Review

Glutaredoxins in fungi

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Abstract

Glutaredoxins (GRXs) can be subdivided into two subfamilies: dithiol GRXs with the CPY/FC active site motif, and monothiol GRXs with the CGFS motif. Both subfamilies share a thioredoxin-fold structure. Monothiol GRXs exist with a single Grx domain while others have a thioredoxin-like domain (Trx) and one or more Grx domains in tandem. Most fungi have both dithiol and monothiol GRXs with different subcellular locations. GRX-like molecules also exist in fungi that separate in one residue from one of the canonical active site motifs. Additionally, Omega-class glutathione transferases are active as GRXs. Among fungi, the GRXs more extensively studied are those from *Saccharomyces cerevisiae*. This organism contains two dithiol GRXs (ScGrx1 and ScGrx2) with partially overlapping functions in defence against oxidative stress. In this function, they cooperate with glutathione transferases Gtt1 and Gtt2. While ScGrx1 is cytosolic, two pools exist for ScGrx2, a major one at the cytosol and a minor one at mitochondria. On the other hand, *S. cerevisiae* cells have two monothiol GRXs with the Trx-Grx structure (ScGrx3 and ScGrx4) that locate at the nucleus and probably regulate the activity of transcription factors such as Aft1, and one monothiol glutaredoxin with the Grx structure (ScGrx5) that localizes as the mitochondria matrix, where it participates in the synthesis of iron-sulfur clusters. The function of yeast Grx5 seems to be conserved along the evolutionary scale.

Key words: glutaredoxin, glutathione transferase, iron-sulfur cluster, oxidative stress, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, thioredoxin, transcriptional regulation

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GRX, glutaredoxin; GSH, reduced glutathione; HEDS - β -hydroxyethyl disulfide

Introduction

Glutaredoxins (GRXs) were originally defined as small (aprox. 10 kDa) heat-stable proteins active as thiol transferases, that are responsible for the reduction of protein disulfides or glutathione-protein mixed disulfides. The reaction involves reduced glutathione (GSH) as hydrogen donor, with the participation of cysteine residues of the enzyme active site (Holmgren 1989; Holmgren and Aslund 1995). Initial observations on GRXs demonstrated their ability to reduce ribonucleotide reductase in *Escherichia coli*, although the list of biological activities in which GRXs are involved through their enzymatic activity has now grown considerably (Fernandes and Holmgren 2004). In addition to the GRX enzyme, the glutaredoxin system also comprises NADPH, GSH and glutathione reductase. Electrons flux from NADPH to glutathione reductase, then to GSH, and finally to the GRX molecule. Classical GRXs usually have the conserved CPYC motif (CPFC in some molecules) at the active site, with these two cysteines playing an essential role in the reaction. For this reason they are also called dithiol GRXs. More recently, a second subfamily of GRXs has been described, based on initial studies in the yeast *Saccharomyces cerevisiae* (Rodríguez-Manzanque et al. 1999). These contain an active site with the CPFS motif, in which the single cysteine residue is required for the biological activity of the protein, without the absolute requirement for other cysteine residues; for those reasons they have been called monothiol GRXs. GRX molecules of both classes have a 'thioredoxin-fold' domain structure, in common with other protein families such as thioredoxins, protein disulfide isomerases, glutathione peroxidases and glutathione transferases (Martin 1995). The thioredoxin-fold domain is formed by a four or five-stranded β -sheet flanked by three or more α -helices on either side of the β -sheet. Thioredoxins form part of a second system regulating the protein redox state, their functions overlapping in part with those of glutaredoxins (Holmgren 1989; Vlamis-Gardikas and Holmgren 2002). In the

thioredoxin system, NADPH acts directly as hydrogen donor, with the participation of thioredoxin reductase to regenerate active thioredoxin.

Dithiol and monothiol GRXs are widely present in prokaryotic and eukaryotic organisms, both unicellular and pluricellular ones (Bellí et al. 2002; Fernandes and Holmgren 2004; Lemaire 2004; Vilella et al. 2004). Comparative analysis of sequenced genomes has revealed that other GRX-like molecules are present in a variety of organisms. The GRX-like molecules do not contain the conserved active site motifs of dithiol or monothiol GRXs, but still display extensive homology with these and have a putative thioredoxin-fold domain in the molecule (Lemaire 2004; Rouhier et al. 2004; Deponte et al. 2005). This fact points to the evolutionary and functional complexity of the GRX family. Many of the recent functional studies on dithiol and monothiol GRXs have been done in *S. cerevisiae* because of the facilities that this organism provides to carry out *in vitro* and *in vivo* approaches in parallel. Review on *S. cerevisiae* GRXs (especially on dithiol ones) and their role in protein redox regulation has been published recently (Carmel-Harel and Storz 2000; Wheeler and Grant 2004). Here we focus our attention on the dithiol and monothiol GRXs of *S. cerevisiae* and other yeasts, particularly on the more recent studies on them, and we also make a comparative analysis of GRX-like proteins in fungal species in general, on the basis of completed genome sequencing projects.

GRXs and GRX-like molecules in fungi

In silico analysis of dithiol GRXs

GSH is present in yeasts and filamentous fungi at intracellular concentrations of 10 mM or even higher, participating in processes such as defence against oxidative and other external stresses, detoxification of heavy metals and xenobiotics, regulation of signal transduction pathways and other metabolic processes, or maintenance of different cell structures (Pocsi et al. 2004). In such diverse biological functions, GSH acts as redox

donor for enzymes such as glutathione peroxidases, glutathione transferases or glutaredoxins among others.

Because of the general character of GSH as redox regulator in fungi, it was expectable that GRXs also would be widespread among fungi. A number of fungal genomes have been totally or partially sequenced (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/genomes; and Fungal Genome Initiative, www.broad.mit.edu). Therefore, we made a BLASTp search for fungal molecules with the sequence characteristics of classical dithiol GRXs (that is, with the CPY/FC active site sequence), using the glutaredoxin 1 of *S. cerevisiae* (ScGrx1) as query sequence. Dithiol GRXs with large amino acid homology to ScGrx1 are present in fungal species covering a wide evolutionary range (Figure 1). A number of residues that are essential for the active three-dimensional conformation of other dithiol GRXs (Xia et al. 1992; Sun et al. 1998; Nordstrand et al. 1999) are conserved in the fungal molecules. This is the case for the lysine residue proximal to the active site (position 24 in ScGrx1), that is required for interaction with the GSH molecule, and also the two consecutive glycine residues at the C-region (positions 86 and 87 in Grx1) that are needed for the formation of the GSH cleft of the GRX molecule. Sequence divergence occurs mainly at both ends of the molecules. In some organisms, long N-terminal extensions exist in these GRX molecules (Figure 1). Several fungal species contain more than one classical dithiol GRX, including *S. cerevisiae*. *Candida albicans* cells have three putative dithiol GRX. *Schizosaccharomyces pombe* Grx2 (SpGrx2) and also one (XP720835) of the three *C. albicans* molecules deviate from the consensus active site sequence, which is CPFC in them. This same sequence has been found in dithiol GRXs from pig liver (Gan and Wells 1987) and vaccinia virus (Johnson et al. 1991). *In silico* analyses were done for predicting the subcellular locations of the listed dithiol GRXs (Table 1). In addition, location of the *S. cerevisiae* and *S. pombe* dithiol GRXs has been experimentally determined (see next section). In several cases no significant prediction results were reached, while in others more than one location could be proposed. For those GRXs

for which a single location was unequivocally proposed, there were representatives of cytosolic, nuclear and mitochondrial molecules (Table 1). It should be remarked that more one location is in fact feasible for GRXs, as has been experimentally demonstrated for *S. cerevisiae* Grx2 (ScGrx2) (Pedrajas et al. 2002). Of the three species for which a mitochondrial dithiol GRX is proposed in Table 1, *A. gossypii* and *C. neoformans* cells do not seem to have additional dithiol GRXs; therefore, either there are two intracellular pools of the respective GRX, with one of these pools at the cytosol (as occurs with ScGrx2), or there are not dithiol GRXs in other cell compartments besides mitochondria.

Some dithiol GRXs with measurable thiol transferase activity deviate from the consensus active site motif CPY/FC. This is the case for human glutaredoxin 2 (HsGrx2), which contains the sequence CSYC at the active site. This GRX has two isoforms derived from alternative exon usage; one isoform is targeted to the mitochondria while the other one is located at the nucleus (Lundberg et al. 2001; Gladyshev et al. 2001). This unusual active site results in lower thiol transferase activity on the standard enzyme substrate β -hydroxyethyl disulfide (HEDS), although the enzyme is effective in the monothiol (deglutathionylation) reaction (Lundberg et al. 2001). Recently, it has been demonstrated that HsGrx2 contains an iron/sulphur cluster that acts as sensor of oxidant conditions; degradation of the cluster under oxidative stress causes activation of the protein (Lillig et al. 2005). None of the fungal proteins displaying homology with known dithiol GRXs have the CSYC motif. However, *S. cerevisiae* and evolutionarily close fungal species contain open reading frames coding for ca. 100 amino acids length proteins with a conserved CPDC motif and displaying moderate homology with classical dithiol GRXs along the entire length (Figure 2). For these GRX-like molecules a cytosolic location is predicted based on the WoLF PSORT and PSORT II programs, except for *A. gossypii* AAS53948, which could be targeted to the mitochondria. None of these putative proteins has been enzymatically or functionally analysed, but their existence in yeast organisms widens the protein types

containing a CxxC motif whose primary structure resembles that of experimentally studied dithiol GRXs.

In silico analysis of monothiol GRXs

Monothiol GRXs with the CGFS active site can be divided into two classes: those with a single glutaredoxin domain [Grx class, *S. cerevisiae* Grx5 (ScGrx5) as representative] and those with an N-terminal thioredoxin-like domain followed by a glutaredoxin domain [Trx-Grx class, *S. cerevisiae* Grx3 and Grx4 (ScGrx3, ScGrx4) and human PICOT protein as representatives] (Witte et al. 2000; Bellí et al. 2002; Vilella et al. 2004). A BLASTp search was carried out among fungal proteins using ScGrx5 as query. Homologues pertaining to the Grx and Trx-Grx classes with the CGFS motif are present in a wide range of fungal species. Figure 3 shows a multiple alignment of the single-Grx domain proteins. Homology extends along most of the amino acid sequence, except for the N-terminal region. In ScGrx5 the initial 29 amino acids correspond to a mitochondrial targeting sequence (Rodríguez-Manzaneque et al. 2002). Since there is not strict sequence conservation for mitochondrial targeting signals, we suspected that by analogy to ScGrx5, the fungal proteins in Figure 3 could be mitochondrially located. Location prediction analysis using the WoLP PSORT and TargetP 1.1 programs indicated with high probability that all those proteins would be mitochondrial, except for *A. fumigatus* XP752229. Therefore, it seems that evolutionary distant fungal species have conserved a mitochondrial CGFS monothiol glutaredoxin of the Grx class.

The Trx-Grx class of molecules is also widespread among fungal species (Table 2). Taking ScGrx3 and ScGrx4 as reference (Molina et al. 2004), all the sequences listed share with them the presence of an N-terminal region that is reminiscent of a thioredoxin sequence and which includes a WAxPC motif. Most of the molecules are predicted to be cytosolic (Table 2). This is however not the case for the Trx-Grx molecules which have been experimentally studied and determined to be

located at the nucleus: ScGrx3 and ScGrx4 (Lopreiato et al. 2004; Molina et al. 2004) and *S. pombe* Grx4 (SpGrx4) (Chung et al. 2005).

BLASTp search against dithiol ScGrx1 also reveals a number of fungal sequences with some homology to the *S. cerevisiae* proteins (E values $<10^{-2}$) which contain a CPxS motif, with x being Y, F, L or H (Table 3). The motif is reminiscent of the dithiol GRX site CPYS. These putative proteins have a common GRX-like module with a variable N-terminal extension that does not display similarity to known sequences. In most of these proteins, the N-terminal region includes a transmembrane domain positioned at the extreme of the polypeptide that is part of a processed signal peptide or acts as an unprocessed membrane anchor (Table 3). Thus, the sequences probably correspond to secreted or membrane-associated proteins. Figure 4 shows the alignment of three of these proteins with the ScGrx1 and ScGrx2 sequences. The *S. cerevisiae* product CAA84956 (coded by ORF *YBR014c*) has the CPYS motif reminiscent of the CPYC sequence. *In vitro* studies with dithiol GRXs mutated in their active site (second cysteine changed by serine) have shown that these derivatives keep their deglutathionylating activity (Bushweller et al. 1992). This opens the possibility that the proteins listed in Table 3 represent a class of GRX-like molecules with hybrid properties between dithiol and monothiol GRXs.

Summarizing, analysis of fungal genomes reveals the diversity of proteins whose sequences contain the core Grx module. Thus, besides the standard dithiol and monothiol GRXs respectively with the active site motifs CPYC and CGFS, other GRX-like proteins exist that await functional characterization and have cysteine-containing motifs that deviate in one residue from the dithiol or monothiol standard motifs. The N-terminal cysteine is nevertheless conserved in all cases.

Dithiol glutaredoxins of *S. cerevisiae* and other fungi

From the initial studies in *E. coli* dithiol GRXs and their role in regulating activity of ribonucleotide reductase [reviewed by Holmgren (1989)], many studies have been done on bacterial and eukaryotic molecules of this class. Functional studies on fungal dithiol GRXs are more recent and have focused on *S. cerevisiae* and *S. pombe*.

Expression and function of *S. cerevisiae* dithiol GRXs

S. cerevisiae possesses two dithiol GRXs (ScGrx1 and ScGrx2) with the CPYC active site motif (Luikenhuis et al. 1998) (Figure 5). Although their primary sequences share 64% identity, their functions are only partially overlapping. Thus, ScGrx1 confers protection against the superoxide anion and hydroperoxides, while ScGrx2 is specialized in the protection against hydroperoxides (Luikenhuis et al. 1998). Expression of the corresponding genes is induced by several stress conditions under the control of STRE elements located at the respective gene promoters (Grant et al. 2000). Transcriptional factors Msn2 and Msn4 that recognise the STRE elements mediate expression of *GRX1* and *GRX2* (Gash et al. 2000). However, differences are again observed between both genes. Thus, hydroperoxide stress induces expression of *GRX1* and *GRX2* at about similar levels, while *GRX1* expression is higher compared to *GRX2* in response to osmotic and heat stress. On the contrary, stationary phase entry causes higher upregulation of *GRX2* compared to *GRX1* (Grant et al. 2000). This partial functional specialization of both GRXs might relate to differential location. While both ScGrx1 and ScGrx2 molecules are present at the cytosol, a fraction of Grx2 molecules also localize at the mitochondria, probably due to alternative translation initiation for the two cytosolic and mitochondrial ScGrx2 forms (Pedrajas et al. 2002).

ScGrx1 and ScGrx2 also could be involved in resistance against chemicals such as the herbicide 2,4-dichlorophenoxyacetic acid, that generates the highly oxidant hydroxyl radicals (Teixeira et al. 2004). This widens the spectra of oxidant species

against which ScGrx1 and/or ScGrx2 exert protection in *S. cerevisiae* cells. The role of the two Grx proteins in oxidative stress defence can be explained by the observation that both of them have glutathione peroxidase activity in *in vitro* assays (Collinson et al. 2002; Collinson and Grant 2003). In accordance with this, *in vivo* overexpression of ScGrx1 or ScGrx2 confers protection against hydrogen peroxide and organic hydroperoxides (*tert*-butyl hydroperoxide and cumene hydroperoxide) in a manner that is dependent on glutathione transferases Gtt1 and Gtt2 (Collinson et al. 2002). Glutathione transferases (GSTs) conjugate GSH to a variety of exogenous and endogenous electrophilic compounds, including environmental xenobiotics, anticancer drugs and products of oxidative stress (Sheehan et al. 2001; Hayes et al. 2005). GSH-conjugated molecules are then excreted or transported into vacuoles, resulting in detoxification of the respective chemicals. The primary sequence of the two *S. cerevisiae* GSTs Gtt1 and Gtt2 diverges significantly from most GST classes, although they display significant enzyme activity against standard GST substrates (Choi et al. 1998). The complex relationship between Grx and Gtt proteins in *S. cerevisiae* with respect to oxidative stress responses is complicated by the fact that ScGrx1 and ScGrx2 are also active as GSTs in a manner that is dependent on the most N-terminal of the two cysteines at the active site (Collinson and Grant 2003). The ScGrx1 and ScGrx2 GRXs would act cooperatively with Gtt1 and Gtt2 GSTs in the defence against hydroperoxides, as the quadruple mutant *grx1 grx2 gtt1 gtt2* displays enhanced sensitivity to several hydroperoxides compared with the double *grx1 grx2* or *gtt1 gtt2* mutants (Collinson and Grant 2003). The precise role of the glutaredoxins and GSTs in the defence mechanism is not known, although ScGrx1/ScGrx2 could convert the hydroperoxides into the corresponding alcohols. These could then be conjugated to GSH by the Gtt proteins, to be finally transported into the vacuole and detoxified (Collinson et al. 2002). Interestingly, *S. cerevisiae* cells have not standard glutathione peroxidases. Instead, they contain three phospholipid hydroperoxide glutathione peroxidases (Gpx1-3) that have activity on phospholipid hydroperoxides but also on

non-phospholipid hydroperoxides (Avery and Avery 2001). The cooperativity between ScGrx1/ScGrx2 and the GSTs Gtt1 and Gtt2 is also manifested by the growth deficiency of the quadruple mutant in the presence of the xenobiotic compound 1-chloro-2,4-dinitrobenzene (CDNB) (Collinson and Grant 2003). This mutant phenotype would demonstrate the GST activity of ScGrx1/ScGrx2 on CDNB in wild type cells followed by elimination of the glutathionylated derivative. Alternatively, the phenotype could be the indirect consequence of GSH depletion in CDNB-treated mutant cells thus resulting in intracellular oxidative stress.

According to the above studies, the role of *S. cerevisiae* dithiol GRXs in oxidative stress defence would occur through their glutathione peroxidase activity. In contrast to bacterial dithiol GRXs (Holmgren 1989, Fernandes and Holmgren 2004), no specific targets for the thiol oxidoreductase activity of ScGrx1 and ScGrx2 have been described up to now. However, both proteins would carry out important redox functions during normal growth in the absence of an external stress, since at least one of them or one of the two cytosolic thioredoxins (Trx1, Trx2) is required for viability of *S. cerevisiae* cells (Draculic et al. 2000). Also, mutants lacking both glutathione reductase and thioredoxin reductase are inviable (Trotter and Grant 2003). These facts point to overlapping constitutive functions between the redox-regulating thioredoxin and glutaredoxin systems. Nevertheless, important differences exist between the two systems in relation to intracellular redox homeostasis. Using the intracellular glutathione redox potential [which is related to the ratio between GSH and oxidized (GSSG) glutathione] as a measure of the redox cell environment, it has been shown that simultaneous lack of Trx1 and Trx2 causes a more dramatic shift towards an intracellular oxidized state that simultaneous lack of ScGrx1 and ScGrx2 (Draculic et al. 2005). Thus, a *grx1 grx2* mutant maintains a GSH/GSSG ratio close to wild type cells, although the absolute concentration of them both in the mutant approximately doubles that of wild type cells.

The arsenate reductase Acr2 is an *in vitro* target of ScGrx1; in the reaction, GSH would act as electron donor for the conversion of As(V) into As(III), with the formation of an intermediate mixed disulfide between Acr1 and oxidized glutathione (Mukhopadhyay et al. 2000). The GRX molecule would finally restore active Acr2 by deglutathionylating the molecule, through a monothiol reaction mechanism involving only the N-terminal cysteine of the GRX. Since this reaction can also be carried out by heterologous *E. coli* GRXs (Mukhopadhyay et al. 2000), the physiological significance of the *in vitro* observation waits studying the sensitivity of *grx1* and/or *grx2* mutants to arsenate. With respect to another heavy metal, cadmium, no role seems to have ScGrx1/ScGrx2 in defence against it (Rai and Cooper 2005), in spite of the fact that GSTs have been reported to participate in heavy metal detoxification (Hayes et al. 2005).

Dithiol GRXs in other fungi

Among other fungal species, only the classical dithiol GRXs of *S. pombe* have been studied in some detail. Fission yeast also contains two dithiol GRXs, SpGrx1 and SpGrx2 (Cho et al. 2000; Chung et al. 2004). While SpGrx1 contains the conserved active site CPYC motif, SpGrx2 contains the rarer CPFC motif. The two *S. pombe* GRXs share 35% identity each other, and are almost 40% identical to the *S. cerevisiae* species. As for *S. cerevisiae*, the two fission yeast GRXs exhibit some specificity in the defence against oxidants. Thus, the *grx1* null mutant is hypersensitive to hydrogen peroxide, while the *grx2* mutant has increased sensitivity to superoxide (Chung et al. 2004). Expression of the *grx1*⁺ gene is upregulated by oxidative and saline stresses and by heat shock, while expression of *grx2*⁺ remains almost unchanged in these conditions (Chung et al. 2004; Kim et al. 2005). On the other hand, glucose depletion upregulates expression of *grx2*⁺ (Kim et al. 2005a). The Sty1 MAP kinase pathway acting on the Atf1 and Pap1 transcription factors participates in the regulatory response of *grx1*⁺ and *grx2*⁺ against the several stresses, a situation that differs from *S.*

cerevisiae, where no MAP kinase pathway has been shown to regulate *GRX1* and *GRX2* expression. While SpGrx1 is clearly cytosolic, discrepancies exist about the subcellular location of SpGrx2. One study (Chung et al. 2004) proposed a mitochondrial location for SpGrx2, while the other study (Kim et al. 2005a) proposed a nuclear location. None of these two studies demonstrated the existence of a cytosolic pool of SpGrx2 molecules. In summary, while the role in the defence against oxidants seems to be well conserved between the classical dithiol GRXs of two species as separated in evolution as *S. cerevisiae* and *S. pombe*, other aspects related to regulatory pathways of expression and perhaps also to subcellular location have diverged between both yeasts.

Monothiol glutaredoxins in *S. cerevisiae* and other fungi

Monothiol GRXs with the CGFS active site motif are conserved along evolution (Bellí et al. 2002; Herrero and Ros, 2002; Lemaire 2004; Vilella et al. 2004). As above indicated, some of them are of the single Grx type, while others are of the Trx-Grx type. ScGrx5 and ScGrx3/ScGrx4 are representatives of both types. In addition, some monothiol GRXs contain two or more domains in tandem (Bellí et al. 2002).

Because monothiol GRXs were identified more recently than dithiol GRXs, very few of them have been characterized and consequently little is known about the substrates and biochemical reactions catalyzed by the members of this group. None of them are active in the glutathione-HEDS mixed disulfide assay, which measures the ability to reduce mixed disulfides formed between glutathione and HEDS (Tamarit et al. 2003; Rahlfs et al. 2001; Deponate et al. 2005; Fernandes et al. 2005). However, mutant dithiolic GRXs remain active in the glutathione-HEDS assay when the second conserved cysteine residue of the active site is replaced by a serine (Bushweller et al. 1992). This fact suggests that monothiol and dithiol GRXs play different roles in the cell. ScGrx5 is the most studied monothiol GRX. *In vitro* studies have shown that this

protein can reduce glutathiolated carbonic anhydrase. This reaction is initiated by the formation of a mixed disulfide between GSH and Cys60, located in the CGFS motif, and results in the formation of a disulfide bond between Cys60 and a second non-conserved cysteine (Cys117) located near the putative glutathione binding site (Tamarit et al. 2003). The significance of this disulfide bond is still not clear, as Cys117 is not essential for the biological function of ScGrx5 and is not conserved in all the members of the group (Belli et al. 2002; Molina-Navarro et al. 2006). However, formation of this disulfide bond has also been observed in the ScGrx5 homologue from *E. coli* (EcGrx4) (Fernandes et al. 2005). The reducer of both proteins is also not clear. GSH can reduce the intramolecular disulfide bond of ScGrx5, but at very low rates. Thioredoxin and thioredoxin reductases can also reduce the oxidized forms of ScGrx5 (Tamarit et al. 2003) or EcGrx4 (Fernandes et al. 2005) respectively, but the significance of this reaction for the biological function of both proteins has not been addressed yet.

Location and functions

Both ScGrx5 and EcGrx4 are monothiol GRXs with a single Grx domain. None of the proteins containing Trx domains has been purified and characterized. This means that the biochemical function of these N-terminal extensions in monothiol GRXs is still unknown. It is not clear whether or not this domain would be redox active and required for the catalytic function of the protein, because only one of the two cysteines conserved in the active site from thioredoxins is found in monothiol GRXs. Additional roles not related to catalysis have been suggested for this domain. In ScGrx3 and ScGrx4 it is required for nuclear localization of the protein (Molina et al. 2004; Ojeda et al. 2006) and in the human PICOT protein it is required for the interaction with protein kinase C (Witte et al. 2000).

The different monothiol GRXs have different localization inside the cell that may define specific functions. ScGrx5 is localized in the mitochondrial matrix and is required for the maturation of iron-sulfur containing proteins (Rodriguez-Manzanique et al.

2002). Consequently, its absence causes inability for respiratory growth and also hypersensitivity to external oxidants (Rodríguez-Manzanique et al. 1999, 2002). Its absence also activates many genes involved in iron acquisition and induces iron overload (Bellí et al. 2004). As a consequence cells lacking ScGrx5 are exposed to continuous oxidizing conditions that can result in damage to specific proteins (Rodríguez-Manzanique et al. 1999; Shenton et al. 2002). The precise function of ScGrx5 in iron-sulfur biogenesis is a matter of discussion. Mühlenhoff and coworkers have proposed that it may be involved in the transfer of iron-sulfur clusters from scaffold proteins to apo-proteins (Muhlenhoff et al. 2003). However, *in silico* modeling of the process of iron-sulfur maturation suggests that ScGrx5 may function in the initial assembly of iron-sulfur clusters into scaffold proteins (Alves et al. 2004).

As mentioned in the preceding paragraph, ScGrx5 is located at the mitochondria. In contrast, ScGrx3 and ScGrx4 are located at the nucleus (Molina et al. 2004). These two GRXs are larger than ScGrx5 because they contain a Trx domain at the N-terminus, which connects with the glutaredoxin domain through a linking region (Bellí et al. 2002; Vilella et al. 2004). ScGrx3 and 4 targeted to the mitochondria (using the ScGrx5 targeting sequence) can partially rescue the defects observed in a *grx5* null mutant, while ScGrx2 cannot perform this function. Nevertheless, it was shown that monothiol GRXs can interchange their activities when compartment barriers are surpassed (Molina et al. 2004). It is worth noting that, in contrast to ScGrx 1 and 2, ScGrx3 and ScGrx4 do not show an important role in oxidative stress response (Rodríguez-Manzanique et al. 1999). These results indicate the evolutionary divergence between dithiol and monothiol GRXs.

A further insight concerning the role of these two glutaredoxins was recently provided (Ojeda et al. 2006). This work showed that both, ScGrx3 and ScGrx4 are required for Aft1-mediated iron regulation. Aft1 together with Aft2 are two transcriptional factors that are activated in iron-deficient cells and regulate iron uptake and homeostasis in *S. cerevisiae* cells (Yamaguchi-Iwai et al. 1995; Blaiseau et al.

2001; Rutherford et al. 2001). Mutant cells lacking both ScGrx3 and ScGrx4 display constitutive expression of genes involved in iron homeostasis. To accomplish their role, both GRXs physically interact with Aft1 and the Cys residue of the CFGS sequence in the glutaredoxin domain is crucial for such regulation (Ojeda et al. 2006).

S. pombe contains three genes encoding for monothiol glutaredoxins (*grx3*⁺, *4*⁺ and *5*⁺). As for *S. cerevisiae*, SpGrx5 localizes at the mitochondria, SpGrx4 at the nucleus and SpGrx3 at the nuclear rim and endoplasmic reticulum (Chung et al. 2005). SpGr3 is in fact one of the GRX-like proteins listed in Table 3 with the CPYS motif; therefore, it can not be classified into the CGFS monothiol GRXs. *S. pombe* null *grx5* mutants are highly sensitive to oxidants such as hydrogen peroxide and superoxide generating compounds. As these defects are also observed in *S. cerevisiae* Δ *grx5* strains, it is very plausible that SpGrx5 may also be involved in iron-sulfur biogenesis. Also, nitrosative and osmotic stresses enhances the transcription and synthesis of SpGrx5, a process depending on the transcription factor Pap1 (homologous to *S. cerevisiae* Yap1) (Kim et al. 2005b). Deletion mutants in *grx3*⁺ do not show significant deficiencies in growth or resistance to oxidants. In contrast, *grx4* null mutants are not viable, thus indicating a critical role of such GRX in *S. pombe* (Chung et al. 2005).

Protein homologues

The Grx domain that defines the group of monothiol GRXs has also been termed PICOT homology domain (PICOT-HD), as it can be found in the human PICOT protein (Isakov et al. 2000). Human PICOT (for PKC interacting cousin of thioredoxin) protein is a 37.5 kDa protein, which is expressed in T lymphocytes. It colocalizes with PKC θ which is thought to play an important role in T cell receptor-induced activation (Witte et al. 2000). PICOT protein has an N-terminal Trx domain followed by two tandem PICOT-HD repeats, in the C-terminal region. In *A. thaliana*, the domain is repeated threefold while twofold multiplication has been described in human, mouse and rat (Isakov et al. 2000). The secondary structure of such domain includes three α helices

and an intervening β strand between each pair of α helices (Isakov et al. 2000). This overall structure resembles the one predicted for GRXs, especially ScGrx5, in which it corresponds to the majority of the peptide (Bellí et al. 2002). The Trx domain lacks the conserved sequence CGPC in the catalytic centre. Instead of catalytic activity, this domain is required for interaction with the above mentioned PKC θ . This interaction does not lead to phosphorylation of PICOT protein (it is not a substrate), but to inactivation of the kinase. Such inactivation occurs in cellular stress responses which are associated to the function of activator protein 1 (AP-1) and nuclear factor κ B (NF- κ B). In vitro, PICOT protein also associates to PKC ζ but not to PKC α (Witte et al. 2000), thus indicating some specificity of PICOT interactions.

In bacteria, *E. coli* have three dithiol GRXs (EcGrx1, EcGrx2 and EcGrx3) and one monothiol GRX (EcGrx4) encoded by *grxD* (Fernandes et al. 2005; Fladvad et al. 2005). It has a molecular weight of 12.7 kDa and shares a high degree of homology to ScGrx5, displaying the conserved CFGS motive at the catalytic centre. Also, as for ScGrx5, it shows no detectable activity in the glutathione-HEDS assay. The glutathionylated form of EcGrx4 acts as a substrate for thioredoxin reductase. This is a specific reaction of the reductase since it does not react with glutathionylated forms of EcGrx1, EcGrx2 or EcGrx3 (Fernandes et al. 2005). The function of EcGrx4 is still unknown, but it shows a three-fold increase in stationary phase, thus suggesting an important role of EcGrx4 as redox-active protein in this phase. The up-regulation seems to be mediated by ppGpp (Fernandes et al. 2005).

In *Plasmodium falciparum* a GRX-like protein has been described that contains a PICOT-HD domain, named PfGLP-1. This protein has a hypothetical mitochondrial targeting sequence with a possible cleavage site at position 43. The mature protein would have a molecular weight of 15 kDa. It lacks a classical CPYC motif. Instead it has a CGFS sequence and shares a high homology with yeast monothiol GRXs (41,5% with ScGrx5, 36,2% with ScGrx3 and 33,3% with ScGrx4) (Rahlf et al. 2001).

As for yeast monothiol GRXs, PfGLP-1 protein has no detectable activity on the glutathione-HEDS assay. Although little is known about its physiological function, it seems likely that, according with the homology with yeast monothiol GRXs, PfGLP-1 would be involved in the redox balance in *P. falciparum*, which could be relevant for the parasite, as this organism depends on robust antioxidant defence mechanisms for infectiveness (Rahlfs et al. 2001).

The insights of ScGrx5 function observed in yeast have been of enormous importance to unravel the function of Grx5 homologues in vertebrates such as zebrafish and mice. These findings also uncovered the connection between iron-sulfur cluster formation and haem biosynthesis in vertebrates (Wingert et al. 2005). In this context, the results showed that lack of zebrafish *grx5* expression, was responsible for the hypochromic anemia in *shiraz* (sir) zebrafish mutants. Such disease is characterized by hypochromic erythrocytes produced as a consequence of impaired haemoglobin synthesis. Such metabolic blockage can be reverted by expression of yeast, mouse or human Grx5 homologues in *shiraz* zebrafish mutants (Wingert et al. 2005). These results clearly indicated that Grx5 function has been evolutionary conserved as the proteins homologues of ScGrx5 from *E. coli*, *Synechocystis sp.*, chicken and zebrafish are able to perform the functions of ScGrx5 and, accordingly, they can rescue the biogenesis of iron-sulfur clusters when expressed in a $\Delta grx5$ *S. cerevisiae* mutant strain and targeted to the mitochondria of the latter (Molina-Navarro et al. 2006; Wingert et al. 2005).

Glutaredoxin activity of Omega class GSTs

GSTs are divided into a large number of classes based on primary sequence, immunological properties and substrate specificity (Armstrong 1997; Hayes et al. 2005). Omega class GSTs display low or null activity against standard GST substrates

such as CDNB, but instead, they are active as glutaredoxins when using HEDS as *in vitro* substrate (Board et al. 2000; Girardini et al. 2002; Whitbread et al. 2005). In most GST classes, an N-terminal tyrosine or serine residue is essential for the nucleophilic attack on the enzyme substrate. In contrast, Omega class GSTs (which lack this Ser or Tyr residue) form a mixed disulphide involving GSH and an N-terminal cysteine residue of the GST molecule (Board et al. 2000). This added to the fact that (as occurs with other GSTs) the Omega class molecules are members of the thioredoxin family, indicates that a structural and biochemical similarity exists between GRXs and Omega-class GSTs.

Omega class GSTs are widely distributed among living organisms, although only a few species have been studied in some detail [reviewed in Whitbread et al. (2005)]. The human molecule hGSTO1-1 modulates the activity of calcium channels in different cell types (Dulhunty et al. 2001) and it also seems to have some role in the post-translational processing of interleukin-1 β (Laliberte et al. 2003). Recently, it has been demonstrated that human Omega GSTs catalyse the reduction of pentavalent methylated arsenic species, thus mediating the pathway of arsenic biotransformation (Schmuck et al. 2005). *S. cerevisiae* cells have three proteins (Gto1, Gto2 and Gto3) with the primary structure and enzymatic properties of Omega class GSTs (Garcerá et al. 2006), that is, they have glutaredoxin, dihydroascorbate reductase and dimethylarsinic acid reductase activities. Importantly, they are active as thiol transferases on HEDS using a single cysteine residue, through a deglutathionylating monothiol mechanism of action (Garcerá et al. 2006). This extends the number of proteins in *S. cerevisiae* that act as thiol transferases employing monothiol or dithiol mechanisms. Homologues of Gto proteins exist in many fungal species. The function of these Gto proteins in *S. cerevisiae* is not known, but while Gto2 and Gto3 are cytosolic, Gto1 is located at the peroxisome (our unpublished observations), probably acting as redox regulators of specific proteins.

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Legends to the Figures

Figure 1. Clustal W multiple alignment of fungal dithiol glutaredoxins with the CPY/FC active site motif (underlined). Aligned sequences have an E value $< 10^{-10}$ in a BLAST search against *S. cerevisiae* Grx1 (using the BLASTp program of <http://www.ncbi.nlm.gov/blast>, with default parameters). Entrez accession number (<http://www.ncbi.nlm.nih/entrez>) is indicated for each protein sequence, followed by the name of the organism. Tools of the European Bioinformatics Institute were used for multiple alignment (<http://www.ebi.ac.uk/cgi-bin/clustalw>), with default parameters. Residues showing identity or similarity are displayed in black and grey boxes respectively.

Figure 2. Clustal W multiple alignment of fungal sequences containing the conserved motif CPDC (underlined) and showing homology to *S. cerevisiae* ScGrx1 (E values between 5×10^{-7} and 5×10^{-3} in a BLASTp search). See legend of Figure 1 for more details.

Figure 3. Clustal W multiple alignment of fungal monothiol glutaredoxins with the CGFS active site motif (underlined). Aligned sequences have an E value $< 10^{-28}$ in a BLASTp search against *S. cerevisiae* ScGrx5. See legend of Figure 1 for more details.

Figure 4. Clustal W multiple alignment of several of the fungal sequences in Table 3 containing the CPxS motif (underlined) and the sequences of *S. cerevisiae* ScGrx1 and ScGrx2. See legend of Figure 1 for more details.

Figure 5. Scheme of the intracellular location and functions of dithiol and monothiol glutaredoxins in *S. cerevisiae*. See the text for more details.

Table 1. Experimentally determined or predicted location of fungal dithiol GRXs

Organism	Protein ^a	Subcellular location ^b
<i>Saccharomyces cerevisiae</i>	Grx1	Cyt (Pedrajas et al., 2002)
	Grx2	Cyt and Mit (Pedrajas et al., 2002)
<i>Candida albicans</i>	XP719021	Mit
	XP720834	Cyt, Nuc
	XP720835	Nuc
<i>Kluyveromyces lactis</i>	CAH01104	Mit
<i>Debaryomyces hansenii</i>	CAG90415	NP
	CAG86752	Nuc
<i>Ashbia gossypii</i>	AAS54082	Mit
<i>Yarrowia lipolytica</i>	XP501474	Cyt
<i>Aspergillus nidulans</i>	XP661819	Cyt, Nuc
<i>Aspergillus fumigatus</i>	XP700359	NP
<i>Neurospora crassa</i>	CAB88564	Cyt, Nuc
<i>Giberella zeae</i>	XP382273	Nuc
<i>Chaetomium globosum</i>	EAQ86182	Cyt
<i>Cryptococcus neoformans</i>	EAL21121	Mit
<i>Schizosaccharomyces pombe</i>	Grx1	Cyt (Chung et al., 2004; Kim et al., 2005a)
	Grx2	Mit or Nuc (Chung et al., 2004; Kim et al., 2005a)
<i>Ustilago maydis</i>	XP761095	NP

^a The accepted name of the protein is indicated based on the literature; otherwise, the Entrez protein accession number is indicated (<http://www.ncbi.nlm.nih.gov/entrez>)

^b Cyt, cytosolic; Mit, mitochondrial; Nuc, nuclear. Protein location was predicted using PSORT II (<http://psort.ims.u-tokyo.ac.jp>), WoLP PSORT (<http://wolfpsort.seq.cbrc.jp>) and TargetP 1.1 (<http://www.cbs.dtu.dk/services/targetp>). For some proteins more than one possible location was predicted with significance by the different programs. For others, no significant prediction was reached (NP). For experimentally determined locations, the original reference is indicated

Table 2. Fungal monothiol glutaredoxins with the Trx-Grx domain structure^a

Organism	Protein ^b	Length (amino acids)	Motif ^c	Subcellular location ^d
<i>Saccharomyces cerevisiae</i>	Grx3	285	CGFS	Nuc (Molina et al. 2004)
	Grx4	244	CGFS	Nuc (Lopreiato et al. 2004, Molina et al. 2004)
<i>Candida glabrata</i>	CAG59644	260	CGFS	Cyt, Nuc
	CAG62263	242	CGFS	Cyt
<i>Candida albicans</i>	XP720477	253	CGFS	Cyt, Nuc
<i>Kluyversomyces lactis</i>	CAH01848	264	CGFS	Cyt
<i>Debaryomyces hansenii</i>	CAG88249	238	CGFS	Cyt
<i>Ashbia gossypii</i>	AAS54601	237	CGFS	Cyt
<i>Yarrowia lipolytica</i>	XP500838	250	CGFS	Cyt
<i>Aspergillus nidulans</i>	XP680836	275	CGFS	Cyt
<i>Aspergillus fumigatus</i>	XP755829	271	CGFS	Cyt
<i>Aspergillus oryzae</i>	BAE60595	297	CGFS	NP
<i>Neurospora crassa</i>	XP964063	266	CGFS	Cyt
<i>Gibberella zeae</i>	XP381493	253	CGFS	Cyt
<i>Chaetomium globosum</i>	EAQ92505	260	CGFS	Cyt
<i>Cryptococcus neoformans</i>	AAW42462	373	CGFS	Nuc
<i>Schizosaccharomyces pombe</i>	Grx4	244	CGFS	Nuc (Chung et al. 2005)
<i>Ustilago maydis</i>	XP760370	266	CGFS	Cyt, Nuc
<i>Mortierella alpina</i>	CAB56513	275	CQFS	Cyt

^a Listed proteins display homology with *S. cerevisiae* ScGrx5 in a BLASTp search (E value < 10⁻¹⁶)

^b The accepted name of the protein is indicated based on the literature; otherwise, the Entrez protein accession number is indicated (<http://www.ncbi.nlm.nih.gov/entrez>)

^c The putative active site sequence is shown based on functional studies with *S. cerevisiae* ScGrx3 and ScGrx4 (Molina et al. 2004) and multiple alignments

^d Cyt, cytosolic; Mit, mitochondrial; Nuc, nuclear. Protein location was predicted using PSORT II (<http://psort.ims.u-tokyo.ac.jp>), WoLP PSORT (<http://wolfpsort.seq.cbrc.jp>) and TargetP 1.1 (<http://www.cbs.dtu.dk/services/targetp>). For some proteins more than one possible location was predicted with significance by the different programs. For others, no significant prediction was reached (NP). For experimentally determined locations, the original reference is indicated

Table 3. Fungal glutaredoxins-like proteins with the conserved CPxS motif^a

Organism	Protein ^b	Length (amino acids)	Motif ^c	Other characteristics ^d
<i>Saccharomyces cerevisiae</i>	CAA84956	203	CPYS	TM(10-27), SP(32-33)
<i>Candida glabrata</i>	CAG60384	226	CPFS	No TM, SP(22-23)
<i>Candida albicans</i>	XP711401	229	CPYS	TM(7-24), SP(30-31)
<i>Kluyveromyces lactis</i>	CAG99837	211	CPFS	TM(7-24), SA
<i>Debaryomyces hansenii</i>	CAG88133	221	CPYS	TM(7-24), SP(30-31)
<i>Ashbya gossypii</i>	AAS52701	216	CPLS	TM(13-32), SA
<i>Yarrowia lipolytica</i>	XP505978	209	CPHS	TM(7-29), SA
<i>Neurospora crassa</i>	CAE76344	254	CPYS	TM(9-26), SP(28-29)
<i>Giberella zeae</i>	XP381346	283	CPHS	TM(7-24), SP(27-28)
<i>Chaetomium globosum</i>	EAQ89239	272	CPYS	TM(7-24), SA
<i>Cryptococcus neoformans</i>	AAW41978	382	CPYS	No TM, No SA or SP
<i>Schizosaccharomyces pombe</i>	Grx3	166	CPYS	TM(7-24), SA

^a Listed proteins display homology with *S. cerevisiae* ScGrx1 in a BLASTp search (E value < 10⁻²)

^b The accepted name of the protein is indicated based on the literature; otherwise, the Entrez protein accession number is indicated (<http://www.ncbi.nlm.nih.gov/entrez>)

^c Conserved motif in a multiple alignment analysis including *S. cerevisiae* ScGrx1 and ScGrx2 (see also Figure 4)

^d TM: transmembrane domain as predicted by the TMHMM program (Krogh et al. 2001); in parenthesis the transmembrane amino acid region. SP (signal peptide, in parenthesis the predicted amino acid cleavage site) and SA (signal anchor) domains were predicted with the SignalP 3.0 program using a hidden Markov model (Bendtsen et al. 2004). TMHMM and SignalP 3.0 programs are accessible at <http://www.cbs.dtu.dk/services>

Figure 1
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CAA42381	<i>S. cerevisiae</i>	Grx1	-----MVSQE	5
AAT92915	<i>S. cerevisiae</i>	Grx2	-----MVSQE	5
XP720834	<i>C. albicans</i>		MDYFQTEKTTETROQRQOKKRPVRNSIICITLSLSYQPNFVMSLIGW-LSSNWFQNEPITPE	60
XP720835	<i>C. albicans</i>		-----MIDK-----MSSILAWGFNLWYQPPPTAQ	25
XP719021	<i>C. albicans</i>		-----MFRITLLTKRLFNTSTMVSSQ	20
CAH01104	<i>K. lactis</i>		-----MPSAA	5
CAG90415	<i>D. hansenii</i>		-----MVSQH	5
CAG86752	<i>D. hansenii</i>		-----MFEY-IKSNWFVSPVPSPV	17
AAS54082	<i>A. gossypii</i>		-----MVSPS	5
XP501474	<i>Y. lipolytica</i>		-----MATES	5
XP661819	<i>A. nidulans</i>		-----MSS	3
XP750359	<i>A. fumigatus</i>		-----MSA	3
CAB88564	<i>N. crassa</i>		-----MSDA	4
XP382273	<i>G. zeae</i>		-----MSQ	3
EAQ86182	<i>C. globosum</i>		-----MEA	3
EAL21121	<i>C. neoformans</i>		-----MSFTALHRLSTLSTHIIRAPISTRYFATTQTAKIGSSISTPNMTAD	46
AAF19628	<i>S. pombe</i>	Grx1	-----MSS	3
CAB52428	<i>S. pombe</i>	Grx2	-----MTSI	4
XP761095	<i>U. maydis</i>		-----MA	2
CAA42381	<i>S. cerevisiae</i>	Grx1	TIKHVKDILIAENEIPVASRTYCHYCHAAALNTLFEKLKVPKSKVLVLQNDMKE-GADITAA	65
AAT92915	<i>S. cerevisiae</i>	Grx2	TVAHVKDILIGQKEVFVAAMTYCHYKATLSTLFQELNVPKSKALVLEDEMSN-GSEIDAA	65
XP720834	<i>C. albicans</i>		LKKEIESNINSHKVLVYSRSTYCHYCTSTKTL--QSL--NQDYKVIELDQI-PKGSATNG	116
XP720835	<i>C. albicans</i>		TEKEIEHTINSHKIVYSRTYCHYCDQTKHLNEQYP--QESYEVINLNL-DGLTTCNQ	83
XP719021	<i>C. albicans</i>		VKNKVEQLIKTKPVFIASRSTYCHYKATKSTIRAI---TKD-AYILELDEV-DGAEIDEA	76
CAH01104	<i>K. lactis</i>		TIARVQGLINSSKIPVASRTYCHYCAATLKTLEEKVKVDKKLATVLQNLQLED-GSDITDA	65
CAG90415	<i>D. hansenii</i>		TTDKVQQLIKTKPVFIASRSTYCHYCAKNTISSI---TKD-AYIILEDEV-EDGSEIDEA	61
CAG86752	<i>D. hansenii</i>		IKAEVQNLIDSNKILIFLSRSTYCHYCDSTKDLI--KSI--TSDFKVVELNTS-ANGRTIDA	73
AAS54082	<i>A. gossypii</i>		VIKQVQALIQQNRVFIASRSTYCHYCAAKRTLLSEKRVPASAVKLLLEDTMGEECAVIAA	66
XP501474	<i>Y. lipolytica</i>		AIKTIKEHIAKDKVIVASRSTYCHYCKQTKQLLSQF---KEAKPVILELDEL-DGAEIDAA	62
XP661819	<i>A. nidulans</i>		AKVKAQQIIDENGVVVFSRSTYCHYCKASKSLSEL---GAK-YYALELDTI-DGADIDNA	59
XP750359	<i>A. fumigatus</i>		AKTKAQNLINENAVVVFSRSTYCHYCNASKKTLKDL---GAK-FYALELDEI-DGCREIDNA	59
CAB88564	<i>N. crassa</i>		ATQKAKQLINDNAVVVFSRSTYCHYCSNTKQILDGL---NAK-YATYELNQE-SDGSDVDA	60
XP382273	<i>G. zeae</i>		ASTKVQQLIDNNSVVVFSRSTYCHYCKQTKKTLDDL---NTE-YELLELDEV-ADGSALDA	59
EAQ86182	<i>C. globosum</i>		AQKKAQALIDENAVMIFSRSTYCHYCRDAKSVFSTR---DVK-YKAVELNQM-DGDDIDDA	59
EAL21121	<i>C. neoformans</i>		VKSLVDKAIADNKVVVFSRTYCHYCKRAKSYLAED---TKDIEILELDER-EDCAATDAY	102
AAF19628	<i>S. pombe</i>	Grx1	VESFVDSAVADNDVVVFASRSTYCHYCHATEKVIADK---KIKAQVYQIDLM-NDGDEIDSY	59
CAB52428	<i>S. pombe</i>	Grx2	AKAFVEKAISSNNPVTVFSRSTYCHYCKAAKNTLTXY---SAPYKAYELDKI-ENGSDIDAY	60
XP761095	<i>U. maydis</i>		ANQAAEKLISEHLNAVFSRSTYCHYCSQAKSVIEKLG-LDKSKVGILELDMGSGESDIDAY	63
CAA42381	<i>S. cerevisiae</i>	Grx1	IYEINGKRTVNNIYINGKKHGGNSDLQELRETG---ELEELLEPILAN-----	110
AAT92915	<i>S. cerevisiae</i>	Grx2	PEEISGKRTVNNVYINGKKHGGNSDLTLKKNQ---KLAEILKPVFQ-----	109
XP720834	<i>C. albicans</i>		IQELTGKRTVNNVFINGKKHGGNSDIQALHSQG---KLKPLFG-----	156
XP720835	<i>C. albicans</i>		IYANTGKYVHIIIFINGQHVGGNSEVQQLHTNG---KLQELLNPQKY-----	127
XP719021	<i>C. albicans</i>		IIEITGKRTVNNVFIGGQHGGNSDVQALKSSD---KLDDKIKAAAL-----	119
CAH01104	<i>K. lactis</i>		IAEITGKRTVNNIFINGKKHGGNSDLQELNNSG---DLDKLLASL-----	107
CAG90415	<i>D. hansenii</i>		IYELTGKRTVNNVFIOGHHGGNSDVQELSSGD---KLESKIKAVL-----	104
CAG86752	<i>D. hansenii</i>		IPEMTGKRTVNNIFINRKHGGNSDLQALQGAG---KLKSLVN-----	113
AAS54082	<i>A. gossypii</i>		IQELSGKRTVNNIYINGRHVGGNSDLKALKASC---ELDQLLEALAE-----	111
XP501474	<i>Y. lipolytica</i>		IAEITGKRTVNNVFIGGQHGGNSDLQVLAQKD---ELADKIKAAAL-----	105
XP661819	<i>A. nidulans</i>		PEEISGKRTVNNIYIAKKHGGNSDLQGIKK-----DLPALLKDAGAL-----	102
XP750359	<i>A. fumigatus</i>		IYEMTGKRTVNNIFIOGKHGGNSDLQAKSA---QLPALLKEAGAL-----	102
CAB88564	<i>N. crassa</i>		ILKLTGKRTVNNIFIGKQHGGNSDLAVVYKNGKNGKKIQELLQKAGAL-----	109
XP382273	<i>G. zeae</i>		PEKISGKRTVNNVYIKQHHGGNSDLQSLNSGG---KLJNLLKEANALKA---	106
EAQ86182	<i>C. globosum</i>		IQKMTGKRTVNNIFIGGTHGGSSDLANNVVSSGKDGKSIDVLLKEAGAL-----	109
EAL21121	<i>C. neoformans</i>		IKKELNGKTVHVVYINKEFIGGSSDLLKLSHEQ---VKQKISAAAASA-----	146
AAF19628	<i>S. pombe</i>	Grx1	ILKKTGKRTVNNIFIQKHVGGNSDLQALFKKG---ELDSLFNATA-----	101
CAB52428	<i>S. pombe</i>	Grx2	IHEKTKKSTVHSIFFRNQFIGGNSDLNKLRSSG---TLTKMIAELKENKSSIL	110
XP761095	<i>U. maydis</i>		ILDKTSKRTVNNIFINQKHGGCSDLIDDAQKSG---KLQQLLQ-----	102

FIGURE 1

Figure 2
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FIGURE 2

Figure 3
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NP015266 S.cerevisiae Grx5	-----MFLPKFNPISFSP-----ILRAKTLLR-----YQNRMYLSTEIRKAIRDAIESA	45
AA006877 S.kluyveri	-----MFGR-ISTR-ALLRP-----AFTHRIPSVLSRFLSTETKQAIRESAIESA	43
CAG62640 C.glabrata	-----MFATRLLGKSALLG-----TTSTRMVFR-----SQMARYMSTETTKAIEGAIASA	45
XP711746 C.albicans	-----MFRRLSLSTSRFINKS-----SSSLSPGLLLNNFRGNFQQSRFISTELKDALDKAVTTS	54
CAH02350 K.lactis	-----MFSRFLATKSPLLRP-----AFRS-----HLSPQRFFISQETTKAIEDAIESA	42
AAS51973 A.gossypii	-----MFATYSASILRSTLP-----LR-----SVGVRLLSQETTRRAIEGAISSA	39
XP505699 Y.lipolytica	MAFMQRSIFALRAATP-AMRAP-----AFRI-----APSMRRFISTETKDAIRTAIGTA	48
XP661908 A.nidulans	-----MFSRTAISFTLRSLFRPQL--SPQFPN-QLPSVL--QARLLSTETKAAIDKAVASA	52
XP752229 A.fumigatus		
CAD71022 N.crassa	-----MLTRSLFSRQLFAAASRPPI--APKAVSSAFRPVLFYQNRFLSDATRQAIIDKAVASA	55
XP384743 G.zeae	-----MFARTVAS-SLRQAARPMVAVRSTASAFRTPIAPLSPFQIRLLSDQTRSAIDKAVSSA	56
AAW41655 C.neoformans	-----MVFAKLGLRLTLRSLPQ-----SQVARSTTILAQRRLFLSAEARKLIDGAVKSN	47
CAC21468 S.pombe_Grx5	-----MNSMFRFWIPKTSIS-----MQLRMLSTQTRQALEQAVKED	36
XP759014 U.maydis	----MSAVFRQSLGLLRSAPARGS----LLCNPTTRACFTPTTYSLTRLLSTEARAKIDSAVKSN	56
NP015266 S.cerevisiae Grx5	PVVLEMKGTETFFKCGFSRATIGLLGNQGDPAKFAAYNVIEDFELREGCKEFSNWTPIQVYV	109
AA006877 S.kluyveri	PVVLEMKGTETFFKCGFSKATINMLGQQGVDPMKFAAYNVIEDFELREGCKEFSNWTPIQVYV	107
CAG62640 C.glabrata	PVVLEMKGTETFFKCGFSRATISMLGQQGVDPKFAAYNVIEDFELREGCKEFSNWTPIQVYV	109
XP711746 C.albicans	PVVLEMKGTETFFKCGFSRATIQLGQQGVDPKFAAYNVIEDFELREGCKEYSSNWTPIQVYV	108
CAH02350 K.lactis	PVVLEMKGTETFFKCGFSRATINMLGQQGVDPVKFAAYNVIEDFELREGCKEISENWTPIQVYV	106
AAS51973 A.gossypii	PVVLEMKGTETFFKCGFSKAAIEILGRQGVDPKFAAFNVIEDSELREGCKEYSENWTPIQVYV	103
XP505699 Y.lipolytica	PVVLEMKGTETFFKCGFSRATIQVLGHQGVDPQKFAAFNVIEDFELREGCKEFSQNWTPIQVYI	112
XP661908 A.nidulans	PVVLEMKGTETFFKCGFSRASIQILGLQGVDPKKFVAANVIEDFELREGCKEYSDNWTPIQVYL	116
XP752229 A.fumigatus	-----MKGTETFFKCGFSRASIQILGLQGVDPKKFVAANVIEDFELREGCKEYSDNWTPIQVYL	59
CAD71022 N.crassa	PVVLEMKGTETFFKCGFSRASIQVLGLQGVDPNKFAAFNVIEDFELREGCKEYSDNWTPIQVYI	109
XP384743 G.zeae	PVVLEMKGTETFFKCGFSRAAIQILGLQGVDPKFAAFNVIEDFELREGCKEYSDNWTPIQVYV	120
AAW41655 C.neoformans	PLVVEMKGTEDAEKCGFSRAVCQILDVQGVPRENLKTYNCIEDFELREGCKEYSENWTPIQVYI	111
CAC21468 S.pombe_Grx5	PVIVLEMKGTETTRMCGFSLKAIQILSLENVADSKLVYNAVSNDELREGCKEFSDNWTPIQVYI	100
XP759014 U.maydis	PLVISMKGTEHDMKCGFSRAVIQVMQVQGVKPELLKTENCIEDFELREGCKEYSDNWTPIQVYV	120
NP015266 S.cerevisiae_Grx5	NKEEYGGCCVITSMARSGEIAADLLEEAQALVP-----EEEEETKDR-----	150
AA006877 S.kluyveri	NKEEYGGCCIVVMMAQTGELAKLLEDADALVP-----EEEE-----	143
CAG62640 C.glabrata	NKEEYGGCCVITSMARSGEIAADVLEEANALVP-----EEKEEGTD-----	149
XP711746 C.albicans	NGEEYGGCCIIITSMAGNGEIAELLEESNALIP-----EENEPTTSEENQETVQSANIKPNRN	175
CAH02350 K.lactis	NKEEYGGCCIVVMMAQTGELAKLLEESDALVP-----EEED-----	142
AAS51973 A.gossypii	NKEEYGGCCILTMMAQSGEITTMLEEASVLVP-----DTE-----	138
XP505699 Y.lipolytica	NKEEYGGCCIVVMS--SQSGEIAEMLQQQDOLLAP-----EEEEHME-----	151
XP661908 A.nidulans	NKEEYGGCCILMSMHQNGEISKLEEKGVLV-----AE-----	149
XP752229 A.fumigatus	DKEEYGGCCILMSMHQNGEIAKLEEKGVLV-----AAD-----	93
CAD71022 N.crassa	DKEEYGGCCIIIVSMHNGEIAKLEEKDVLVKGEEGAAREQTEKKE-----	165
XP384743 G.zeae	EKEEYGGCCILVSMHNGEIAKLEDEKKVIL-----EGEPEQKE-----	159
AAW41655 C.neoformans	KGEYVGGCCILLSMHSGEIEDLLIKEGLAPP-----LPEGPEGSA-----	152
CAC21468 S.pombe_Grx5	NGEYVGGCCILLASMHKSGEIHKLKEINALAPEQPKDSEETTKKD-----	146
XP759014 U.maydis	DGEYVGGCCIVINSMHSGEIEDLLKFNGLLP-----INEEAAKAQA-----	162

FIGURE 3

Figure 4
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FIGURE 4

Figure 5
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FIGURE 5