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The Peroxiredoxin and Glutathione Peroxidase Families in *Chlamydomonas reinhardtii*

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ABSTRACT

Thiol/selenol peroxidases are ubiquitous nonheme peroxidases. They are divided into two major subfamilies: peroxiredoxins (PRXs) and glutathione peroxidases (GPXs). PRXs are present in diverse subcellular compartments and divided into four types: 2-cys PRX, 1-cys PRX, PRX-Q, and type II PRX (PRXII). In mammals, most GPXs are selenoenzymes containing a highly reactive selenocysteine in their active site while yeast and land plants are devoid of selenoproteins but contain nonselenium GPXs. The presence of a chloroplastic 2-cys PRX, a nonselenium GPX, and two selenium-dependent GPXs has been reported in the unicellular green alga *Chlamydomonas reinhardtii*. The availability of the Chlamydomonas genome sequence offers the opportunity to complete our knowledge on thiol/selenol peroxidases in this organism. In this article, Chlamydomonas PRX and GPX families are presented and compared to their counterparts in Arabidopsis, human, yeast, and Synechocystis sp. A summary of the current knowledge on each family of peroxidases, especially in photosynthetic organisms, phylogenetic analyses, and investigations of the putative subcellular localization of each protein and its relative expression level, on the basis of EST data, are presented. We show that Chlamydomonas PRX and GPX families share some similarities with other photosynthetic organisms but also with human cells. The data are discussed in view of recent results suggesting that these enzymes are important scavengers of reactive oxygen species (ROS) and reactive nitrogen species (RNS) but also play a role in ROS signaling.

LIFE in an oxygen-rich environment has to deal with the danger of oxidative stress. During normal cell metabolism, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly produced, essentially by respiratory and photosynthetic electron transfer chains. These highly reactive molecules can react with many cell components and damage DNA, proteins, and lipids. Thus, their concentration has to be strictly controlled. For this purpose, aerobic organisms are equipped with nonenzymatic (ascorbate, glutathione, tocopherol, and carotenoid) or enzymatic (catalase, ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, and peroxiredoxin) antioxidant systems to remove ROS from the cells. To control their concentrations, ROS and RNS have to be sensed. It has been established in many organisms that ROS, and especially hydrogen peroxide, are signaling molecules that diffuse across membranes and induce specific signal transduction pathways. Furthermore, ROS can also be produced on purpose by cells in response to several stimuli to function as second messengers inside the cell. Finally, ROS and RNS can control enzyme activities by triggering several post-translational modifications such as disulfide bond formation, thiol oxidation to sulfenic/sulfinic/sulfonic acid, glutathionylation, nitrosylation, or carbonylation.

Thiol/selenol peroxidases have emerged, during recent years, as important scavengers of ROS/RNS but they have also been shown to play some role in ROS signaling (Rouhier and Jacquot 2005; Dietz *et al.* 2006; Herbette *et al.* 2007). These nonheme peroxidases are ubiquitous and present in several subcellular compartments. They are divided into two major subfamilies: peroxiredoxins (PRXs) and glutathione peroxidases (GPXs). PRXs were initially identified in yeast and mammals as thiol-specific antioxidants protecting glutamine synthetase from oxidation (Kim *et al.* 1989; Chae *et al.* 1993, 1994a,b; Lim *et al.* 1994; for a historical review see Rhee *et al.* 2005b). Since then, PRXs have been identified in many organisms and constitute a ubiquitous family of thiol-dependent peroxidases catalyzing the reduction of hydrogen peroxide, alkylhydroperoxides, and peroxynitrite to water, the corresponding alcohol, or nitrite, respectively (Nogoceke *et al.* 1997; Bryk *et al.* 2000; Hillas *et al.* 2000). Pe-

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roxiredoxins are divided into four types: 2-cys PRX, 1-cys PRX, PRX-Q, and type II PRX (PRXII). They share a common reaction mechanism where the catalytic cysteine, called the peroxidatic cysteine, is oxidized by hydroperoxides to sulfenic acid and subsequently regenerated to its reduced form by different molecules, such as thioredoxin (TRX), glutaredoxin (GRX), or glutathione, depending on the type of PRX considered (Rouhier and Jacquot 2005).

Glutathione peroxidase was discovered in human as an erythrocyte enzyme protecting hemoglobin from oxidative damage (Mills 1957). The enzyme was shown to catalyze the glutathione-dependent reduction of hydrogen peroxide and diverse alkylhydroperoxides to water or the corresponding alcohol. Initial studies on human GPX1 revealed that it is a selenoenzyme containing a highly reactive selenocysteine (Se-Cys) residue in its active site (Flohé *et al.* 1973; Rotruck *et al.* 1973). Since then, GPXs have been shown to constitute a small multigenic family in mammals. Most human GPX contains a Se-Cys residue at its active site. Once oxidized, these Se-Cys-containing GPXs (selenoGPXs) are regenerated to their reduced form by reduced glutathione (GSH). By contrast, yeast, land plants, and some animals, are devoid of selenoproteins. However, these organisms contain seleno-independent GPXs, also named non-selenium GPX (NS-GPX), where the Se-Cys residue of the active site is replaced by a cysteine (Herbette *et al.* 2007). Mammals contain several NS-GPXs in addition to the classical selenoGPXs. It was found that many NS-GPXs are not reduced by GSH but are efficiently reduced by thioredoxins in *Plasmodium falciparum* (Sztajer *et al.* 2001), *Saccharomyces cerevisiae* (Tanaka *et al.* 2005), Drosophila (Maiorino *et al.* 2007), and land plants (Herbette *et al.* 2002; Jung *et al.* 2002; Navrot *et al.* 2006). Consequently, NS-GPX may be considered as atypical PRXs although their sequence is more closely related to the sequence of standard selenoGPXs.

In *Chlamydomonas reinhardtii*, one PRX and three GPXs have been studied so far. Like human cells, Chlamydomonas likely contains both selenoGPXs and NS-GPXs. Now, the availability of Chlamydomonas genome sequence (Merchant *et al.* 2007) offers the opportunity to complete our knowledge on thiol/selenol peroxidases in this organism. A total of seven PRXs and five GPXs could be identified in the genome of *C. reinhardtii* due to their sequence homology to PRXs and GPXs of other organisms. Phylogenetic analysis revealed that Chlamydomonas PRXs and GPXs share similarities with members of both plant and animal thiol/selenol peroxidase families as observed for many other Chlamydomonas gene families (Merchant *et al.* 2007).

THE PEROXIREDOXIN FAMILY

Phylogenetic analysis of peroxiredoxins: The PRX family is divided into four subgroups on the basis of sequence analysis: 2-cys PRX, PRXII, 1-cys PRX, and PRX-Q. 2-cys PRXs contain two conserved cysteine residues, both essential for catalysis. Type II PRXs, also named atypical 2-cys PRXs, contain two cysteine residues but the position of one of these cysteines is not conserved (Bréhélin *et al.* 2003; Rouhier and Jacquot 2005). PRX-Qs also contain two cysteine residues while 1-cys PRXs contain only one conserved cysteine residue.

Six isoforms of PRX (I–VI) have been identified in mammals. HsPRXI–HsPRXIV are 2-cys PRXs located in the cytosol (HsPRXI–HsPRXII), mitochondria (HsPRXIII), or secreted (HsPRXIV) (Rhee *et al.* 2005b). HsPRXV is a type II PRX localized in the cytosol, mitochondria, and peroxisomes (Knoops *et al.* 1999) while HsPRXVI is a 1-cys PRX located in the cytosol (Choi *et al.* 1998; Kang *et al.* 1998). Higher plants contain more PRX isoforms than mammals (Rouhier and Jacquot 2005). For example, the genome of *Arabidopsis thaliana* contains 10 distinct PRX genes. Among these PRXs, 2 belong to the 2-cys PRX subgroup, 6 are type II PRXs, 1 is a 1-cys PRX, and 1 is a PRX-Q. Both 2-cys PRXs are located in chloroplasts (König *et al.* 2002). The plastidial localization of 2-cys PRXs was confirmed experimentally in several organisms (Baier and Dietz 1997; Genot *et al.* 2001; König *et al.* 2002). 1-Cys PRXs harbor a nuclear bipartite signal allowing targeting to the nucleus and/or the cytosol (Stacy *et al.* 1999; Haslekas *et al.* 2003) while type II PRXs are found in several subcellular compartments. Arabidopsis type II PRXs AtPRXIIB, AtPRXIIC, and AtPRXIID are in the cytosol, AtPRXIIE is chloroplastic, and AtPRXIIF is targeted to mitochondria (Bréhélin *et al.* 2003; Finkemeier *et al.* 2005). AtPRXIIA is an atypically long PRX that does not seem to be expressed (Horling *et al.* 2002; Bréhélin *et al.* 2003). The fourth type of PRX, PRX-Q, corresponds to proteins targeted to chloroplasts in higher plants. The enzyme was reported to be associated with PSII particles on the stromal side of thylakoid membranes and proposed to play a role in the protection of PSII from oxidative stress (Lamkemeyer *et al.* 2006). However, another study suggested that the enzyme is targeted to the thylakoid lumen (Petersson *et al.* 2006). Clearly, further work is required to determine the precise subcellular localization and function of PRX-Q in chloroplasts.

Analysis of the genome of Chlamydomonas allowed us to identify seven PRX genes (Table 1; Figure 1). CrPRX1 and CrPRX2 are 2-cys PRXs, CrPRX3–5 are type II PRXs, CrPRX6 belongs to the PRX-Q subgroup, and CrPRX7 is a fusion protein between a 2-cys PRX and a TRX. No 1-cys PRX has been identified in the Chlamydomonas genome. The PRX genes are distributed in different scaffolds and predicted to be localized in various compartments. The phylogenetic analysis of Chlamydomonas PRXs and PRX families from Arabidopsis, human, yeast, and Synechocystis sp. clearly shows that the members of each subgroup are more

TABLE 1

The peroxiredoxin family in *Chlamydomonas reinhardtii*

Name	PRX type	Scaffold	Putative subcellular localization	Number of ESTs	Protein length (aa)
PRX1	2-cys PRX	3:2064657–2066515	Chloroplast	458	235
PRX2	2-cys PRX	5:332956–334928	Cytosol/flagella	128	198
PRX3	Type II	4:617682–619635	Cytosol/flagella	2	161
PRX4	Type II	7:1092036–1094611	Mitochondrion	21	226
PRX5	Type II	1:570296–572738	Chloroplast	43	194
PRX6	PRX-Q	8:1690047–1692411	Chloroplast	22	196
PRX7	2-cys PRX/TRX fusion	105:86535–91084	Mitochondrion	0	301

Scaffold indicates the location of the corresponding gene in *Chlamydomonas reinhardtii* genome version 3.0 (http://genome.jgi-psf.org/Chlre3). Subcellular localizations are deduced from the analysis of the protein N-terminal sequence or the localizations demonstrated for homologous proteins in higher plants or proteomic analyses. The number of ESTs was determined by BLAST alignment of the transcript sequence with NCBI DBest database of November 12, 2007 and supplemented with JGI EST sequences.

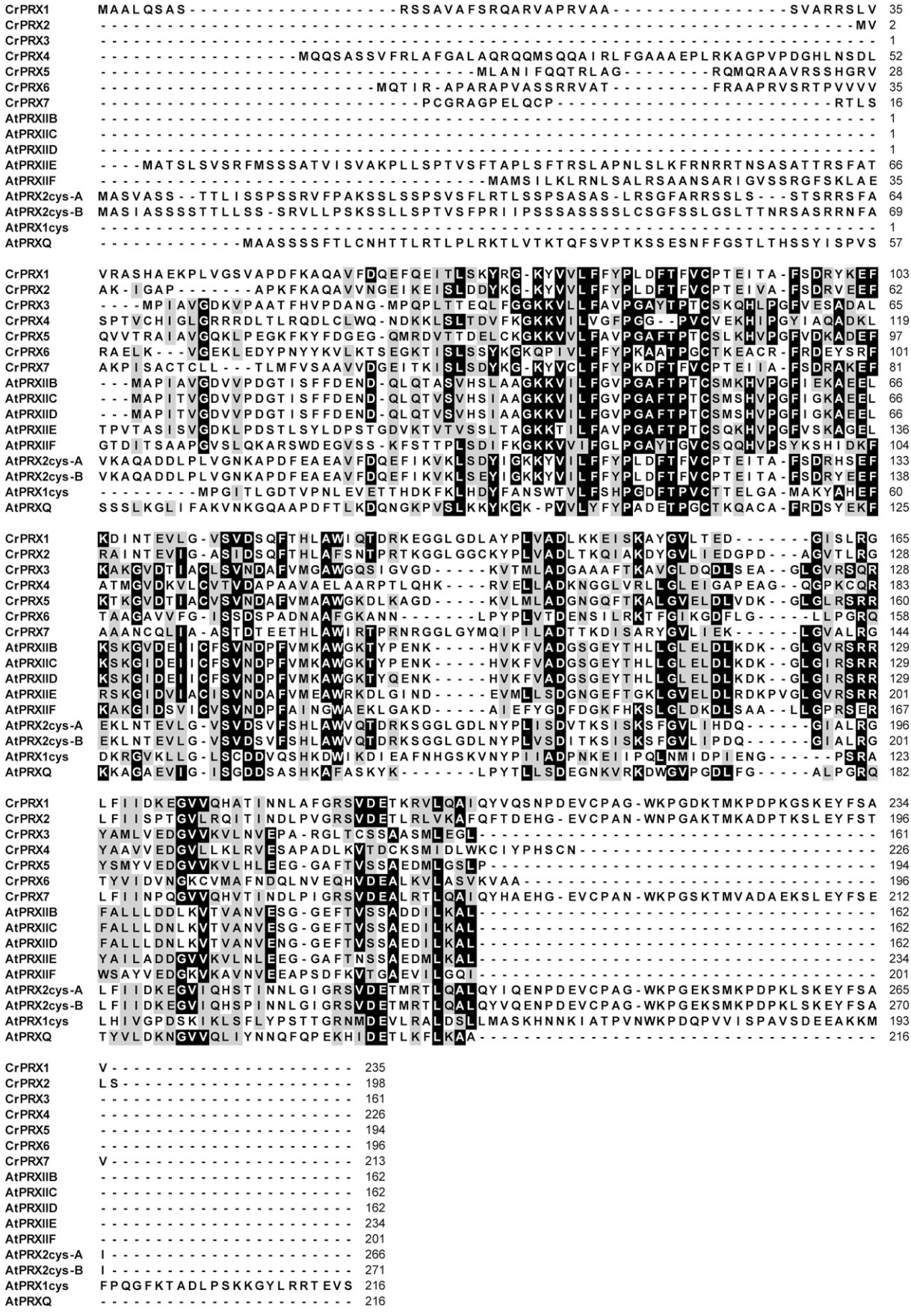
closely related to members of the same subgroup from other organisms than to PRXs from the same organism but from different subgroups (Figure 2).

CrPRX1 was the first PRX identified in Chlamydomonas (Goyer *et al.* 2002). CrPRX1 is homologous to the two 2-cys PRXs from Arabidopsis (Figure 2), of which both were shown to be localized in the chloroplast (König *et al.* 2002). The presence of *PRX2* in the Chlamydomonas genome is more surprising. Even though this gene encodes a typical 2-cys PRX it lacks any targeting sequence and the protein is likely to be located in the cytosol. Still, all 2-cys PRXs from higher plants are chloroplastic proteins while in mammals, 2-cys PRXs are found in cytosol, mitochondria, or are secreted by the cell. A cytosolic 2-cys PRX suggests that the Chlamydomonas PRX family shares some similarities with animal systems as observed for many gene families in the genome of Chlamydomonas (Merchant *et al.* 2007). This is supported by the phylogenetic analysis presented in Figure 2. While CrPRX1 is clearly related to higher plant chloroplastic 2-cys PRXs, CrPRX2 is found within a different subgroup along with cytosolic human PRXs (PRXI–IV). CrPRX2 was found in the flagellar proteome (Pazour *et al.* 2005), suggesting that despite the absence of any obvious N-terminal extension, the enzyme might be present in both the cytosol and the flagella. Genome data indicate that the genome of Chlamydomonas contains a third gene related to 2-cys PRX, named *PRX7*. This gene encodes a hybrid protein containing an N-terminal 2-cys-PRX domain fused to a C-terminal TRX h domain. The enzyme is probably mitochondrial. Interestingly, some bacteria contain fusion proteins composed of a type II PRX followed by a GRX module. These proteins can use GSH to reduce a wide range of peroxide substrates (Pauwels *et al.* 2003; Rouhier and Jacquot 2003; Cha *et al.* 2004). Moreover, artificial hybrid proteins between poplar type II peroxiredoxin and glutaredoxin or thioredoxin h were recently engineered and shown to be functional, indicating that the two modules can function together (Rouhier *et al.* 2006). This suggests that CrPRX7 may function as a peroxidase using electrons provided by mitochondrial NADPH-thioredoxin reductase. However, the *CrPRX7* gene model is uncertain and no EST corresponding to this putative PRX was found in EST databases, suggesting that this gene might not be expressed.

Chlamydomonas also contains three type II PRXs: CrPRX3, CrPRX4, and CrPRX5. CrPRX4 is related to Arabidopsis mitochondrial PRXIIF (Figure 2) and exhibits an N-terminal extension that could function as a mitochondrial targeting sequence (Figure 1). CrPRX3 and CrPRX5, the two other Chlamydomonas type II PRXs, are distantly related to Arabidopsis chloroplastic PRXIIE. However, only CrPRX5 contains a putative chloroplast transit peptide (Figure 1) and CrPRX3 is more likely a cytosolic type II PRX like Arabidopsis PRXIIA–D. However, as with CrPRX2, CrPRX3 was also found in the flagellar proteome (Pazour *et al.* 2005) and thus might also be present in this compartment.

Surprisingly, in the genome of Chlamydomonas, no gene encoding a 1-cys PRX was identified while this type of PRX appears ubiquitous and was found in many organisms including higher plants and cyanobacteria (Figure 2) (Rouhier and Jacquot 2005; Stork *et al.* 2005). However, this type of PRX also appears to be absent from the genomes of *Ostreococcus tauri* and *Volvox carteri*. This strongly suggests that 1-cys PRXs are not present in green algae.

Chlamydomonas PRX6 is a typical PRX-Q, closely related to higher plant PRX-Q (Figures 1–2). The protein contains an N-terminal extension that might correspond to a transit peptide for targeting to the chloroplast. Therefore, PRX6 is likely to be the functional homolog of higher plant PRX-Q. Initially identified in *Sedum lineare*, as a homolog of *Escherichia coli* bacterioferritin comigratory protein, PRX-Q is the most recently characterized PRX type (Kong *et al.* 2000). In cyanobacteria, an unexpected multiplicity of *PRX-Q*


CrPRX1 MAALQSAS-----RSSAVAFSRQARVAPRVAA-----SVARRSLV 35
CrPRX2 -----MV 2
CrPRX3 ----- 1
CrPRX4 -----MQQSASSVFRLAFGALAQRQQMSQQAIRLFGAAAEPLRKAGPVPDGHLNSDL 52
CrPRX5 -----MLANIFQQTRLAG--------RQMQRAAVRSSHGRV 28
CrPRX6 -----MQTIR-APARAPVASSRRVAT---------FRAAPRVSRTPVVVV 35
CrPRX7 -----PCGRAGPELQCP-----RTLS 16
AtPRXIIB ----- 1
AtPRXIIC ----- 1
AtPRXIID ----- 1
AtPRXIIE ----MATSLSVSRFMSSSATVISVAKPLLSPTVSFTAPLSFTRSLAPNLSLKFRNRRTNSASATTRSFAT 66
AtPRXIIF -----MAMSILKLRNLSALRSAANSARIGVSSRGFSKLAE 35
AtPRX2cys-A MASVASS--TTLISSPSSRVFPAKSSLSSPSVSFLRTLSSPSASAS-LRSGFARRSSLS---STSRRSFA 64
AtPRX2cys-B MASIASSSSTTLLSS-SRVLLPSKSSLLSPTVSFPRIIPSSSASSSSLCSGFSSLGSLTTNRSASRRNFA 69
AtPRX1cys ----- 1
AtPRXQ ----------MAASSSSFTLCNHTTLRTLPLRKTLVTKTQFSVPTKSSESNFFGSTLTHSSYISPVS 57
CrPRX1 VRASHAEKPLVGSVAPDFKAQAVFDQEFQEITLSKYRG-KYVVLFFYPLDFTFVCPTEITA-FSDRYKEF 103
CrPRX2 AK-IGAP-------APKFKAQAVVNGEIKEISLDDYKG-KYVVLFFYPLDFTFVCPTEIVA-FSDRVEEF 62
CrPRX3 ----MPIAVGDKVPAATFHVPDANG-MPQPLTTEQLFGGKKVLLFAVPGAYTPTCSKQHLPGFVESADAL 65
CrPRX4 SPTVCHIGLGRRRDLTLRQDLCLWQ-NDKKLSLTDVFKGKKVILVGFPGG--PVCVEKHIPGYIAQADKL 119
CrPRX5 QVVTRAIAVGQKLPEGKFKYFDGEG-QMRDVTTDELCKGKKVVLFAVPGAFTPTCSLKHVPGFVDKADEF 97
CrPRX6 RAELK---VGEKLEDYPNYYKVLKTSEGKTISLSSYKGKQPIVLFFYPKAATPGCTKEACR-FRDEYSRF 101
CrPRX7 AKPISACTCLL---TLMFVSAAVVDGEITKISLSDYKG-KYVCLFFYPKDFTFVCPTEIIA-FSDRAKEF 81
AtPRXIIB ---MAPIAVGDVVPDGTISFFDEND-QLQTASVHSLAAGKKVILFGVPGAFTPTCSMKHVPGFIEKAEEL 66
AtPRXIIC ---MAPITVGDVVPDGTISFFDEND-QLQTVSVHSIAAGKKVILFGVPGAFTPTCSMSHVPGFIGKAEEL 66
AtPRXIID ---MAPITVGDVVPDGTISFFDEND-QLQTVSVHSIAAGKKVILFGVPGAFTPTCSMSHVPGFIGKAEEL 66
AtPRXIIE TPVTASISVGDKLPDSTLSYLDPSTGDVKTVTVSSLTAGKKTILFAVPGAFTPTCSQKHVPGFVSKAGEL 136
AtPRXIIF GTDITSAAPGVSLQKARSWDEGVSS-KFSTTPLSDIFKGKKVVIFGLPGAYTGVCSQQHVPSYKSHIDKF 104
AtPRX2cys-A VKAQADDLPLVGNKAPDFEAEAVFDQEFIKVKLSDYIGKKYVILFFYPLDFTFVCPTEITA-FSDRHSEF 133
AtPRX2cys-B VKAQADDLPLVGNKAPDFEAEAVFDQEFIKVKLSEYIGKKYVILFFYPLDFTFVCPTEITA-FSDRYEEF 138
AtPRX1cys ---------MPGITLGDTVPNLEVETTHDKFKLHDYFANSWTVLFSHPGDFTPVCTTELGA-MAKYAHEF 60
AtPRXQ SSSLKGLIFAKVNKGQAAPDFTLKDQNGKPVSLKKYKGK-PVVLYFYPADETPGCTKQACA-FRDSYEKF 125
CrPRX1 KDINTEVLG-VSVDSQFTHLAWIQTDRKEGGLGDLAYPLVADLKKEISKAYGVLTED-------GISLRG 165
CrPRX2 RAINTEVIG-ASIDSQFTHLAFSNTPRTKGGLGGCKYPLVADLTKQIAKDYGVLIEDGPD---AGVTLRG 128
CrPRX3 KAKGVDTIACLSVNDAFVMGAWGQSIGVGD-----KVTMLADGAAAFTKAVGLDQDLSEA--GLGVRSQR 128
CrPRX4 ATMGVDKVLCVTVDAPAAVAELAARPTLQHK----RVELLADKNGGLVRLLGLEIGAPEAG--QGPKCQR 183
CrPRX5 KTKGVDTIACVSVNDAFVMAAWGKDLKAGD-----KVLMLADGNGQFTKALGVELDLVDK--GLGLRSRR 160
CrPRX6 TAAGAVVFG-ISSDSPADNAAFGKANN-------LPYPLVTDENSILRKTFGIKGDFLG-----LLPGRQ 158
CrPRX7 AAANCQLIA-ASTDTEETHLAWIRTPRNRGGLGYMQIPILADTTKDISARYGVLIEK------LGVALRG 144
AtPRXIIB KSKGVDEIICFSVNDPFVMKAWGKTYPENK-----HVKFVADGSGEYTHLLGLELDLKDK--GLGVRSRR 129
AtPRXIIC KSKGIDEIICFSVNDPFVMKAWGKTYPENK-----HVKFVADGSGEYTHLLGLELDLKDK--GLGIRSRR 129
AtPRXIID KSKGIDEIICFSVNDPFVMKAWGKTYQENK-----HVKFVADGSGEYTHLLGLELDLKDK--GLGIRSRR 129
AtPRXIIE RSKGIDVIACISVNDAFVMEAWRKDLGIND-----EVMLLSDGNGEFTGKLGVELDLRDKPVGLGVRSRR 201
AtPRXIIF KAKGIDSVICVSVNDPFAINGWAEKLGAKD-----AIEFYGDFDGKFHKSLGLDKDLSAA--LLGPRSER 167
AtPRX2cys-A EKLNTEVLG-VSVDSVFSHLAWVQTDRKSGGLGDLNYPLISDVTKSISKSFGVLIHDQ------GIALRG 196
AtPRX2cys-B EKLNTEVLG-VSVDSVFSHLAWVQTDRKSGGLGDLNYPLVSDITKSISKSFGVLIPDQ------GIALRG 201
AtPRX1cys DKRGVKLLG-LSCDDVQSHKDWIKDIEAFNHGSKVNYPIIADPNKEIIPQLNMIDPIENG------PSRA 123
AtPRXQ KKAGAEVIG-ISGDDSASHKAFASKYK-------LPYTLLSDEGNKVRKDWGVPGDLFG-----ALPGRQ 182
CrPRX1 LFIIDKEGVVQHATINNLAFGRSVDETKRVLQAIQYVQSNPDEVCPAG-WKPGDKTMKPDPKGSKEYFSA 234
CrPRX2 LFIISPTGVLRQITINDLPVGRSVDETLRLVKAFQFTDEHG-EVCPAN-WNPGAKTMKADPTKSLEYFST 196
CrPRX3 YAMLVEDGVVKVLNVEPA-RGLTCSSAASMLEGL----------------------------------- 161
CrPRX4 YAAVVEDGVLLKLRVESAPADLKVTDCKSMIDLWKCIYPHSCN----------------------------- 226
CrPRX5 YSMYVEDGVVKVLHLEEG-GAFTVSSAEDMLGSLP---------------------------------- 194
CrPRX6 TYVIDVNGKCVMAFNDQLNVEQHVDEALKVLASVKVAA------------------------------- 196
CrPRX7 LFIINPQGVVQHVTINDLPIGRSVDEALRTLQAIQYHAEHG-EVCPAN-WKPGSKTMVADAEKSLEYFSE 212
AtPRXIIB FALLLDDLKVTVANVESG-GEFTVSSADDILKAL---------------------------------- 162
AtPRXIIC FALLLDNLKVTVANVESG-GEFTVSSAEDILKAL---------------------------------- 162
AtPRXIID FALLLDNLKVTVANVENG-GEFTVSSAEDILKAL---------------------------------- 162
AtPRXIIE YAILADDGVVKVLNLEEG-GAFTNSSAEDMLKAL---------------------------------- 234
AtPRXIIF WSAYVEDGKVKAVNVEEAPSDFKVTGAEVILGQI---------------------------------- 201
AtPRX2cys-A LFIIDKEGVIQHSTINNLGIGRSVDETMRTLQALQYIQENPDEVCPAG-WKPGEKSMKPDPKLSKEYFSA 265
AtPRX2cys-B LFIIDKEGVIQHSPINNLGIGRSVDETMRTLQALQYVQENPDEVCPAG-WKPGEKSMKPDPKLSKEYFSA 270
AtPRX1cys LHIVGPDSKIKLSFLYPSTTGRNMDEVLRALDSLLMASKHNNKIATPVNWKPDQPVVISPAVSDEEAKKM 193
AtPRXQ TYVLDKNGVVQLIYNNQFQPEKHIDETLKFLKAA---------------------------------- 216
CrPRX1 V----------------------- 235
CrPRX2 LS---------------------- 198
CrPRX3 ----------------------- 161
CrPRX4 ----------------------- 226
CrPRX5 ----------------------- 194
CrPRX6 ----------------------- 196
CrPRX7 V----------------------- 213
AtPRXIIB ----------------------- 162
AtPRXIIC ----------------------- 162
AtPRXIID ----------------------- 162
AtPRXIIE ----------------------- 234
AtPRXIIF ----------------------- 201
AtPRX2cys-A I----------------------- 266
AtPRX2cys-B I----------------------- 271
AtPRX1cys FPQGFKTADLPSKKGYLRRTEVS 216
AtPRXQ ----------------------- 216

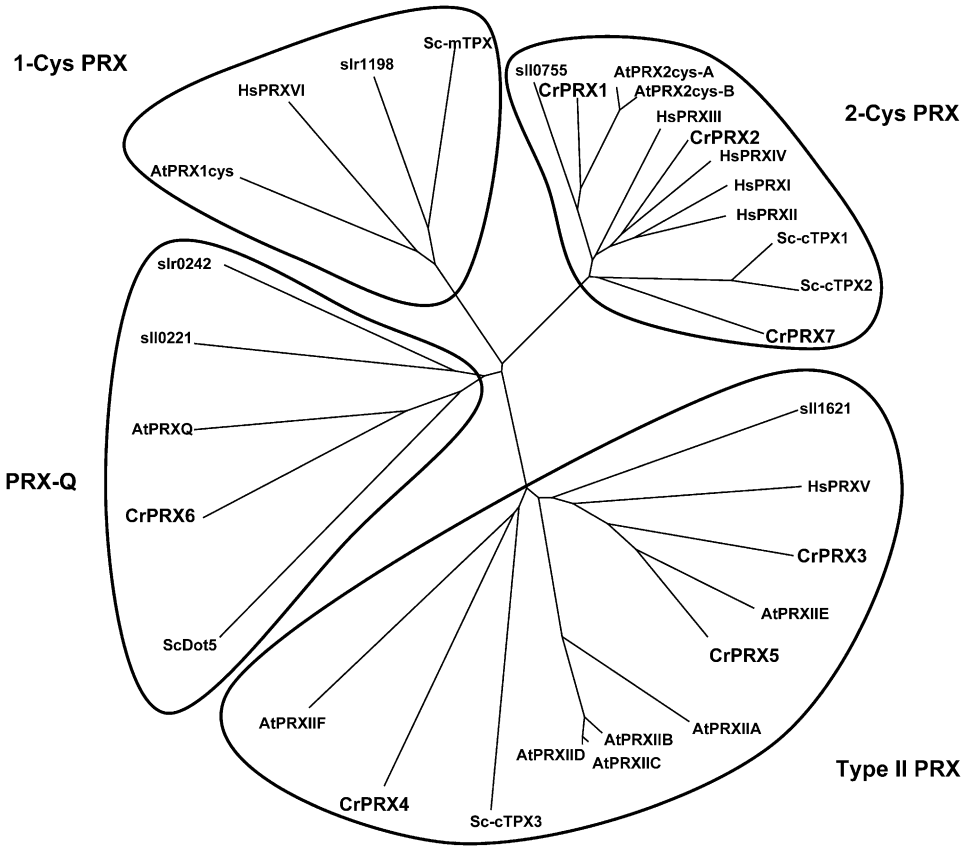


FIGURE 2.—Phylogenetic tree of the peroxiredoxin families in *Chlamydomonas reinhardtii* and diverse organisms. The unrooted tree was constructed with the ClustalX program. Gaps were excluded. Accession numbers and abbreviations are as follows: At, *Arabidopsis thaliana* as in Figure 1; Cr, *Chlamydomonas reinhardtii* as in Table 1; Hs, *Homo sapiens* PRXI, Q06830; PRXII, P32119; PRXIII, P30048; PRXIV, Q13162; PRXV, P30044; PRXVI, P30041; Sc, *Saccharomyces cerevisiae* cTPX1, P34760; cTPX2, Q04120; cTPX3, P38013; m-TPX, P34227; ScDot5, P40553; sll or slr, Synechocystis *PCC6803* sll0221; sll1621; slr0242; and slr1198.

genes was reported with two genes in Synechocystis sp., four in *Synechococcus elongatus* PCC 7942, and four in Anabaena sp. PCC 7201 (STORK *et al.* 2005; LATIFI *et al.* 2007). Several of these cyanobacterial PRX-Q constitute a distinct subgroup within the PRX-Q family in which the second cysteine is more distant in the primary sequence, compared to higher plants. This subgroup is also present in photosynthetic bacteria (WAKITA *et al.* 2007). These atypical PRX-Q exhibit a thioredoxin dependent peroxidase activity and appear to play a major role in the protection of these organisms against oxidative stress (LATIFI *et al.* 2007; WAKITA *et al.* 2007).

Activity of peroxiredoxins: All PRX isoforms share a conserved cysteine, named the peroxidatic cysteine (WOOD *et al.* 2002), which is converted, by nucleophilic attack, to sulfenic acid (SOH) (CHAE *et al.* 1994a; BRYK *et al.* 2000). This oxidized cysteine residue is regenerated by intra- or intermolecular disulfide-bond formation (WOOD *et al.* 2003b). Depending on the PRX type and the organism considered, this disulfide bond can be reduced by diverse thiol reductants such as TRX, GRX, and cyclophilins in eukaryotes (CHAE *et al.* 1994b; LEE *et al.* 2001; ROUHIER *et al.* 2001) or AhpF in prokaryotes (POOLE 2005).

Mammalian 2-cys PRXs are obligate homodimers, independent of the oxidation state of the two conserved cysteines, with a head-to-tail arrangement (SCHRODER *et al.* 2000). An intermolecular disulfide bond is formed at the active site during catalysis (CHAE *et al.* 1994a). 2-cys PRXs are regenerated by TRX reduction of this intermolecular disulfide bond (CHAE *et al.* 1994b). Upon reduction, 2-cys PRX dimers can aggregate into decameric doughnut-shaped structures (SCHRODER *et al.* 2000; WOOD *et al.* 2002).

Chloroplastic CrPRX1 was initially trapped on a monocysteinic TRX column aimed at identifying new TRX targets and subsequently purified and sequenced (GOYER *et al.* 2002). In a similar way, CrPRX2 was identified as a cytosolic TRX-bound protein in a large-scale proteomic study (LEMAIRE *et al.* 2004), suggesting that this 2-cys PRX is likely to be reduced by cytosolic TRX. Since the reaction mechanism of plant chloro-

FIGURE 1.—Multiple sequence alignment of peroxiredoxins from *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*. The proteins were aligned with the ClustalW program. Residues in solid boxes correspond to 50% identity. Residues in shaded boxes correspond to 50% conservation with the BLOSUM62 matrix. Accession nos. and abbreviations are as follows: Cr, *Chlamydomonas reinhardtii*, accession numbers as in Table 1, only the N-terminal domain of CrPRX7 was used for the alignment; At, *Arabidopsis thaliana* Prx IIA, At1g65990; Prx IIB, At1g65980; Prx IIC, At1g65970; Prx IID, At1g60740; Prx IIE, At3g52960; Prx IIF, At3g06050; 2-Cys Prx A, At3g11630; 2-Cys Prx B, At5g06290; 1-Cys Prx, At1g48130; and Prx Q, At3g26060.

plastic 2-cys PRXs is similar to the one described for yeast and mammalian 2-cys PRXs that involves the formation of an intermolecular disulfide bond reduced by TRX (Cheong *et al.* 1999; König *et al.* 2002, 2003), CrPRX1 might have the same reaction mechanism too. Four types of TRXs (f, m, x, and y) are present in both Chlamydomonas and higher plant chloroplasts (Lemaire *et al.* 2003b; 2007). TRX x was shown to be the most efficient chloroplastic TRX for reduction of Arabidopsis 2-cys PRXs (Collin *et al.* 2003, 2004). A similar specificity was found for CrPRX1, which is more efficiently reduced by Chlamydomonas TRX x (S. D. Lemaire, unpublished results) than by TRX m, f, or y (Lemaire *et al.* 2003a). Besides TRX, chloroplastic 2-cys PRXs can also be reduced by CDSP32, a drought-induced TRX-like protein containing two TRX modules (Broin *et al.* 2002) and by NTRC, a chloroplastic bipartite enzyme containing an NADPH-TRX reductase fused to its own TRX module (Moon *et al.* 2006; Pérez-Ruiz *et al.* 2006). Interestingly, genes encoding homologs of CDSP32 and NTRC are present in the Chlamydomonas genome and might therefore also act as reductants for PRX (Lemaire *et al.* 2007). Chloroplastic 2-cys PRXs can reduce a broad range of hydroperoxides but also peroxynitrites (Goyer *et al.* 2002; König *et al.* 2003; Sakamoto *et al.* 2003). In Chlamydomonas, CrPRX1 was shown to catalyze H_2O_2 and tertiary butyl-hydroperoxide (tBOOH) reduction and to protect DNA against ROS-induced degradation (Goyer *et al.* 2002). Chloroplastic 2-cys PRXs also appear to form decameric structures like their homologs from nonphotosynthetic organisms (König *et al.* 2003). This oligomeric form of 2-cys PRX reversibly associates with thylakoid membranes (König *et al.* 2002).

Type II PRXs have also been characterized extensively at the biochemical level. Human type II PRX, HsPRXV, contains three cysteine residues. Once oxidized to SOH the peroxidatic cys47 forms a disulfide with cys151 that can be reduced by TRX (Evrard *et al.* 2004). Chlamydomonas type II CrPRX4 is related to Arabidopsis mitochondrial PRXIIF, which can reduce a broad range of substrates but more efficiently H_2O_2 than alkyl-hydroperoxides (Horling *et al.* 2003). Analysis of knockout Arabidopsis plants suggested that PRXIIF is essential for redox homeostasis and root growth under stress conditions (Finkemeier *et al.* 2005). The most efficient reductants for this PRX appear to be GRXs (Finkemeier *et al.* 2005; Gama *et al.* 2007). Similarly, Arabidopsis PRXIIB is not regenerated by TRX but can efficiently catalyze peroxide reduction in the presence of GSH and GRX (Bréhélin *et al.* 2003).

In contrast, poplar cytosolic PRXII can use either GRXs or TRXs as proton donors (Rouhier *et al.* 2001). Only one cysteine appears essential for the activity of poplar PRXIIC (Rouhier *et al.* 2002). This enzyme is a homodimer that dissociates into monomers when the peroxidatic cysteine undergoes glutathionylation (Noguera-Mazon *et al.* 2006). Therefore, glutathionylation might be required for the GRX-dependent reduction of the enzyme as previously observed for mammalian 1-cys PRX, which interacts with ΠGST and undergoes glutathionylation (Manevich *et al.* 2004). Alternatively, glutathionylation, which occurs under oxidative stress, might constitute a mechanism of regulation of the activity of PRXs, as observed for many other enzymes (reviewed in Michelet *et al.* 2006).

Chlamydomonas type II PRX5 may be the functional homolog of higher plant plastidial PRXIIE, whose physiological electron donor remains unknown since this PRX is reduced by neither GRX nor TRX (Bréhélin *et al.* 2003). However, only "classical" GRX, containing a CPYC active site, have been tested on PRXIIE while this type of GRX does not appear to be present in chloroplasts from higher plants or Chlamydomonas (Lemaire 2004; Rouhier *et al.* 2004a). Most GRXs located in chloroplasts belong to the CGFS type, a subgroup distinct from classical GRXs and with distinct biochemical properties (Tamarit *et al.* 2003; Cheng *et al.* 2006). Therefore, PRXIIE might possibly be reduced by this type of GRX in chloroplasts. However, like CrPRX1 and CrPRX2, CrPRX5 was found among putative TRX targets bound to TRX affinity columns (Lemaire *et al.* 2004), suggesting that this type II PRX might be reduced by TRX.

In higher plants, the chloroplast targeted PRX-Q can reduce a broad range of peroxides (Rouhier *et al.* 2004b; Lamkemeyer *et al.* 2006). Once oxidized to the sulfenic form, the peroxidatic cysteine is regenerated by formation of an intramolecular disulfide bond that exhibits a redox potential of −325 mV at pH 7.0 (Rouhier *et al.* 2004b). This disulfide bond is much more efficiently reduced by TRX y than by all other chloroplastic TRX types (Collin *et al.* 2004; Rouhier *et al.* 2004b). Arabidopsis mutant lines deficient in PRX-Q are not distinguishable from wild-type plants but the enzyme has been proposed to play a role in the protection of the photosynthetic apparatus under oxidative stress (Lamkemeyer *et al.* 2006).

Expression of peroxiredoxins: The analysis of EST abundance data indicates that *CrPRX1* and *CrPRX2* are, by far, the most highly expressed PRX genes in Chlamydomonas (Table 1). In higher plants, chloroplastic 2-cys PRXs are also the most highly expressed PRXs at the mRNA level (Horling *et al.* 2003; Rouhier and Jacquot 2005). However, in higher plants the number of ESTs is only 2-fold higher for 2-cys PRXs compared to PRX-Q or several type II PRXs while this difference is close to 10-fold in Chlamydomonas. This suggests an important role for 2-cys PRXs in the alga.

In higher plants, the expression of 2-cys PRXs is regulated by light and ascorbate treatment at both the mRNA and protein levels (Horling *et al.* 2001, 2003). The transcript levels are also decreased under salt stress (Horling *et al.* 2002). In Arabidopsis, the promoter appears to be under the control of ABA and photosys-

tem I (PSI) redox state (Baier *et al.* 2004). Chlamydomonas *PRX1* mRNA accumulation was shown to be regulated by light, oxygen concentration, and the redox state of the photosynthetic electron transfer chain (Goyer *et al.* 2002). Genetic analyses suggested that chloroplastic 2-cys PRXs participate in the protection of the photosynthetic apparatus (Baier and Dietz 1999). All these data indicate that Chlamydomonas PRX1 is the functional homolog of higher plant 2-cys PRXs and is likely to play a similar role in the single chloroplast of the green alga.

EST data suggest that *CrPRX5* is the most highly expressed type II PRX in Chlamydomonas (Table 1). The corresponding transcript was shown to be increased during sulfur starvation (Zhang *et al.* 2004). Analyses at the mRNA and protein levels in Arabidopsis and poplar indicate that PRXIIB is expressed in all tissues while PRXIIC and PRXIID are almost only expressed in pollen (Rouhier *et al.* 2001; Bréhélin *et al.* 2003). The transcript level of *AtPRXIIB* is increased under phosphorus starvation and by salt and tBOOH treatments, while *AtPRXIIC* mRNA level is strongly affected by phosphorus deprivation, salts, ascorbate, and oxidative stress treatments (Horling *et al.* 2002, 2003; Kandlbinder *et al.* 2004). In Arabidopsis, PRXIIF expression is constitutive, suggesting a housekeeping function (Finkemeier *et al.* 2005; Dietz *et al.* 2006) while PRXIIE is mainly expressed in reproductive tissues and its transcript level is increased under high light, decreased by salt or ascorbate treatments but unaffected by oxidative stress (Horling *et al.* 2002, 2003; Bréhélin *et al.* 2003).

In poplar, PRX-Q protein is mainly found in leaves and the protein level is increased by pathogen attack (Rouhier *et al.* 2004b). Overexpression of PRX-Q in tobacco was shown to increase tolerance to fungal diseases (Kiba *et al.* 2005). In Arabidopsis, the accumulation of *PRX-Q* mRNA is increased under high light or oxidative stress and decreased by salt stress, ascorbate, or under low light (Horling *et al.* 2002, 2003). The transcript levels of Chlamydomonas PRX-Q, *CrPRX6*, was reported to be induced in Chlamydomonas cells exposed to copper stress (Jamers *et al.* 2006) but also during sulfur starvation (Zhang *et al.* 2004).

THE GLUTATHIONE PEROXIDASE FAMILY

Phylogenetic analysis of glutathione peroxidases: Phylogenetic studies of the GPX gene family usually distinguish three main groups. Group 1 contains human GPX1 and GPX2; group 2 contains human GPX3, GPX5, and GPX6; and a third group, named the phospholipid hydroperoxide GPX (PHGPX) group, includes human GPX4 and GPXs from various other organisms including plants (Brigelius-Flohé *et al.* 1994; Herbette *et al.* 2007). Mammals contain both selenocysteine-containing GPXs and nonselenium GPXs (NS-GPXs). The analysis of the genome of *C. reinhardtii* revealed the presence of five GPX genes (Table 2) that, according to sequence homology, all belong to the PHGPX group (Figure 4). However, two Chlamydomonas GPXs, CrGPX1 and CrGPX2, are selenoproteins whereas CrGPX3, -4, and -5 are NS-GPXs. This is different from the situation in plants but similar to that of mammals, which contain both selenocysteine-containing GPXs and nonselenium GPXs (NS-GPXs). For example, humans contain five selenoGPXs (GPX1–4, -6) and two NS-GPXs (GPX5, -7). Selenocysteine is a rare amino acid that can be incorporated cotranslationally into proteins (Papp *et al.* 2007). It is encoded by the opal codon UGA, which is not recognized as a stop codon due to the presence of a *cis*-acting mRNA structure called the *se*leno*c*ysteine *i*nsertion *s*equence (SECIS) element. The Se-Cys insertion machinery also requires several *trans*-acting factors including the Se-Cys tRNA (reviewed in Allmang and Krol 2006). Selenoproteins have been identified in archaea, bacteria, and eukaryotes but not in all organisms. There is no overlap between the sets of selenoproteins present in prokaryotes and eukaryotes. Selenoproteins are generally involved in catabolic pathways in prokaryotes while in eukaryotes they participate in biosynthetic pathways or antioxidant processes such as TRX reductase, methionine sulfoxide reductase, or GPX (Herbette *et al.* 2007).

CrGPX1 was the first selenoprotein identified in Chlamydomonas (Fu *et al.* 2002; Novoselov *et al.* 2002). Early studies had indicated that addition of sodium selenite to Chlamydomonas cultures led to a strong increase in GPX activity in total soluble extracts (Yokota *et al.* 1988). Purification of the corresponding protein revealed that the enzyme was associated with selenium and exhibited biochemical properties comparable to mammalian selenoGPXs (Shigeoka *et al.* 1991). Although these studies suggested the existence of selenoGPX in Chlamydomonas, no DNA or protein sequence was determined. The existence of a UGA codon in the coding sequence or the incorporation of Se-Cys in the protein were shown more than 10 years later by two research groups. Cloning of the CrGPX1 cDNA confirmed the presence of an in-frame UGA codon and mass spectrometry demonstrated that this codon allows Se-Cys incorporation into the protein (Fu *et al.* 2002). In the same year, another research group identified 10 selenoproteins in Chlamydomonas and confirmed the existence of a selenocysteyl-tRNA that recognized specifically the UGA codon and showed that Chlamydomonas SECIS elements are similar, but not identical, to those of animals, indicating a common origin for the animal and Chlamydomonas selenoprotein insertion system (Novoselov *et al.* 2002). The newly identified selenoproteins included CrGPX1 but also the second Chlamydomonas selenoGPX, CrGPX2.

NS-GPXs show high sequence similarity with selenoGPXs but the active site selenocysteine is replaced by

TABLE 2

The glutathione peroxidase family in *Chlamydomonas reinhardtii*

Name	Se-Cys	Scaffold	Putative subcellular localization	No. of ESTs	Protein length (aa)
GPX1	Yes	7:731611–733930	Mitochondrion and/or chloroplast	25	200
GPX2	Yes	95:205010–208056	Mitochondrion and/or chloroplast	3	258
GPX3	No	19:607308–609738	Mitochondrion	15	200
GPX4	No	10:678260–680851 or 10:676669–680804	Cytosol or membrane anchored	6 or 8	206 or 536
GPX5	No	4:1975794–1977833	Cytosol (short form), chloroplast (long form)	59	162/211

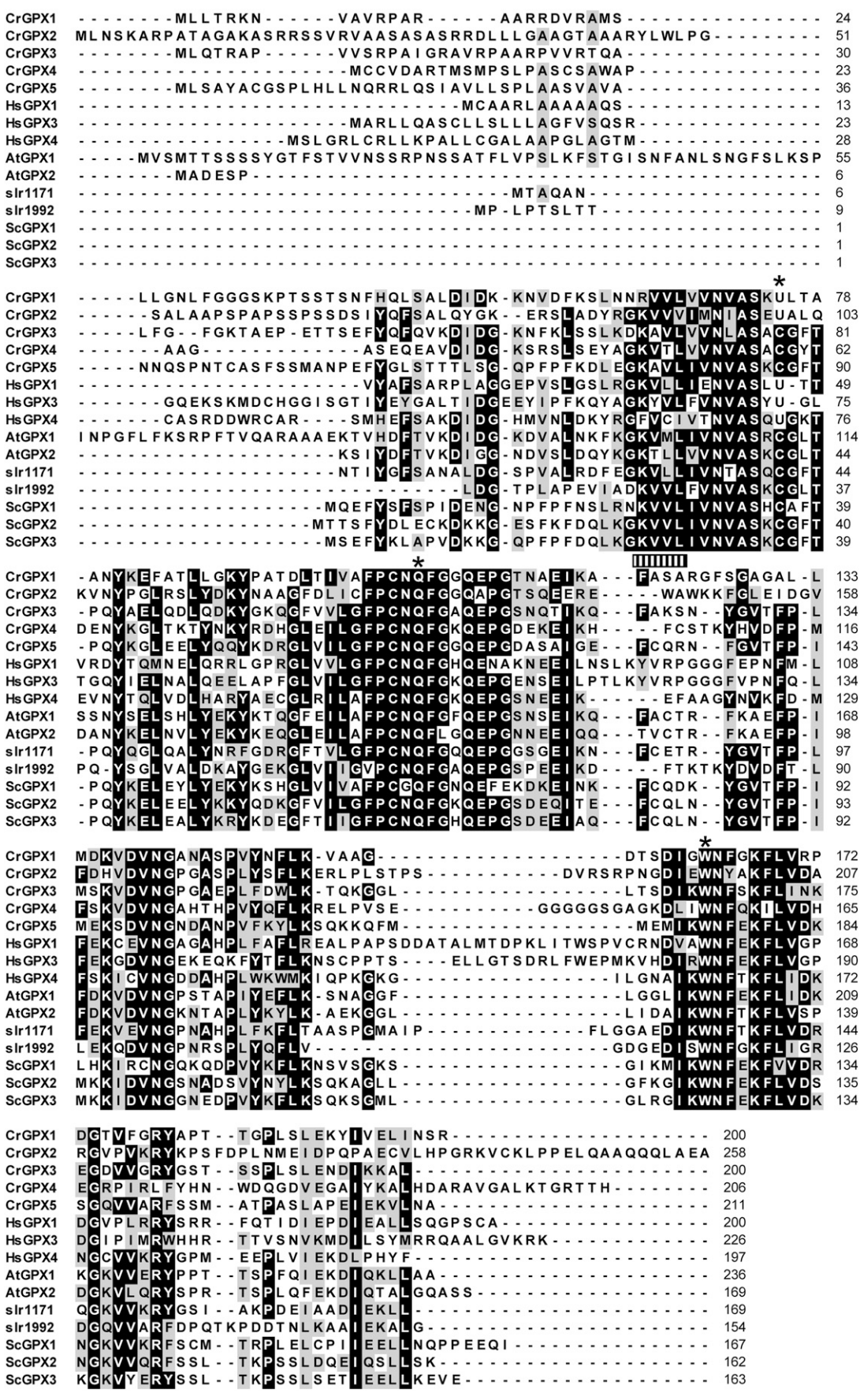
Scaffold indicates the location of the corresponding gene in *Chlamydomonas reinhardtii* genome version 3.0 (http://genome.jgi-psf.org/Chlre3). Subcellular localizations are deduced from the analysis of the protein N-terminal sequence by several prediction programs (Predotar, TargetP, SignalP, and PSORT available at http://www.expasy.ch). The number of ESTs was determined by BLAST alignment of the transcript sequence with NCBI DBest database of November 12, 2007, and supplemented with JGI EST sequences.

a cysteine. Some organisms, such as yeast and higher plants, are devoid of selenoproteins since they do not possess the SECIS machinery. Phylogenetic analyses of selenoGPXs and NS-GPXs from different organisms suggest that Se-Cys utilization has been independently lost during evolution by many groups of organisms, including higher plants, yeast, and some animals (Copeland 2005). It has been suggested that the rise in atmospheric oxygen concentration disfavored the use of the easily oxidized Se-Cys and selected for NS-GPX evolution in plants. The Se-Cys utilization system has probably been lost in plants over a billion years ago, when the Chlorophytes, including Chlamydomonas, diverged from the Streptophytes including land plants and their close relatives (Merchant *et al.* 2007). Consequently, these organisms only contain NS-GPXs. Eight NS-GPXs are present in *A. thaliana* while six have been identified in the genome of *Populus trichocarpa* (Rodriguez Milla *et al.* 2003; Rouhier and Jacquot 2005; Navrot *et al.* 2006). But why did green algae like Chlamydomonas retain the Se-Cys machinery? Unlike higher plants Chlamydomonas is an aquatic organism living in an environment with lower oxygen levels, where the selection pressure against Se-Cys might have been more limited. Recent comparative analysis of selenoprotein-rich and deficient organisms revealed that many aquatic organisms retained their selenoproteome, whereas the selenoproteomes of some terrestrial organisms were reduced or completely lost, suggesting that aquatic life indeed supports selenium utilization (Lobanov *et al.* 2007).

Most NS-GPXs, including those from higher plants, yeast, and cyanobacteria belong to the PHGPX group (Herbette *et al.* 2007). PHGPXs were shown to be able to reduce phospholipid hydroperoxides directly in membranes and are expressed as monomers because they lack subunit interaction sites present in GPXs of the other groups (Thomas *et al.* 1990; Maiorino *et al.* 1991; Imai and Nakagawa 2003). These sites correspond to the gaps visible on GPX sequence alignments (Figure 3). The PHGPX group also contains selenoGPXs, including Chlamydomonas CrGPX1 and CrGPX2 and human GPX4/PHGPX whose sequence was proposed to represent the ancestor of the mammalian GPX gene family (Brigelius-Flohé *et al.* 1994; Herbette *et al.* 2007). However, CrGPX1 and CrGPX2 are not closely related to human selenoGPXs and form a distinct subgroup within the PHGPX group (Figure 4).

The phylogenetic tree of the GPX family presented in Figure 4 suggests that the PHGPX group can be further divided into two distinct groups. The first one (group 3) comprises human GPX4, plant GPXs, and several Chlamydomonas GPXs (CrGPX1, CrGPX2, and CrGPX4). Chlamydomonas GPX4 is related, like higher plant GPXs, to human GPX4, suggesting that it might display PHGPX activity, as already shown for several higher plant NS-GPXs (Herbette *et al.* 2002). The second group (group 4) includes NS-GPXs from Chlamydomonas (CrGPX3 and CrGPX5), yeast, and Synechocystis and can be further subdivided into 2 subgroups (4a and 4b), each containing a Chlamydomonas NS-GPX (Figure 4). Subgroup 4a contains

Figure 3.—Multiple sequence alignment of glutathione peroxidases from *Chlamydomonas reinhardtii* and diverse organisms. The proteins were aligned with the ClustalW program. Residues in solid boxes correspond to 50% identity. Residues in shaded boxes correspond to 50% conservation with the BLOSUM62 matrix. U stands for selenocysteine residues. Accession numbers and abbreviations are as follows: Cr, *Chlamydomonas reinhardtii*, accession numbers as in Table 2; At, *Arabidopsis thaliana* GPX1, At2g25080; GPX2, At2g31570; Hs, *Homo sapiens* GPX1, P07203; GPX3, P22352; GPX4, P36969; Sc, *Saccharomyces cerevisiae* GPX1, P36014; GPX2, P38143; GPX3, P40581; sll or slr, Synechocystis *PCC6803* slr1171; and slr1992. The asterisks indicate the position of the conserved residues involved in the catalytic mechanism of selenoGPX (First Cys, Gln, Trp). The striped bar corresponds to the region containing the second partially conserved cysteine involved in the catalytic mechanism of NS-GPX.


CrGPX1 -------MLLTRKN------VAVRPAR------AARRDVRAMS---------- 24
CrGPX2 MLNSKARPATAGAKASRRSSVRVAASASASRRDLLLGAAGTAAARYLWLPG--------- 51
CrGPX3 -------MLQTRAP------VVSRPAIGRAVRPAARPVVRTQA---------- 30
CrGPX4 ----------------MCCVDARTMSMPSLPASCSAWAP---------- 23
CrGPX5 -------MLSAYACGSPLHLLNQRRLQSIAVLLSPLAASVAVA---------- 36
HsGPX1 ----------------------------MCAARLAAAAAQS---------- 13
HsGPX3 ---------------------MARLLQASCLLSLLLAGFVSQSR---------- 23
HsGPX4 ----------------MSLGRLCRLLKPALLCGALAAPGLAGTM---------- 28
AtGPX1 -----MVSMTTSSSSYGTFSTVVNSSRPNSSATFLVPSLKFSTGISNFANLSNGFSLKSP 55
AtGPX2 -------MADESP---------- 6
sIr1171 ----------------------------MTAQAN---------- 6
sIr1992 ----------------------------MP-LPTSLTT---------- 9
ScGPX1 ---------- 1
ScGPX2 ---------- 1
ScGPX3 ---------- 1
CrGPX1 -----LLGNLFGGGSKPTSSTSNFHQLSALDIDK-KNVDFKSLNNRVVLVVNVASKULTA 78
CrGPX2 ------SALAAPSPAPSSPSSDSIYQFSALQYGK--ERSLADYRGKVVVIMNIASEUALQ 103
CrGPX3 -----LFG--FGKTAEP-ETTSEFYQFQVKDIDG-KNFKLSSLKDKAVLVVNLASACGFT 81
CrGPX4 -------AAG----------ASEQEAVDIDG-KSRSLSEYAGKVTLVVNVASACGYT 62
CrGPX5 -----NNQSPNTCASFSSMANPEFYGLSTTTLSG-QPFPFKDLEGKAVLIVNVASKCGFT 90
HsGPX1 ----------------------VYAFSARPLAGGEPVSLGSLRGKVLLIENVASLU-TT 49
HsGPX3 -------GQEKSKMDCHGGISGTIYEYGALTIDGEEYIPFKQYAGKYVLFVNVASYU-GL 75
HsGPX4 -------CASRDDWRCAR----SMHEFSAKDIDG-HMVNLDKYRGFVCIVTNVASQUGKT 76
AtGPX1 INPGFLFKSRPFTVQARAAAEKTVHDFTVKDIDG-KDVALNKFKGKVMLIVNVASRCGLT 114
AtGPX2 ----------------------KSIYDFTVKDIGG-NDVSLDQYKGKTLLVVNVASKCGLT 44
sIr1171 ----------------------NTIYGFSANALDG-SPVALRDFEGKVLLIVNTASQCGFT 44
sIr1992 ----------------------------LDG-TPLAPEVIADKVVLFVNVASKCGLT 37
ScGPX1 --------------------MQEFYSFSPIDENG-NPFPFNSLRNKVVLIVNVASHCAFT 39
ScGPX2 ------------------MTTSFYDLECKDKKG-ESFKFDQLKGKVVLIVNVASKCGFT 40
ScGPX3 --------------------MSEFYKLAPVDKKG-QPFPFDQLKGKVVLIVNVASKCGFT 39
CrGPX1 -ANYKEFATLLGKYPATDLTIVAFPCNQFGGQEPGTNAEIKA---FASARGFSGAGAL-L 133
CrGPX2 KVNYPGLRSLYDKYNAAGFDLICFPCNQFGGQAPGTSQEERE-----WAWKKFGLEIDGV 158
CrGPX3 -PQYAELQDLQDKYGKQGFVVLGFPCNQFGAQEPGSNQTIKQ---FAKSN--YGVTFP-L 134
CrGPX4 DENYKGLTKTYNKYRDHGLEILGFPCNQFGKQEPGDEKEIKH-----FCSTKYHVDFP-M 116
CrGPX5 -PQYKGLEELYQQYKDRGLVILGFPCNQFGGQEPGDASAIGE---FCQRN--FGVTFP-I 143
HsGPX1 VRDYTQMNELQRRLGPRGLVVLGFPCNQFGHQENAKNEEILNSLKYVRPGGGFEPNFM-L 108
HsGPX3 TGQYIELNALQEELAPFGLVILGFPCNQFGKQEPGENSEILPTLKYVRPGGGFVPNFQ-L 134
HsGPX4 EVNYTQLVDLHARYAECGLRILAFPCNQFGKQEPGSNEEIK------EFAAGYNVKFD-M 129
AtGPX1 SSNYSELSHLYEKYKTQGFEILAFPCNQFGFQEPGSNSEIKQ---FACTR--FKAEFP-I 168
AtGPX2 DANYKELNVLYEKYKEQGLEILAFPCNQFLGQEPGNNEEIQQ---TVCTR--FKAEFP-I 98
sIr1171 -PQYQGLQALYNRFGDRGFTVLGFPCNQFGQQEPGGSGEIKN---FCETR--YGVTFP-L 97
sIr1992 PQ-YSGLVALDKAYGEKGLVIIGVPCNQFGAQEPGSPEEIKD-----FTKTKYDVDFT-L 90
ScGPX1 -PQYKELEYLYEKYKSHGLVIVAFPCGQFGNQEFEKDKEINK---FCQDK--YGVTFP-I 92
ScGPX2 -PQYKELEELYKKYQDKGFVILGFPCNQFGKQEPGSDEQITE---FCQLN--YGVTFP-I 93
ScGPX3 -PQYKELEALYKRYKDEGFTIIGFPCNQFGHQEPGSDEEIAQ---FCQLN--YGVTFP-I 92
CrGPX1 MDKVDVNGANASPVYNFLK-VAAG----------DTSDIGWNFGKFLVRP 172
CrGPX2 FDHVDVNGPGASPLYSFLKERLPLSTPS----------DVRSRPNGDIEWNYAKFLVDA 207
CrGPX3 MSKVDVNGPGAEPLFDWLK-TQKGGL----------LTSDIKWNFSKFLINK 175
CrGPX4 FSKVDVNGAHTHPVYQFLKRELPVSE----------GGGGGSGAGKDLIWNFQKILVDH 165
CrGPX5 MEKSDVNGNDANPVFKYLKSQKKQFM----------MEMIKWNFEKFLVDK 184
HsGPX1 FEKCEVNGAGAHPLFAFLREALPAPSDDATALMTDPKLITWSPVCRNDVAWNFEKFLVGP 168
HsGPX3 FEKGDVNGEKEQKFYTFLKNSCPPTS----ELLGTSDRLFWEPMKVHDIRWNFEKFLVGP 190
HsGPX4 FSKICVNGDDAHPLWKWMKIQPKGKG----------ILGNAIKWNFTKFLIDK 172
AtGPX1 FDKVDVNGPSTAPIYEFLK-SNAGGF----------LGGLIKWNFEKFLIDK 209
AtGPX2 FDKVDVNGKNTAPLYKYLK-AEKGGL----------LIDAIKWNFTKFLVSP 139
sIr1171 FEKVEVNGPNAHPLFKFLTAASPGMAIP----------FLGGAEDIKWNFTKFLVDR 144
sIr1992 LEKQDVNGPNRSPLYQFLV----------GDGEDISWNFGKFLIGR 126
ScGPX1 LHKIRCNGQKQDPVYKFLKNSVSGKS----------GIKMIKWNFEKFVVDR 134
ScGPX2 MKKIDVNGSNADSVYNYLKSQKAGLL----------GFKGIKWNFEKFLVDS 135
ScGPX3 MKKIDVNGGNEDPVYKFLKSQKSGML----------GLRGIKWNFEKFLVDK 134
CrGPX1 DGTVFGRYAPT--TGPLSLEKYIVELINSR---------- 200
CrGPX2 RGVPVKRYKPSFDPLNMEIDPQPAECVLHPGRKVCKLPPELQAAQQQLAEA 258
CrGPX3 EGDVVGRYGST--SSPLSLENDIKKAL---------- 200
CrGPX4 EGRPIRLFYHN--WDQGDVEGAIYKALHDARAVGALKTGRTTH---------- 206
CrGPX5 SGQVVARFSSM--ATPASLAPEIEKVLNA---------- 211
HsGPX1 DGVPLRRYSRR--FQTIDIEPDIEALLSQGPSCA---------- 200
HsGPX3 DGIPIMRWHHR--TTVSNVKMDILSYMRRQAALGVKRK---------- 226
HsGPX4 NGCVVKRYGPM--EEPLVIEKDLPHYF---------- 197
AtGPX1 KGKVVERYPPT--TSPFQIEKDIQKLLAA---------- 236
AtGPX2 DGKVLQRYSPR--TSPLQFEKDIQTALGQASS---------- 169
sIr1171 QGKVVKRYGSI--AKPDEIAADIEKLL---------- 169
sIr1992 DGQVVARFDPQTKPDDTNLKAAIEKALG---------- 154
ScGPX1 NGKVVKRFSCM--TRPLELCPIIEELLNQPPEEQI---------- 167
ScGPX2 NGKVVQRFSSL--TKPSSLDQEIQSLLSK---------- 162
ScGPX3 KGKVYERYSSL--TKPSSLSETIEELLKEVE---------- 163

Chlamydomonas GPX3 and two Synechocystis GPXs. The cyanobacterial enzymes display PHGPX activity and can be reduced by NADPH but not by GSH (Gaber *et al.* 2001). Subgroup 4b contains Chlamydomonas GPX5 (formerly named GPXH) and several yeast NS-GPXs, indicating a stronger homology of CrGPX5 with the yeast proteins than with other Chlamydomonas GPXs.

Subcellular localization of Chlamydomonas glutathione peroxidases: The subcellular localization of Chlamydomonas GPXs has not been determined experimentally and appears difficult to predict on the basis of protein sequence analyses. Several prediction programs suggest that some Chlamydomonas GPXs might be targeted to mitochondria or chloroplasts (Table 2). Both selenoGPXs, CrGPX1 and CrGPX2, harbor N-terminal extensions predicted to target the proteins to mitochondria or chloroplasts, depending on the program used. CrGPX3 exhibits an N-terminal extension predicted to target the protein to mitochondria by most programs. However, proteins that contain an N-terminal extension and that are related to cyanobacterial enzymes, like GPX3, are often targeted to chloroplasts. The N-terminal extension of CrGPX4 is very short, suggesting that the protein is cytosolic although some programs suggest a possible chloroplast localization. An alternate gene structure is also possible for GPX4 (Table 2) and would encode a protein of 536 amino acids where the GPX domain would be fused to an upstream domain containing an N-terminal transmembrane anchor. For CrGPX5/GPXH the situation seems to be even more complex since two forms of CrGPX5/GPXH may be expressed in Chlamydomonas. A short form corresponds to the cDNA originally cloned and encodes a protein with the "MANPE" N-terminal sequence (Leisinger *et al.* 1999). However, some ESTs are longer than the original cDNA and contain an upstream in-frame ATG codon that could allow synthesis of a longer form of GPX5/GPXH with the "MLSAY" N-terminal sequence (Figure 3). Synthesis of this long form would require the translation to start very close to the 5′end of the transcript. The short form is a 162-amino-acid protein and is most probably cytosolic. The long form is a polypeptide of 211 amino acids containing an N-terminal extension predicted to target the protein to the chloroplast. A putative localization of Chlamydomonas GPX isoforms in different subcellular compartments is in agreement with the situation in higher plants, where GPXs are predicted to be present in the cytosol, mitochondria, chloroplasts, or secreted (Rodriguez Milla *et al.* 2003; Navrot *et al.* 2006). In poplar, for example, the targeting of GPX1 to chloroplasts and of GPX3.2 to both chloroplasts and mitochondria has been confirmed experimentally (Navrot *et al.* 2006). Similarly, the targeting of the different Chlamydomonas GPXs to individual compartments will have to be experimentally demonstrated.

Activity of glutathione peroxidases: All GPX isoforms contain three strictly conserved amino acids that are involved in the catalytic mechanism as shown for mammalian selenoGPXs (Maiorino *et al.* 1995). These residues, which are all conserved in the five Chlamydomonas GPX isoforms, are one cysteine, replaced by a Se-Cys in selenoGPXs, a Gln and a Trp (marked by asterisks in Figure 3). The first step of the catalytic mechanism of GPXs involves a primary oxidation of the catalytic Cys or Se-Cys residue to sulfenic or selenenic acid, respectively. In selenoGPXs, nucleophilic attack of the selenenic acid by GSH forms a selenylsulfide that reacts with a second GSH molecule to regenerate a reduced selenol at the Se-Cys residue and liberate oxidized glutathione (GSSG) (Johansson *et al.* 2005). The low pK_a of Se-Cys (pK_a = 5.2) compared to cysteine (pK_a = 8.3) confers a high reactivity to selenoproteins employing the reactivity of Se-Cys during their catalytic cycle, such as GPXs (Johansson *et al.* 2005). Indeed, the selenol group is mainly in its anion selenolate form at physiological pH while the thiol of a Cys residue is only partly ionized, making Se-Cys more reactive than Cys. Consistently, site-directed replacement of Se-Cys by Cys considerably decreases the activity of selenoGPXs (Rocher *et al.* 1992; Maiorino *et al.* 1995). In the case of NS-GPX, the sulfenic acid forms a disulfide with a second cysteine of the enzyme (striped bar in Figure 3) and this disulfide can be reduced in a second step (Navrot *et al.* 2006; Herbette *et al.* 2007). Many studies have shown that most NS-GPXs use thioredoxin rather than glutathione as an electron donor. This TRX dependence has been established for GPXs from *P. falciparum* (Sztajer *et al.* 2001), yeast (Tanaka *et al.* 2005), and Drosophila (Maiorino *et al.* 2007). Human GPX3, a selenoGPX was also shown to use either TRX or GRX as an electron donor (Bjornstedt *et al.* 1994). This is consistent with the localization of this GPX in plasma where the concentration of glutathione is very low. Early studies on plant GPXs from diverse sources had shown that several NS-GPXs could be reduced by TRX (Herbette *et al.* 2002; Jung *et al.* 2002). A systematic analysis demonstrated that all poplar GPXs use TRX rather than GSH as a reductant (Navrot *et al.* 2006). Similar results were also reported for several Arabidopsis GPXs (Iqbal *et al.* 2006). This suggests that in higher plants, all GPX isoforms are reduced by TRX. More generally, most NS-GPXs, despite their sequence homology with selenoGPX, are functionally TRX-dependent peroxidases. Therefore, their catalytic mechanism is similar to the one described for some PRX, like PRX-Q, suggesting that NS-GPXs may be considered as a novel class of PRX as proposed by several groups (Rouhier and Jacquot 2005; Tanaka *et al.* 2005; Herbette *et al.* 2007).

Four types of TRX (f, m, x, and y) are present in the chloroplast of Chlamydomonas (Lemaire *et al.* 2003b) and other photosynthetic organisms (Lemaire *et al.* 2007), but only y-type TRXs appear to be able to reduce

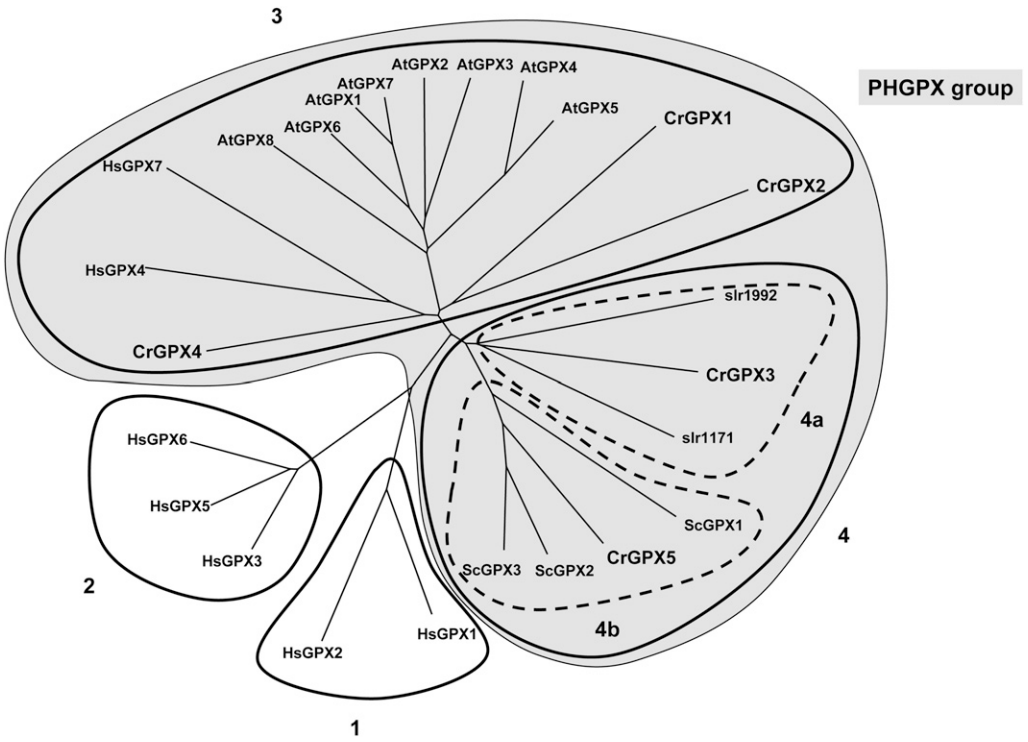


FIGURE 4.—Phylogenetic tree of the glutathione peroxidase families in *Chlamydomonas reinhardtii* and diverse organisms. The unrooted tree was constructed with the ClustalX program. Gaps were excluded. Accession numbers and abbreviations are as follows: At, *Arabidopsis thaliana* GPX1, At2g25080; GPX2, At2g31570; GPX3, At2g43350; GPX4, At2g48150; GPX5, At3g63080; GPX6, At4g11600; GPX7, At4g31870; GPX8, At1g63480; Cr, *Chlamydomonas reinhardtii* as in Table 2; Hs, *Homo sapiens* GPX1, P07203; GPX2, P18283; GPX3, P22352; GPX4, P36969; GPX5, O75715; GPX6, P59796; GPX7, Q96SL4; Sc, *Saccharomyces cerevisiae* GPX1, P36014; GPX2, P38143; GPX3, P40581; sll or slr, Synechocystis *PCC6803* slr1171; and slr1992.

poplar chloroplastic GPXs (NAVROT *et al.* 2006). In *C. reinhardtii*, no GPX activity was tested with TRX so far, but CrGPX5/GPXH was retained on a TRX h1 affinity column loaded with Chlamydomonas soluble extracts (LEMAIRE *et al.* 2004) indicating that, as most NS-GPXs, this GPX is likely reduced by TRX. Indeed, activity measurement using a recombinant CrGPX5/GPXH protein expressed in *E. coli* failed to detect any significant activity with reduced GSH, showing that this was probably the wrong reductant (LEISINGER *et al.* 1999). Furthermore, CrGPX5/GPXH is related to yeast NS-GPXs, which were shown to be efficiently reduced by TRX (TANAKA *et al.* 2005). Conversely, a GPX related to CrGPX5/GPXH, from the halotolerant strain Chlamydomonas sp. *W80* can use GSH as an electron donor to reduce lipid hydroperoxides but not H_2O_2 or phospholipid hydroperoxides (TAKEDA *et al.* 2003). The specific activity of the enzyme was 36-fold higher than for higher plant NS-GPXs. On the other hand, it was >10-fold lower than the activity of mammalian PHGPX enzymes and >100-fold lower than the activity of purified CrGPX1, of which both are SelenoGPXs (FU *et al.* 2002). This shows that the specificity for GSH or TRX is not depending on the active site (Cys or Se-Cys) but rather on the substrate binding capacity. TRXs possess the so-called thioredoxin fold, a conserved secondary structure containing α-helices and β-sheets and a CxxC motif (FOMENKO and GLADYSHEV 2003). A derivative of this motif (CxxT) is found in all Chlamydomonas NS-GPX including CrGPX5/GPXH but is absent in the SelenoGPXs CrGPX1 and CrGPX2. This suggests that GSH might be the substrate for CrGPX1 and CrGPX2, whereas CrGPX3–5 might be reduced by TRX. However, the resolving cysteine involved in the regeneration of NS-GPX by TRX is not strictly conserved in all NS-GPX (striped bar region in Figure 3). This cysteine is absent in CrGPX3, which therefore may not be reduced by TRX. Nevertheless, the residue appears to be present in CrGPX4 and CrGPX5, suggesting that these two latter GPXs are likely to be reduced by TRX.

Expression of glutathione peroxidases: In higher plants, GPX genes appear to be expressed in most tissues, although some accumulate only low mRNA levels (RODRIGUEZ MILLA *et al.* 2003; NAVROT *et al.* 2006). Several plant hormones (salicylic acid, jasmonic acid, abscissic acid, and auxin) could increase GPX mRNA levels in Arabidopsis but with different patterns for each individual gene, suggesting the involvement of multiple signaling pathways (RODRIGUEZ MILLA *et al.* 2003). In Chlamydomonas, several examples suggest that the expression of GPXs is regulated by diverse stress conditions. An induction of *CrGPX1* expression was observed under sulfur starvation (ZHANG *et al.* 2004) and *CrGPX3* and *CrGPX4* transcript levels were shown to be upregulated during manganese deficiency probably due to the resulting oxidative stress (ALLEN *et al.* 2007). Similarly, the mRNA levels of *CrGPX5/GPXH*, which appear to be the most highly expressed GPX on the basis of EST abundance (Table 2), were increased in Chlamydomonas cells exposed to copper stress (JAMERS *et al.* 2006).

The expression of *CrGPX5/GPXH* under different stress conditions has been extensively studied by differ-

ent research groups at the mRNA level (Fischer *et al.* 2006; Ledford *et al.* 2007). The *CrGPX5/GPXH* gene was originally isolated in a screen for Chlamydomonas peroxidase and a strong accumulation of the corresponding transcript was observed in response to oxidative stress (Leisinger *et al.* 1999). A more detailed analysis revealed a specific transcriptional upregulation of *CrGPX5/GPXH* transcripts by singlet oxygen either generated by exogenous photosensitizers such as rose bengal or neutral red (Leisinger *et al.* 2001), evolving in a xantophyll-deficient mutant in the light (Ledford *et al.* 2004), or produced in the photosystem II reaction center during high light illumination (Fischer *et al.* 2006). Other ROS, like superoxide radicals or H_2O_2, only slightly increased the expression of the *CrGPX5/GPXH* gene. *GPX5/GPXH* mRNA levels also slowly but continuously increased upon exposure to organic hydroperoxides like tBOOH or cumene hydroperoxide (CuOOH). It was shown that the induction of the *CrGPX5/GPXH* gene by singlet oxygen and organic hydroperoxides involves two different regulation mechanisms of which one is a transcriptional activation and the other a mRNA stabilization process (Fischer *et al.* 2007a). Since the formation of organic hydroperoxides via lipid peroxidation is stimulated directly by singlet oxygen, it was suggested that *CrGPX5/GPXH* expression is regulated by both primary (singlet oxygen) and secondary effects (lipid hydroperoxides) of high light stress (Fischer *et al.* 2007a). The upregulation by singlet oxygen might be a preventive response to combat expected membrane damage because the CrGPX5/GPXH protein may be involved in the removal of lipid hydroperoxides from membranes. Indeed, acclimation to low concentrations of singlet oxygen or overexpression of the *GPX5/GPXH* gene increased the resistance of *C. reinhardtii* to photooxidative stress conditions (Ledford *et al.* 2007). Similarly, overexpression of a CrGPX5 homologous protein from Chlamydomonas W80 in tobacco plants reduced the level of unsaturated fatty acid hydroperoxides and thus enhanced tolerance to oxidative stress (Yoshimura *et al.* 2004). Furthermore, the *CrGPX5/GPXH* gene seems to encode both a chloroplastic and a cytosolic protein, indicating a role in defense against oxidative stress in multiple cellular compartments. Recent findings showed that singlet oxygen produced in the photosystem II reaction center can diffuse as far as into the cytoplasm of Chlamydomonas exposed to very high light intensity, which could explain the strong induction of a nuclear gene like the long form of *CrGPX5/GPXH* under this condition (Fischer *et al.* 2007b).

PHYSIOLOGICAL FUNCTION OF GPX AND PRX

Due to their ability to reduce H_2O_2 and organic hydroperoxides, PRXs and GPXs were considered to be mainly involved in protection of cells from oxidative stress caused by the formation of reactive oxygen species during normal metabolism or harsh environmental conditions. In Chlamydomonas, chloroplastic CrPRX1 was shown to protect DNA against ROS-induced degradation and to detoxify hydroperoxides using chloroplastic TRXs as reductants (Goyer *et al.* 2002; S. D. Lemaire, unpublished results). Thus, similar to Arabidopsis 2-Cys PRXs, CrPRX1 might be involved in the protection of the chloroplast from increased ROS formation (Baier and Dietz 1999). Overexpression of the CrGPX5/GPXH protein in Chlamydomonas increased the tolerance against photooxidative stress (Ledford *et al.* 2007) and transgenic tobacco plants overexpressing a GPX protein from a Chlamydomonas sp. *W80* strain were more resistant to oxidative stress caused by paraquat treatment, chilling, and osmotic stress (Yoshimura *et al.* 2004). All these data suggest that also in *C. reinhardtii*, PRXs and GPXs play a major role in the protection of the cell from ROS-induced damage and oxidative stress. However, this raises the question of why Chlamydomonas has seven members of the PRX and five of the GPX family of which two are selenoGPXs, which exhibit a much higher peroxidase activity than NS-GPXs. In higher plants, PRXs and GPXs generally have a low specific activity compared to other cellular peroxidases, such as catalase or ascorbate peroxidases. In the case of PRXs, this lower activity could be compensated by their high affinity for peroxides and their high abundance. Indeed, several PRX isoforms, and especially 2-cys PRXs, are abundant proteins expressed at a high level in plants. Although there may be some discrepancies between mRNA and protein levels, the analysis of EST abundance suggests that 2-cys PRXs are also expressed at high levels in Chlamydomonas while it does not seem to be the case for GPX genes, except *GPX5/GPXH*, which is more highly expressed than other GPXs (Tables 1 and 2). The different GPX and PRX isoforms are located in diverse subcellular compartments and their expression appears to be upregulated under stress conditions. Thus, the level of these ROS scavengers might be even further increased under particular conditions when more ROS are produced. All together, these data suggest that a specific expression and localization of the PRX and GPX proteins in the cell might be a reason for the presence of multiple members of these gene families in one organism.

Another explanation for the presence of multiple members of related gene families in one organism might be linked to different substrate specificities. Compared to other peroxidases, PRXs and GPXs can reduce a broader range of substrates. Therefore, their physiological function may be related to their ability to scavenge reactive species that are not efficiently detoxified by other types of peroxidases. PRXs, for example, can reduce peroxynitrite (Wong *et al.* 2002; Sakamoto *et al.* 2003) while some GPXs can reduce phospholipids and lipid hydroperoxides (Avery and

Avery 2001; Herbette *et al.* 2002; Takeda *et al.* 2003). Several NS-GPXs have been shown to scavenge lipid or phospholipid hydroperoxides *in vivo* in Synechocystis (Gaber *et al.* 2001) and yeast (Avery *et al.* 2004). By contrast, CrGPX1 showed the highest activity with H_2O_2 and could also reduce tBOOH and CuOOH, but not lipid hydroperoxides (Fu *et al.* 2002). All these data suggest that GPXs play a role in the protection against lipid peroxidation and thereby in the maintenance of membrane integrity. Structural studies on poplar GPX5 suggest that this enzyme might interact with Cd^{2+} and could therefore play a role as Cd^{2+} sink in defense against heavy metals (Koh *et al.* 2007). Increased levels of GPX in poplar leaves exposed to high concentrations of cadmium and copper (Navrot *et al.* 2006) and higher tolerance to cadmium of yeast cells overexpressing the GPX3 protein also support a putative role of GPXs in heavy metal tolerance (Avery *et al.* 2004). Still, it is not clear whether this increased tolerance is due to a stimulated oxidative stress defense or to direct detoxification of Cd^{2+} by GPX. GPXs have also been suggested to play a role in defense/response to adverse conditions, sexual reproduction, and host pathogen interaction (reviewed in Herbette *et al.* 2007). Once again, some of these functions may indeed be linked to the peroxidase activity of GPXs.

Besides their antioxidant function, PRXs and GPXs could also have a signaling function. ROS not only are toxic molecules that have to be rapidly scavenged to protect cellular constituents from oxidative damage but also are intracellular messengers that can be produced on purpose and play a key role in signaling pathways of several physiological processes (Apel and Hirt 2004; Laloi *et al.* 2004; Hancock *et al.* 2006; Pitzschke *et al.* 2006; D'Autréaux and Toledano 2007). Thus, controlling the intracellular ROS levels is essential to reach a balance between optimal cellular response and minimal stress. No direct involvement of plant PRXs in ROS signaling has been proven so far but, in mammals, 2-cys PRXs have been suggested to play an important role in H_2O_2 signaling (reviewed in Rhee *et al.* 2005a). During the catalytic cycle, the peroxidatic cysteine of 2-cys PRXs can become overoxidized to sulfinic acid (Rabilloud *et al.* 2002; Yang *et al.* 2002). However, this overoxidation is not irreversible since an enzyme named sulfiredoxin (SRX) can reduce hyperoxidized PRX (Biteau *et al.* 2003; Chang *et al.* 2004). Reduction of sulfinic acid by SRX is specific to 2-cys PRX isoforms (Woo *et al.* 2005). Since the reduction of overoxidized 2-cys PRX by SRX is a slow process, 2-cys PRXs can become transiently inactivated, thereby allowing H_2O_2 to accumulate above the critical level to function as a signal activating downstream responses (Wood *et al.* 2003a). Higher plants also contain 2-cys PRX and SRX in chloroplasts where they might play a role in H_2O_2 signaling, chloroplasts being major sites of production of ROS in the light under normal conditions (Liu *et al.* 2006; Rey *et al.* 2007). A putative SRX gene, more closely related to animal SRX than to higher plant SRXs, was identified in the genome of Chlamydomonas.

A role as intermediate in signal transduction has been demonstrated for several NS-GPXs. In *S. cerevisiae*, GPX3 was shown to be a sensor and transducer of the H_2O_2 signal to the transcription factor Yap1 (Delaunay *et al.* 2002). Upon increased production of H_2O_2 inside the cell, the oxidized GPX3 protein interacts with the transcription factor Yap1 forming an intermolecular disulfide bond that is resolved into an intramolecular disulfide bond in Yap1. Once Yap1 regulatory cysteines are oxidized by GPX3, Yap1 is activated, accumulates in the nucleus, and activates the expression of antioxidants and components of the cellular thiol-reducing pathways (Lee *et al.* 1999; Delaunay *et al.* 2000, 2002). Deactivation of Yap1 is mediated by TRX reduction of the regulatory disulfide, which leads to accumulation of the transcription factor in the cytosol (Kuge *et al.* 2001). *S. cerevisiae* GPX3 may also regulate the activity of methionine-sulfoxide reductase (Kho *et al.* 2006). In Arabidopsis, an NS-GPX was recently shown to also function as a redox transducer by regulating the activity of a protein phosphatase 2C in response to drought stress and abscisic acid (Miao *et al.* 2006).

Overall, these data suggest that GPXs and PRXs may have important roles in signal transduction, control of enzyme activity, and regulation of gene expression. Both types of enzymes may function as thiol oxidoreductases playing a role in redox signaling and oxidant scavenging, just like TRXs and GRXs. In photosynthetic organisms, proteomic approaches have allowed identification of nearly 300 putative targets of TRX and GRX (Buchanan and Balmer 2005; Rouhier *et al.* 2005; Michelet *et al.* 2006; Lemaire *et al.* 2007). In the coming years, similar approaches may be crucial to unravel the cellular targets and the multiple functions of PRXs and GPXs in Chlamydomonas and other organisms.

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