Regulation of Protein S-Thiolation by Glutaredoxin 5 in the Yeast
Saccharomyces cerevisiae*

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The irreversible oxidation of cysteine residues can be prevented by protein S-thiolation, a process by which protein -SH groups form mixed disulfides with low molecular weight thiols such as glutathione. We report here that this protein modification is not a simple response to the cellular redox state, since different oxidants lead to different patterns of protein S-thiolation. SDS-polyacrylamide gel electrophoresis shows that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is the major target for modification following treatment with hydroperoxides (hydrogen peroxide or tert-butylhydroperoxide), whereas this enzyme is unaffected following cellular exposure to the thiol oxidant diamide. Further evidence that protein S-thiolation is tightly regulated in response to oxidative stress is provided by the finding that the Tdh3 GAPDH isoenzyme, and not the Tdh2 isoenzyme, is S-thiolated following exposure to H2O2 in vivo, whereas both GAPDH isoenzymes are S-thiolated when H2O2 is added to cell-free extracts. This indicates that cellular factors are likely to be responsible for the difference in GAPDH S-thiolation observed in vivo rather than intrinsic structural differences between the GAPDH isoenzymes. To begin to search for factors that can regulate the S-thiolation process, we investigated the role of the glutaredoxin family of oxidoreductases. We provide the first evidence that protein S-thiolation in vivo is regulated by a monothiol-glutaredoxin rather than the classical glutaredoxins, which contain two active site cysteine residues. In particular, glutaredoxin 5 is required for efficient dethiolation of the Tdh3 GAPDH isoenzyme.

Sulphydryl (-SH) groups play a remarkably broad range of roles in the cell, with their redox state affecting the activity of many enzymes, receptors, and transcription factors. As a result, all organisms contain complex regulatory machinery to maintain the redox states of -SH groups in both proteins and low molecular weight sulphydryls (reviewed in Refs. 1–3). However, cysteine residues are among the most easily oxidized residues in proteins, and oxidation can result in intermolecular protein cross-linking and enzyme inactivation, eventually leading to cell death (4). Such irreversible oxidation events can be prevented by protein S-thiolation, in which protein -SH groups form mixed disulfides with low molecular weight thiols such as GSH (5, 6).

A variety of proteins that become S-thiolated in response to cellular stress have been detected in mammalian cells. These include key metabolic enzymes such as carbonyl anhydrase, glycogen phosphorylase, creatine kinase, glyceraldehyde-3-phosphate dehydrogenase (GAPDH),1 glutathione transferase, and superoxide dismutase as well as structural and transport proteins such as hemoglobin, actin, and crystallin (reviewed in Ref. 6). Studies have also suggested that this modification may be involved in the regulation of the function and activity of proteins, including the human immunodeficiency virus type 1 protease (7), ubiquitin-conjugating enzymes in bovine retina cells (8), and DNA binding by the transcription factor c-Jun (9). There does not appear to be any one unifying feature of the proteins that become S-thiolated apart from their relative abundance in mammalian cells. Not all -SH-containing proteins are thiolated in response to an oxidative stress, and the specificity of this protein modification indicates that it must be a tightly regulated process. However, the exact role of thiolation in protection against reactive oxygen species-mediated damage is unknown, as are the consequences of this process on cell growth and metabolism.

Recent studies in yeast have shown that protein S-thiolation is important for survival during exposure to oxidative stress conditions. The basal levels of S-thiolation are maintained at low levels, but they are increased following treatment with hydrogen peroxide (10). GAPDH was identified as the major target of protein S-thiolation following exposure to H2O2 (11). This protein modification to GAPDH is tightly regulated, since, despite a high degree of sequence homology (98% similarity, 96% identity), the Tdh3, but not the Tdh2 GAPDH isoenzyme was S-thiolated. Furthermore, activity of both the Tdh2 and Tdh3 GAPDH isoenzymes was reduced following exposure to H2O2, but only Tdh3 activity was restored within a 1-h recovery period, indicating that S-thiolation of the Tdh3 polypeptide was readily reversible. This protein modification was also shown to be physiologically important, since mutants lacking TDH3 showed increased sensitivity to a challenge with H2O2, indicating that the S-thiolated Tdh3 polypeptide is required for survival during conditions of oxidative stress. In contrast, the nonthiolated Tdh2 polypeptide was required for survival during exposure to continuous low levels of oxidants, conditions in which the Tdh3 polypeptide was S-thiolated and hence inactive. These findings supported a model in which both enzymes are required for survival during conditions of oxidative stress, playing complementary roles depending on their ability to undergo S-thiolation (11).

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1 The abbreviations used are: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NEM, N-ethylmaleimide; DTT, dithiothreitol.

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Taken together, these previous studies indicate that protein S-thiolation is a novel form of post-translational modification regulating protein activity in response to growth or other cellular signals. Protein S-thiolation must therefore be reversible, and in vitro studies have shown that dethiolation can occur via direct reduction by GSH as well as enzymatically via both glutaredoxins and thioredoxins (5, 12). Glutaredoxin appears to be the most active dithiolase, and in addition, a recent study has shown a correlation between protein-SSG reduction and glutaredoxin activity in mammalian cells (13). However, little is known about the factors regulating this protein modification in vivo. In this study, we provide the first in vivo evidence that protein dethiolation can be catalyzed by a monothiol-glutaredoxin rather than the classical glutaredoxins, which contain two active site cysteine residues.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Growth Conditions**—The Saccharomyces cerevisiae strains used in this study are described in Table I. The double grx3::kanMX4 grx5::kanMX4 and grx4::kanMX4 grx5::kanMX4 mutant strains were constructed using standard yeast genetic methods. Strains were grown in rich YEPD medium (2% (w/v) glucose, 2% (w/v) bactopeptide, 1% (w/v) yeast extract) or minimal SD medium (0.17% (w/v) yeast nitrogen base without amino acids, 5% (w/v) ammonium sulfate, 2% (w/v) glucose) supplemented with appropriate amino acids and bases: 0.15 mM adenine, 0.2 mM uracil. Media were diluted into fresh YEPD medium and plated in triplicate on YEPD solidified by the addition of 2% (w/v) agar. Sensitivity to oxidants was determined by growing cells to exponential phase in SD medium at 30 °C. Parallel extracts were prepared in buffer containing either 50 mM N-ethylmaleimide (NEM) to prevent thiolation or 50 mM tert-butylhydroperoxide, or diamide, and cell extracts were prepared in the presence of NEM to prevent S-thiolation during the extraction procedure or with DTT to reduce any S-thiolated proteins. The levels of protein S-thiolation were quantified as the difference between the NEM- and DTT-treated extracts. GAPDH Enzyme Assays—GAPDH activity was measured according to McAllister and Holland (14) and expressed as μmol of NADH formed per min per μg of protein.

**RESULTS**

**Effect of Different Oxidants on Protein S-Thiolation**—To characterize the effects of oxidative stress induced by different oxidants on protein S-thiolation, the intracellular pool of low molecular weight sulfydryls was radiolabeled as described under “Experimental Procedures.” Cells were challenged with 2 mM H2O2, tert-butylhydroperoxide, or diamide, and cell extracts were prepared in the presence of NEM to prevent S-thiolation during the extraction procedure or with DTT to reduce any S-thiolated proteins. The levels of protein S-thiolation were quantified as the difference in counts incorporated between the NEM- and DTT-prepared extracts (Fig. 1A). The total level of S-thiolation in unstressed cells was very low but increased following treatment with all three oxidants. H2O2 and tert-butylhydroperoxide caused a similar increase in protein S-thiolation, whereas that from diamide was much lower.

To determine the number and range of proteins that are S-thiolated following treatment with the different oxidants, total cell extracts were examined by SDS-PAGE (Fig. 1B). As expected from the radioactive counts, few S-thiolated proteins were detected in unstressed cells, whereas, several proteins were detected following exposure to oxidants. Treatment with 2 mM H2O2 or tert-butylhydroperoxide induced a similar pattern of protein S-thiolation. In both cases, a prominent protein of ~38 kDa was detected, which we previously identified as GAPDH (11). In addition, several unknown proteins were S-thiolated in response to both oxidants (e.g. of 45, 70, and 100 kDa). These unknown proteins were also detected following treatment with diamide, but surprisingly, diamide did not induce protein S-thiolation of GAPDH. Radioactivity incorpora-
Regulation of Protein S-Thiolation

Protein S-Thiolation Does Not Correlate with Cellular Redox State or Viability—To determine whether this difference in S-thiolation arose as a result of the oxidants having different effects on the cellular oxidation state, we determined whether the increases in protein S-thiolation levels correlated with changes in the cellular redox state. Glutathione is the most abundant low molecular weight sulfydryl, and differences in the ratio of reduced to oxidized glutathione (GSH/GSSG) can be used as a sensitive indicator of the overall redox balance (15). Exponential phase yeast cells grown under normal aerobic conditions have a high glutathione redox ratio, indicating that most glutathione is maintained in a reduced (GSH) form (16, 17). Treatment with both 2 mM H2O2 and tert-butylhydroperoxide reduced the GSH/GSSG ratio, but to different extents (Fig. 2B). H2O2 treatment at this concentration did not increase the levels of GSSG, but it decreased GSH by ~40% (Fig. 2A), reducing the GSH/GSSG ratio from 188 in the control culture to 137. In contrast, tert-butylhydroperoxide or diamide did not affect viability (Fig. 2C), whereas 2 mM H2O2 resulted in greater than 90% loss of cell viability within 1 h. Thus, protein S-thiolation is a regulated process that occurs in response to particular stress conditions. In order to identify the processes responsible for this regulation, we next examined protein S-thiolation of GAPDH as a model protein.

Both Tdh2 and Tdh3 Can Be S-Thiolated in Vitro—Three unlinked genes (TDH1, TDH2, and TDH3) encode isoenzymes of GAPDH in yeast (19). We have previously shown that the Tdh3 isoenzyme, but not the Tdh2 isoenzyme, is S-thiolated in response to oxidative stress (11). This difference in S-thiolation between the Tdh2 and Tdh3 polypeptides might arise due to differences in their structures that affect the accessibility of sulfydryl groups to S-thiolation, or alternatively, the difference might be due to trans-acting factors that regulate the process in the cell. To discriminate between these two possible mechanisms, we examined the ability of Tdh2 and Tdh3 to be S-thiolated in vitro. Specifically, the pool of low molecular weight sulfydryls was radiolabeled in the wild-type strain and in the tdh1 tdh2 and tdh1 tdh3 mutants. No S-thiolation was detected in the absence of oxidative stress (Fig. 3A, +H2O2 lanes). Treatment of cells with H2O2 resulted in S-thiolation of GAPDH in strains containing Tdh3 (wild type and tdh1 tdh2) but not in strains that lacked Tdh3 (tdh1 tdh3), confirming that Tdh3 is the S-thiolated isoenzyme of GAPDH (Fig. 3A, +H2O2 lanes).
Cell-free extracts were prepared from the nonstressed strains and treated with 1 mM H2O2 for 30 min at 30 °C in vitro (Fig. 3B). No S-thiolation was detected in the unstressed extracts, but GAPDH was S-thiolated in response to treatment with H2O2 in extracts from all three strains. Radiolabeling was confirmed to occur as a result of S-thiolation, since it was reversed by treatment with DTT. B, cell-free extracts were prepared from the nonstressed strains and treated with 1 mM H2O2 for 30 min at 30 °C in vitro. No S-thiolation was detected in the unstressed extracts, but GAPDH was S-thiolated in response to treatment with H2O2 in extracts from all three strains. Radiolabeling was confirmed to occur as a result of S-thiolation, since it was reversed by treatment with DTT.

**Regulation of GAPDH Activity in Glutaredoxin Mutants**—We have previously identified two genes, designated *GRX1* and *GRX2*, in yeast that encode glutaredoxins (20). Mutants lacking *GRX1* and *GRX2* were unaffected in mixed disulfide formation (20), and similarly, S-thiolation of GAPDH is unaffected in *grx1* and *grx2* mutants (data not shown). Recently, a new family of glutaredoxin-related proteins has been identified in yeast (*GRX3*–5) that is conserved throughout evolution from bacterial to mammalian species (21). In addition, Grx5 was found to play an important role in protection against oxidative damage (21). These glutaredoxin-like proteins differ from classical glutaredoxins in that they contain a single cysteine residue at their putative active sites. Thus, they would be able to reduce protein-mixed disulfides in a reaction that proceeds via a monothiol mechanism (21, 22). We therefore investigated the role of Grx5 in protein S-thiolation by determining its effect on GAPDH enzyme activity. The wild-type and *grx5* mutant strains were grown to exponential phase, and 2 mM H2O2 was added to induce protein S-thiolation (Fig. 4). Prior to the peroxide treatment, the basal level of GAPDH activity in the *grx5* mutant was ~10-fold higher than in the wild-type strain. Following treatment with H2O2, GAPDH activity was inhibited by ~60 and 89% in the wild-type and *grx5* mutant strain, respectively (Fig. 4A). This reduction in GAPDH activity may have arisen due to irreversible oxidation or S-thiolation of the enzyme active site, both of which would inhibit GAPDH activity. To distinguish between these two possibilities, cells were transferred into fresh SD medium to follow the recovery of GAPDH activity once the H2O2 stress was removed. In the wild-type strain, GAPDH activity recovered within 20 min to ~90% of the basal level, before the oxidant treatment. In contrast, GAPDH activity in the *grx5* mutant was only restored to 30% of the control activity during a 60-min recovery period, indicating that Grx5 is required for efficient dethiolation of S-thiolated GAPDH. The differences in GAPDH activity did not arise due to changes in protein levels. In agreement with the measured enzyme activity, GAPDH protein was elevated in the *grx5* mutant compared with the wild-type strain. In addition, Western blot analysis showed that differences in protein levels between the untreated, H2O2-treated, and recovery extracts could not account for the changes in GAPDH activity detected (Fig. 4B).

**Protein S-Thiolation in the grx5 Mutant**—To further investigate the role of Grx5 in protein S-thiolation, cells in which the pools of low molecular weight thiols had been radiolabeled were challenged with H2O2 at concentrations between 1 and 4 mM for 30 min (Fig. 5). Protein synthesis in the BY4742 strain background appears to be somewhat more resistant to cycloheximide than the CY4 strain. This was seen as the incorporation of label that could not be reversed by DTT and was therefore due to protein synthesis rather than protein S-thiolation. A peak of GAPDH S-thiolation was seen in the wild-type strain at a concentration of 1 mM H2O2. In contrast, significantly higher levels of protein S-thiolation were detected in the *grx5* mutant at all concentrations of H2O2 tested. In particular, S-thiolation of GAPDH was very prominent, and there were elevated S-thiolation of several unknown proteins with approximate sizes of 30, 45, 60, 70, and 100 kDa.

**Loss of GRX5 Results in Delayed Dethiolation of Tdh3**—To examine the role of Grx3–5 in dethiolation, wild-type and glutaredoxin mutant cells were radiolabeled and treated with 2 mM H2O2 for 30 min to induce S-thiolation. Cells were washed to remove the H2O2 and any unincorporated radiolabel and resuspended in fresh minimal SD medium to follow the dethiolation process (Fig. 6). In the wild-type strain, dethiolation of...
GAPDH was \(-80\%\) complete within 5 min. In contrast, dethiolation of GAPDH was significantly slower in the \(grx5\) mutant compared with the wild-type strain and was only \(50\%\) complete after a 10-min recovery period (\(p < 0.001\)). However, dethiolation did occur in the \(grx5\) mutant and was complete within 20 min. These results indicate that Grx5 is required for the efficient dethiolation of GAPDH, but other factors can also catalyze the reaction. These other factors do not appear to be Grx3 or Grx4, since dethiolation of GAPDH occurred with similar kinetics to the wild-type strain in a \(grx3\) and a \(grx4\) mutant (data not shown). To further examine the roles of Grx3 and Grx4, dethiolation was examined in \(grx3\ grx5\) and \(grx4\ grx5\) double mutant cells (Fig. 6B). Loss of \(GRX3\) or \(GRX4\) in the \(grx5\) mutant had little or no effect on dethiolation, which was still complete within the 20-min recovery period.

Loss of \(GRX5\) Lowers the Glutathione Redox Ratio—To determine whether glutaredoxins 3–5 are required to maintain the cellular redox state, the levels and redox ratio of glutathi-
one were measured in grx3–5 mutants (Table II). Previous work has shown that glutathione is unaffected in grx1 or grx2 mutants (20). Similarly, strains lacking GRX3 or GRX4 contained levels of GSH and GSSG that were comparable with the wild type, resulting in a redox ratio of ~140 (Table II). In contrast, the grx5 mutant contained approximately 4-fold higher levels of GSSG, which resulted in a lowered GSH/GSSG redox ratio of 46. The treatment with 2 mM H$_2$O$_2$ for 30 min, which was used to induce protein S-thiolation in previous experiments (Figs. 4–6), did not affect the wild-type strain but caused a small increase in GSSG (27%) and decrease in the GSH redox ratio (17%).

**DISCUSSION**

It has long been recognized that low molecular weight thiols, such as GSH, can interact in a reversible manner with the cysteine –SH groups in many cellular proteins (6, 23). In particular, protein S-thiolation/dethiolation is a dynamic process that occurs in cells under physiological conditions as well as following exposure to an oxidative stress (5, 24, 25). Models have been proposed in which the modification of proteins by S-thiolation does not require an enzymatic activity but proceeds via the reaction of partially oxidized protein sulfydryls (thyl radical or sulfenic acid intermediates) with thiols such as cysteine or GSH or by thiol/disulfide exchange reactions with the oxidized disulfide form of glutathione (GSSG) (5). However, the tight control of GAPDH S-thiolation described in this present study indicates that cellular factors must regulate the modification of Tdh3. The Tdh2 and Tdh3 GAPDH isoenzymes share extensive sequence homology (98% similarity, 96% identity), particularly in the regions surrounding their active site cysteine residues (14, 19), but the Tdh3, and not the Tdh2 isoenzyme, is reversibly S-thiolated following exposure to H$_2$O$_2$. In contrast, both GAPDH isoenzymes could be S-thiolated in *vitro* using cell-free extracts. Thus, there do not appear to be any intrinsic structural differences to account for the difference in protein-mixed disulfide formation between the two GAPDH isoenzymes. Our studies also show that S-thiolation of GAPDH is a specific response to particular oxidants, since it is not increased in response to oxidative stress induced by treatment with diamide. This was despite the fact that diamide readily shifts the cellular redox balance to a more oxidized state and induces the S-thiolation of other unknown proteins. Therefore, S-thiolation in yeast is a controlled process that is regulated by specific cellular factors. The factors that are responsible for the differential S-thiolation of Tdh2 and Tdh3 remain to be identified.

To protect protein -SH groups against irreversible oxidation or to serve an enzyme-regulatory function, protein S-thiolation must be readily reversible. Many studies have demonstrated that S-thiolated proteins formed during oxidative stress are readily dethiolated once the stress is removed (25–27). In addition, the reduction of mixed disulfides is stimulated by both NADH- and NADPH-dependent systems (27, 28), but the physiological electron donors have not been identified. Most studies investigating the factors controlling dethiolation have been performed *in vitro* using radiolabeled protein-mixed disulfides as substrates. These studies have implicated various oxidoreductases including those of the GSH/glutaredoxin and thioredoxin systems (12, 29). Glutaredoxin appears to be the most efficient dethiolase enzyme in these *in vitro* experiments. In addition, a correlation has been demonstrated between protein-S-glutathionylation and the reversible S-glutathiolation of human immunodeficiency virus type 1 protease can be catalyzed by a glutaredoxin *in vitro* (7). We therefore investigated the role of yeast glutaredoxins in protein S-thiolation.

**Yeasts contain two classical glutaredoxin genes, designated GRX1 and GRX2, whose protein products share 40–50% identity and 61–76% similarity with those from bacterial and mammalian species (20). Additionally, a family of glutaredoxin-related proteins has been identified in yeast (encoded by GRX3–5), which is conserved throughout evolution (21). These glutaredoxin-like proteins differ from Grx1 and Grx2 in that they contain a single cysteine residue at their putative active

**TABLE II**

| Strain                | GSH$^a$ | GSSG$^a$ | GSH/GSSG |
|-----------------------|---------|----------|----------|
| Wild type             | 2.65 ± 0.65 | 19.1 ± 5.9 | 139      |
| Wild type + H$_2$O$_2$ | 2.37 ± 0.58 | 17.1 ± 6.9 | 138      |
| grx3                  | 3.47 ± 0.24 | 23.9 ± 3.6 | 145      |
| grx4                  | 3.05 ± 0.39 | 23.1 ± 6.4 | 132      |
| grx5                  | 3.30 ± 0.39 | 71.6 ± 25.5 | 46      |
| grx5 + H$_2$O$_2$     | 3.46 ± 0.44 | 91.1 ± 27.0 | 38      |

$^a$ Data are the means of triplicate experiments.

$^b$ The wild type (BY4742) and grx5 mutant were treated with 2 mM H$_2$O$_2$ for 30 min.
sites. Thus, they would be unable to substitute for glutaredoxins or thioredoxins as disulfide reductases with substrates like ribonucleotide reductase that require a dithiol mechanism (22). However, they could reduce protein-mixed disulfides in a reaction that proceeds via a monothiol mechanism (21, 22). In agreement with this, our studies show that Grx5 is required for dethiolation in vivo and can reduce mixed disulfides formed by GAPDH and other S-thiolated proteins. In contrast, the dithiol glutaredoxins (Grx1, Grx2) are not affected in the formation of mixed disulfides (20) as well as in the S-thiolation/dethiolation of GAPDH (data not shown). Similarly, strains lacking GRX3 or GRX4 are unaffected in the protein S-thiolation/dethiolation process.

This study shows that the grx5 mutant contains higher levels of S-thiolated Tdh3 compared with the wild-type strain following exposure to 1 mM H$_2$O$_2$. Western blot analysis shows that the grx5 mutant contains higher levels of GAPDH protein, so it is possible that the amount of thiolation per molecule of GAPDH may be the same between the grx5 mutant and the wild-type strain. However, it seems unlikely that elevated levels of GAPDH protein can account for the increased GAPDH thiolation observed in the grx5 mutant in response to higher levels of H$_2$O$_2$ (2 and 4 mM). The level of Tdh3 S-thiolation peaks at 1 mM H$_2$O$_2$ in the wild-type strain, whereas thiolation is elevated at concentrations of 2 and 4 mM in the grx5 mutant. In addition, the levels of thiolation for several unknown proteins are elevated in the grx5 mutant compared with the wild-type strain, consistent with a role for Grx5 in dethiolation.

Loss of GRX5 results in a delayed recovery of GAPDH enzyme activity following oxidative treatment, with only a 2-fold recovery detected in the grx5 mutant following a 1-h growth period. In contrast, although the grx5 mutant shows a slower rate of Tdh3 dethiolation, it is still complete within a 20-min recovery period. This difference presumably reflects the presence of three GAPDH isoenzymes in yeast, of which only Tdh3 can be protected against irreversible oxidation by protein S-thiolation. Thus, dethiolation of the Tdh3 isoenzyme in the grx5 mutant would account for the limited recovery of GAPDH activity observed. Oxidation of the Tdh1 and Tdh2 isoenzymes will result in irreversible oxidation. Given that the grx5 mutant is extremely sensitive to oxidative stress (21), these nonthiolated isoenzymes are likely to be readily inactivated. Thus, simply dethiolating Tdh3 in the grx5 mutant does not restore GAPDH activity to the basal levels found prior to the oxidative stress within a 1-h time period. It is also interesting to note that GAPDH protein levels are elevated in the grx5 mutant, and this may arise in a mechanism where GAPDH synthesis is increased to compensate for any oxidized, and hence inactivated, GAPDH that may be formed.

Loss of GRX5 results in extremely slow growth and elevated levels of protein oxidative damage compared with strains lacking GRX1–GRX4 (21). Here, we have shown that the cellular redox balance is also shifted toward a more oxidized form (measured as GSH/GSSG) in a grx5 mutant, whereas it is unaffected by the loss of GRX1–GRX4. Thus, GRX5 appears to be the most important glutaredoxin for protection against oxidative stress. However, the synthetic lethality described for a grx2 grx5 and grx3 grx4 grx5 mutant indicates that there must be some overlapping functions shared by the different glutaredoxins (21). Although this study has indicated a role for Grx5 in the dethiolation of protein-mixed disulfides, other activities must be required, since the reduction of S-thiolated GAPDH can still proceed. In addition, there may also be enzymes that regulate the forward S-thiolation reaction. Mammalian glutaredoxins have been shown to catalyze both the formation and reduction of mixed disulfides (30). However, yeast glutaredoxins do not appear to be required for S-thiolation, since mixed disulfides are formed in grx1–grx5 mutants in response to oxidative stress. Other enzymes that regulate protein S-thiolation may represent already known factors involved in the maintenance of cellular redox homeostasis or as yet unidentified activities. Yeast will provide an ideal model system to search for these factors, given the genetic and biochemical tractability of the organism as well as the ready availability of null mutants.

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