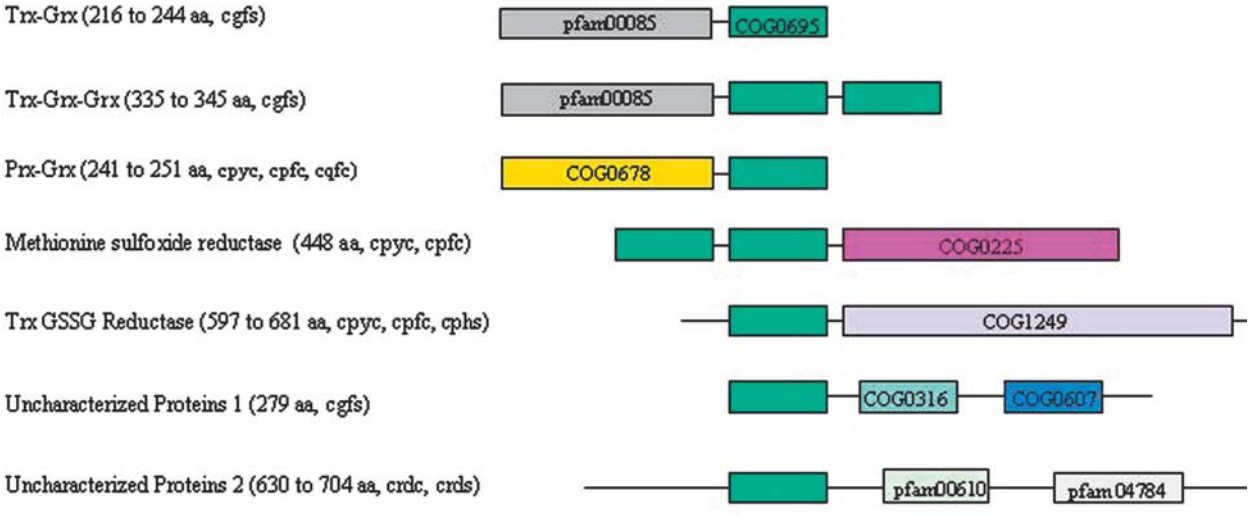

Figure 3. Fusion proteins containing one or many Grx modules. The hybrid proteins were found using the CDART program available at http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi. The pfam or COG entry codes of the different colored domains are indicated. The size of the proteins in amino acids and the sequences of the Grx active site are indicated between parentheses. The accession numbers characteristic for each type of protein are as follows. Trx-Grx XP_311699, *Anopheles gambiae*; AA053174, *Dictyostelium discoideum*; NP_609641, *Drosophila melanogaster*; NP_596647, *Schizosaccharomyces pombe*. Trx-Grx-Grx: NP_741524, *Caenorhabditis elegans*; NP_075629, *Mus musculus*; AAF28844, *Homo sapiens*. Prx-Grx: NP_485581, *Nostoc sp*; NP_407361, *Yersinia pestis*; NP_246286, *Pasteurella multocida*; NP_273984, *Neisseria meningitidis*; NP_232265, *Vibrio cholerae.* Methionine sulfoxide reductase: AF121271, *Gracilaria gracllis.* TGR: AAN63052, *Echinococcus granulosus*; AAK85233, *Schistosoma mansoni*; NP_694802, *Mus musculus*; AAH50032, *Homo sapiens.* Uncharacterized protein 1: NP_638714, *Xanthomonas campestris*; NP_299673, *Xylella fastidiosa.* Uncharacterized protein 2: AAM91894, *Oryza sativa*; P_566405, *A. thaliana*.

Other proteins, uncharacterized so far, contain one Grx module associated with various protein motifs of unknown function. First, some proteins, found in *Xylella fastidiosa* and *Xanthomonas campestris* or *axonopodis* and consisting of 279 amino acids, possess a Grx module followed by a domain of unknown function called IscA (COG0316) and a domain called PspE (COG0607) related to rhodanese sulfurtransferase. Other plant proteins (from *O. sativa* and *A. thaliana*), of larger size (from 630 to 700 amino acids), present a similar architecture. It consists of a Grx domain followed by a domain of unknown function (DEP, pfam00610) found in various signaling proteins such as Dishevelled, Egl-10 or Pleckstrin, and another conserved domain of unknown function (DUF547, pfam04784).

Crosstalk between GSH/Grx and Trx systems

The analysis of *S. cerevisiae* mutant strains for Trx reductase, GR and for the bicysteinic Trx or Grx suggests that the redox state of the Trx system is maintained independent of the GSH/Grx system [47]. Nevertheless, some data indicate that the two systems are dependent on one another. First, the yeast mitochondrial monocysteinic Grx5 is efficiently reduced by *E. coli* Trx compared with GSH [17]. The reverse example is the reduction of a poplar Trx by a poplar Grx or by *E. coli* Grx1, 2 or 3, but not by NTR [85]. Moreover, a cysteine residue in position 72 of human Trx, which does not belong to the active site, is glutathiolated in response to an oxidant, this modification abolishing its activity [48]. It is likely that Grx could regulate the Trx system by the deglutathiolation process. Another example of the complexity of these systems is the GSSG reduction by the Trx system in some organisms such as *D. melanogaster* which lack GR [49]. Finally, as mentioned above, the TGR protein is another example of the interconnection between the two systems because it is able to reduce both Trx and GSSG and to use GSH as a donor for the Grx module [7].

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