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Minireview

The system biology of thiol redox system in *Escherichia coli* and yeast: Differential functions in oxidative stress, iron metabolism and DNA synthesis

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Abstract **By its ability to engage in a variety of redox reactions and coordinating metals, cysteine serves as a key residue in mediating enzymatic catalysis, protein oxidative folding and trafficking, and redox signaling. The thiol redox system, which consists of the glutathione and thioredoxin pathways, uses the cysteine residue to catalyze thiol-disulfide exchange reactions, thereby controlling the redox state of cytoplasmic cysteine residues and regulating the biological functions it subserves. Here, we consider the thiol redox systems of *Escherichia coli* and *Saccharomyces cerevisiae*, emphasizing the role of genetic approaches in the understanding of the cellular functions of these systems. We show that although prokaryotic and eukaryotic systems have a similar architecture, they profoundly differ in their overall cellular functions.**

Keywords: Thiol redox control; Thioredoxin; Glutathione; Oxidative stress; Iron metabolism; Ribonucleotide reductase

1. Introduction

Most life on earth exists in an oxidizing environment. If not properly countered, oxygen would cause most cellular components to become oxidized. Living cells have in fact domesticated the oxidizing power of O_2, not only for the purpose of energy generation in the form of ATP through the proton motive pump in which O_2 acts as terminal electron acceptor, but also in a multitude of enzymatic reactions in which O_2 acts either as a catalyst or as a cofactor. These reactions, many of which are driven by the redox properties of the thiol group of cysteine, include the synthesis of desoxyribonucleotides building blocks of ADN that involves the O_2-initiated reduction of ribonucleotides by ribonucleotide reductase, the oxidative folding of secreted proteins by the FAD-containing thiol oxidase Ero1, and the mitochondrial import of proteins. To be controlled, such O_2-dependent thiol redox reactions need to be reversed by electrons that are mostly provided by the reducing power storage molecules NADH and NADPH and ultimately by glucose oxidation. To move from one to another component, these electrons are usually funneled in the form of protons through a web of redox wires made of redox-active cysteine residues, which can alternatively exist in the reduced thiol (SH) or oxidized disulfide bond (–S–S–) forms. This redox web constitute the thiol redox system made of two distinct branches, the thioredoxin and glutathione pathway. The thiol redox system not only controls O_2-dependent catalytic disulfides, but is also used in the reductive assimilation of sulfate to sulfite and as an antioxidant system in the reduction of H_2O_2.

We consider here the thiol redox systems of *Escherichia coli* and *Saccharomyces cerevisiae* emphasizing on the knowledge brought about by genetic approaches in understanding their cellular functions. The role of the thiol redox system in the secretory pathway will not be considered here. We will show that despite a similar architecture, a virtually identical mode of operation using electrons from NADPH to reduce disulfide bonds by a thiol-disulfide exchange mechanism, and sharing many targets, prokaryotic and eukaryotic systems have overall different cellular functions. One of the most striking differences is the abduction of the *S. cerevisiae* GSH pathway by iron metabolism, a feature that has not been described in prokaryotes. *S. cerevisiae* presumably serves as a good model for understanding the mechanism of thiol redox control and homeostasis in higher eukaryotes.

2. Basic description of cytoplasmic thiol redox control systems

The cytoplasmic thiol redox system consist of the GSH and thioredoxin pathways that can be thought of as electron flow pathways operating by virtue of gradients in redox potentials (see Figs. 1 and 2). Ultimately, the electron source for the system is the oxidation of pentose phosphate through the pentose phosphate pathway that regenerates NADPH from NADP+. NADPH has an extremely low redox potential of −315 mV that allows it to act as the primary hydrogen donor for both systems.

Both thioredoxin and glutaredoxin reduce disulfide bonds by a thiol-disulfide exchange reaction via two vicinal (CXXC) active-site cysteine residues, which either form a disulfide or a dithiol (for reviews see [1–3]). Oxidized thioredoxin is reduced to its dithiol form by the FAD-bound NADPH-dependent thioredoxin reductase, Oxidized glutaredoxin is instead reduced

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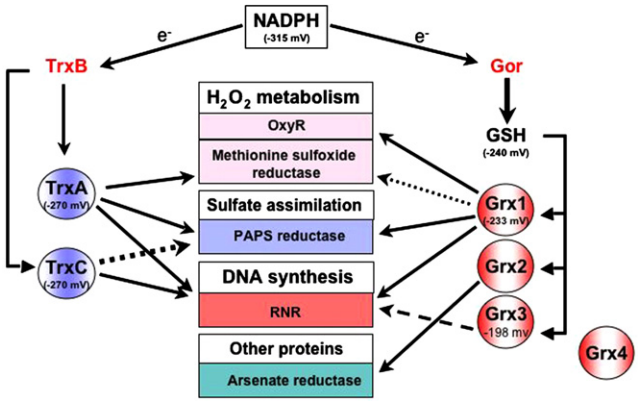

Fig. 1. Components of the *E. coli* thioredoxin and glutaredoxin systems. The figure is modified from Ref. [14]. Only a few thiol redox targets is represented. Black arrows represent electron flows. For references, see text.

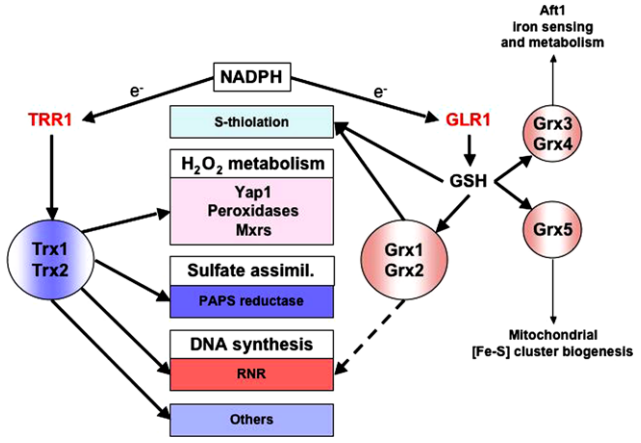

Fig. 2. Overview of the *S. cerevisae* GSH and thioredoxin pathways. For references, see the text.

by glutathione, which, in turn, is reduced by the FAD-bound NADPH-dependent glutathione reductase. As established in bacteria, these pathways operate in parallel and the extent to which electrons flow between them is not clear. Before examining the thiol redox system in eukaryotes, it is first useful to consider some of its aspects in bacteria since it appears fundamentally widely conserved.

Through a saga that have lasted about 40 years since the initial discovery of thioredoxin in 1964 [4] and that is probably not yet finished, biochemical, enzymatic and genetic approaches conducted by several laboratories, and in particular by those of Holmgren and Beckwith, have provided a very detailed and comprehensive view of the *E. coli* thiol redox system that constitute the working model of this system in other organisms. We essentially consider here the genetics of this system for the sake of providing a picture of its in vivo function, bearing in mind that interpretation of genetic data could only be done with the knowledge provided by biochemistry. Thiol redox reactions lead to intermediates that are often labile and therefore difficult to identify biochemically in vivo, leaving to genetics a significant part to elucidate the in vivo functions of the thiol redox system. Nevertheless, as we will see recent biochemical approaches to the in vivo redox state of cysteine residues and to the identification of the targets of thioltransferases, often conducted at the proteome-wide level are nicely complementing the knowledge brought about by genetics.

3. Lessons from bacteria

In *E. coli*, the glutathione pathway is composed of GSH, synthesized by the action of γ-glutamylcysteine synthetase, the rate-limiting enzyme encoded by *GshA*, and glutathione synthase encoded by *GshB* [5], of glutathione reductase encoded by *Gor*, and of three glutaredoxins, Grx1, Grx2 and Grx3 encoded by *GrxA*, *GrxB*, and *GrxC* [6,7] (Fig. 1). Grx4 is a third glutaredoxin of unknown function that differ from the others as being a monothiol glutaredoxin related to yeast monothiol glutaredoxins [8,9]. The thioredoxin system consists of two thioredoxins encoded by *TrxA*, and *TrxC* and thioredoxin reductase encoded by *TrxB* [5,10].

3.1. Thiol redox systems constitute electron flow pathways

A genetic demonstration of the notion that thiol redox systems operate as electron flow pathways came from experiments by Beckwith and colleagues [11]. They took advantage of the periplasmic enzyme alkaline phosphatase (AP), which becomes active when folded by virtue of intramolecular disulfide bonds formation. When expressed in the cytoplasm upon removing its periplasmic localization sequence, AP remains inactive as not being oxidized and properly folded. Through a screen based upon the enzyme's requirement for disulfide bonds for activity, they searched for mutations that would restore the activity of cytoplasmic AP, identifying *TrxB* [11]. They then showed that deleting both *TrxA* and *TrxC* completely eliminated the AP activity caused by the *trxb* mutation [12], indicating that lack of thioredoxin reductase allows cytoplasmic AP disulfide bond formation not by virtue of a defect in its reduction, but by promoting the accumulation of oxidized thioredoxin that acts as an oxidant for AP. Therefore, interrupting the gradient of redox potentials of the pathway upstream of thioredoxin converts the thioredoxin from a thiol-reduction catalyst to a thiol-oxidation catalyst. These data are also an experimental confirmation of the idea that an aerobic environment tends to oxidize biological systems if not countered. Similarly, ectopic expression of thioredoxin in the periplasm converts this enzyme into an oxidant catalyst capable of replacing DsbA in the oxidative protein-folding pathway [13]. This is because as in the previous example, thioredoxin becomes insulated from its reductant thioredoxin reductase.

3.2. The E. coli GSH and thioredoxin pathways are functionally redundant

The two branches of the thiol redox system are functionally redundant in *E. coli* [14] (Table 1). Neither branch alone appears to be required for normal aerobic growth. However, inactivation of both pathways is unviable. What is the cause of this lethality? Two mechanisms were suspected: either a toxic accumulation of disulfide bonds in the cytoplasm, a condition often referred to as disulfide stress, or a defect in the reduction of a protein essential for viability. As we will see, it has taken decades to formally ascribe the essential function of the *E. coli* thiol redox system to the reduction of a unique disulfide bond that forms in the enzyme ribonucleotide

Table 1
Phenotypes of thiol redox mutants in *E. coli*

Mutant	Phenotype	Rescued by	Defects
gsha	nl[a]		
gora	nl		
trxatrxbtrxc	nl		
grxa	nl		
trxagrxa	Special growth requirements	Organic sulfur	Sulfate assimilation
trxagsha	Similar to *trxagrxa*	Similar to *trxagrxa*	Similar to *trxagrxa*
trxbgsha	Barely viable	DTT or anaerobic	Ribonucleotide reduction
trxbgora	Like *trxbgsha*	Like *trxbgsha*	Like *trxbgsha*
trxatrxcgsha	Unviable aerobically	DTT, anaerobiosis	Ribonucleotide reduction
trxatrxcgrxa	Unviable aerobically	Anaerobiosis	Ribonucleotide reduction

Mutations of both thioredoxin and GSH pathways require a source of organic sulfur for growth due to defective sulfate assimilation. For references, see text.
[a]nl: Normal vegetative growth.

reductase (RNR). Reduction of ribonucleotides to deoxyribonucleotides (dNTPs) by RNR is balanced by formation of a disulfide bond in the enzyme that must be reduced for ongoing catalysis (for a review, see [15]).

Mutants lacking both *TrxA* and *GrxA* or *TrxA* and *GshA* are unviable because of a toxic accumulation of 3′-phosphoadenosine 5′phosphosulfate (PAPS) [16], an intermediate metabolite in sulfur assimilation (Table 1). However, this lethal phenotype is fully reversed by a high concentration of organic sulfate or by deletion of *CysA* or *CysC*, which either represses or inactivates the sulfate assimilation pathway, and therefore inhibits PAPS production. PAPS is reduced to sulfite by the action of PAPS reductase, which is itself recycled back by NADPH-dependent reduction by thioredoxin or glutaredoxin [17]. Therefore, TrxA or Grx1, but not TrxC, Grx2 or Grx3, is adequate for reduction of PAPS reductase and sulfate assimilation (see Fig. 1).

The *trxatrxcgrxa* triple mutant is also aerobically unviable [12], but this phenotype is due to defective RNR reduction, as the anaerobic viability of the mutant strain suggests. Under anaerobiosis, *E. coli* uses an alternate RNR that requires formate as the hydrogen donor, instead of the thiol redox system [18,19]. Therefore, TrxA, TrxC, or Grx1 but not Grx2 and Grx3 are required for RNR reduction. Likewise, mutants lacking *TrxB* and either *GshA* or *Gor*, or lacking or *TrxA*, *TrxC*, and *GshA* are unviable aerobically, but not anaerobically [12,14]. It is just recently that the essential function of the *E. coli* thiol redox system has been formally established by genetically suppressing the *trxatrxcgrxc* growth defect [20]. Random mutations in Grx3, a very poor RNR reductant (see above and Fig. 1), were selected for their ability to restore growth to the mutant strain, identifying Grx3 mutations that increased its catalytic efficiency towards RNR reduction. Extragenic suppressors of the *trxatrxcgrxc* growth defect were also sought, revealing further insights into the interaction of Grx3 with RNR. Three independent suppressor mutations were selected that resulted in increased expression of RNR, and which had the effect of allowing reduction of the enzyme by Grx3. Thus, increasing either the catalytic efficiency of Grx3 or the abundance of its low-specificity substrate RNR, allowed sufficient reduction of RNR to rescue the growth of the *trxatrxcgrxc* strain.

Interestingly, a mutation allowing the *trxbgor* strain to grow aerobically occurs at very high frequencies. Such suppressor strain is still able to catalyze disulfide bonds in ectopically expressed AP in the cytoplasm, indicating that disulfide stress is not lethal per se. Cloning of the suppressor mutation identified a new source of disulfide-reduction power and a surprising genetic twist. The suppressor mutation was mapped to the *ahpC* gene encoding a peroxiredoxin and is the result of a reversible expansion of a triplet nucleotide repeat sequence that convert the AhpC protein from a peroxidase to a disulfide reductase.

3.3. Functions of the E. coli thiol redox system

Biochemistry and genetics have clearly established RNR and PAPS reductase as substrates of the thiol redox systems. However, many other substrates do exist for this system (see Fig. 1), which do not show up in genetic approaches either because of lack of an overt phenotype or its masking by a dominant one. A proteome-wide biochemical identification of in vivo oxidized protein thiols in *E. coli* elegantly helped visualize an important number of known, suspected or unsuspected substrates of thioredoxin [21]. Thus, the thiol redox system is of biological importance in many other processes, as for instance in cellular detoxification pathways for various kinds of electrophiles and other toxics. In most aerobic organisms, thiol redox systems have also crucial functions in peroxide scavenging by providing the reducing power to all thiol and selenothiol-peroxidases, the peroxiredoxins and glutathione peroxidases (GPx) (see below). In contrast, *E. coli* does not carry GPx enzymes and bypasses the GSH and thioredoxin pathways by using as major peroxide-scavenging enzyme a peroxiredoxin, AhpC, which carries its own specific NADPH-dependent reductase AhpF [22,23].

4. The *S. cerevisiae* thiol redox system

The yeast thiol redox system is both very similar to the *E. coli* system, also consisting of the thioredoxin and GSH pathways, but much more complex and carrying important differences with its prokaryotic counterpart, which makes it probably closer to higher eukaryotes.

4.1. Description of thiol redox pathways

The yeast GSH pathway consists of GSH, which is synthesized by the action of γ-glutamylcysteine synthetase (*GSH1*) and glutathione synthase (*GSH2*) (for a review see [24,25]), one glutathione reductase (*GLR1*) and of two classes of glutaredoxins (Fig. 2). The catalytic center of the two dithiol glut-

aredoxins Grx1 and Grx2 consists of the CPYC motif [26,27], whereas that of the three monothiol enzymes, Grx3, Grx4 and Grx5, is CGFS [28]. Grx3 and Grx4 carry also an extra N-terminal thioredoxin domain with a WAD/EPCK motif reminiscent of the WCGPCK thioredoxin catalytic motif. *S. cerevisiae* has two thioredoxin pathways, a cytosolic one consisting of two apparently redundant thioredoxin isoforms (Trx1 and Trx2) [29] and a thioredoxin reductase (Trr1) [30], and a mitochondrial one consisting of Trx3 and Trr2 [31] that will not be dealt here.

4.2. The genetics of the S. cerevisiae thioredoxin pathway

Although both single and double *TRX1* and *TRX2* mutants are viable, Δ*trx1*Δ*trx2* has an extended S phase that is not seen in either single mutant [29] (Table 2). This protracted S phase is due to inefficient RNR reduction. This has recently been established on the basis of a significant decrease of the dNTP pools [32,33], an absolute decrease of the RNR reduced form and an increase of its oxidized disulfide bond form as visualized by its in vivo redox state, and on the effect of the overexpression of RNR that accelerates the S phase and restores both the dNTPs pool and the abundance of the reduced RNR form [32]. Viability of Δ*trx1*Δ*trx2* thus indicates that the GSH pathway can operates RNR reduction, but the protracted S phase also indicates that Grx1 and Grx2 are not as efficient as the thioredoxins in RNR reduction. The lethality of a quadruple mutant lacking cytoplasmic thioredoxins and dithiol glutaredoxins [34] supports the redundant role of both dithiol transferase pairs in RNR reduction, although the cause of this lethality has not been formally established. The Δ*trx1*Δ*trx2* is also auxotroph for sulfur amino acids indicating that thioredoxin is the exclusive hydrogen donor for PAPS reductase [29]. Nevertheless growth under a very low oxygen tension relieves methionine auxotrophy [34], indicating that dithiol glutaredoxins can substitute for this function, albeit very inefficiently. Δ*trx1*Δ*trx2* is also unable to use methionine sulfoxide as a source of organic sulfate [35] because of defective reduction of this oxidized form of methionine, is hypersensitive to peroxides [36] and has a deregulated Yap1 pathway [37,38], phenotypes that are related to the role of thioredoxin in reducing methionine sulfoxide reductase [39,40], the yeast thiol peroxidases and Yap1 respectively (see below). A search for in vivo substrates of thioredoxin by purification of thioredoxin-interacting proteins and by a directed two-hybrid assay using as bait a thioredoxin mutant lacking the CGPC motif C-terminal cysteine residue that stabilizes interaction with substrates, identified PAPS reductase (Met16), the peroxiredoxins Tsa1 and Ahp1 [41] amongst a few other proteins, confirming some of the phenotypes of the thioredoxin mutant strain.

Interestingly, cells lacking cytoplasmic thioredoxin reductase [42], do not carry the cell cycle and sulfate assimilation phenotypes of Δ*trx1*Δ*trx2* suggesting that they retain some thioredoxin activity (Spector and Toledano, unpublished observations). However, they are very slow growing [43] especially under aerobic conditions and are hypersensitive to peroxides (see below). The Δ*trr1* slow growth phenotype is at least in part related to toxic accumulation of oxidized thioredoxin, as deletion of both *TRX1* and *TRX2* in Δ*trr1* improves its growth (Spector and Toledano, unpublished observations). Thioredoxin reductases, as thioredoxin, are also required for the response to reductive stress imposed by dithiothreitol [43].

4.3. The genetics of the S. cerevisiae GSH pathway

A major difference with the *E. coli* system is that strains with a deleted *GSH1* and thus lacking GSH are unviable both aerobically and anaerobically, only growing with exogenously added GSH [44–46] (Table 2). GSH has thus an essential function not shared with the thioredoxin pathway. In an attempt to understand this essential function, a search for genetic suppressors of the GSH auxotrophy was conducted, only yielding mutations that restored biosynthesis of very low levels of GSH by the abduction of the proline biosynthetic pathway [47]. The cause of the essential requirement for GSH is very puzzling; it is not related to oxidative stress, or to defective DNA synthesis or sulfate assimilation or to disulfide stress [47], but at least in part to a defect in iron–sulfur cluster ([Fe–S]) assembly as detailed below. The GSH precursor γ-glutamylcysteine can substitute for GSH, although only

Table 2
Phenotypes of thiol redox system mutants in *S. cerevisiae*

Mutant	Phenotype	Rescued by	Defects
Δ*trx1*	nl[a]		
Δ*trx2*	nl		
Δ*trr1*	Slow growth		
Δ*trx1*Δ*trx2*	Protracted S phase, Met auxotrophy	RNR overexpression	Sulfate assimilation, Ribonucleotide reduction
Δ*trr1*Δ*trx1*Δ*trx2*	Similar to Δ*trx1*Δ*trx2*	Similar to Δ*trx1*Δ*trx2*	Similar to Δ*trx1*Δ*trx2*
Δ*glr1*	nl		Accumulates GSSG
Δ*grx1*Δ*grx2*	nl		
Δ*grx1*Δ*grx2*Δ*trx1*Δ*trx2*	Unviable		Ribonucleotide reduction
Δ*trx1*Δ*trx2*Δ*glr1*	Unviable aerobically	Anaerobic weak growth	Complex
Δ*trr1*Δ*glr1*	Unviable aerobically		Complex
Δ*trx1*Δ*trx2*Δ*gsh1*	Unviable aerobically	ND	Complex
Δ*grx2*Δ*grx5*	Unviable		Unknown
Δ*gsh1*	GSH auxotrophy	0.5 μM GSH	Cytoplasmic [Fe–S] assembly
Δ*grx1*Δ*grx2*Δ*grx3*Δ*grx4*	nl		
Δ*grx5*	Slow growth	Grx3 and Grx4 targeted to mitochondria	Mitochondrial [Fe–S] assembly
Δ*grx3*Δ*grx4*Δ*grx5*	Unviable		[Fe–S] assembly
Δ*grx3*Δ*grx4*Δ	Slow growth		Cytoplasmic [Fe–S] assembly

[a]nl: Normal vegetative growth. All defect presented are hypothetical and have not been yet demonstrated experimentally. For references, see text.

partially [44,48], but not DTT or the overexpression of thioredoxin that only have the effect of delaying the time needed for exhaustion of the cellular GSH pool [47,49,50].

Glutathione reductase in contrast is totally dispensable [51], despite the essential requirement for GSH, indicating that GSSG, which accumulates in the Δ*glr1* strain [36], is not deleterious. *S. cerevisiae* probably carries an alternate GSH reducing system since reduced GSH represents up to 40% of the total amount of GSH in Δ*glr1*.

The genetics of glutaredoxins is complex [27,28]. Dithiol and monothiol glutaredoxins have not only important structural differences but also distinct functions, the later sharing with GSH a function in [Fe–S] assembly (see below). Dithiol glutaredoxins presumably catalyze disulfide bond reduction, capable of replacing thioredoxin for RNR reduction, but their specific in vivo substrates are still unknown. The lethality of the quadruple mutant lacking both cytoplasmic thioredoxins and dithiol glutaredoxins [34] indicate that monothiol glutaredoxin cannot substitute for their dithiol counterparts. Grx5 is located in mitochondria where it fulfills its Fe–S assembly function whereas Grx3 and Grx4 fulfill a related function in the cytoplasm. All single glutaredoxin genes mutants are viable, but Δ*grx5* has a unique slow growth phenotype with respect to the other mutants [28]; it is unable to respire, and accumulates iron and oxidized proteins at very high levels, phenotypes linked to the function of Grx5 in [Fe–S] assembly. A strain lacking all glutaredoxins but Grx5 is viable demonstrating the importance of this enzyme, but strains lacking Grx5 together with either Grx2 or Grx3 and Grx4 are lethal. These data suggest that Grx5 has at least two essential functions; one of them, the nature of which is unknown, can be rescued by Grx2 and the other, presumably related to [Fe–S] assembly is rescued by either Grx3 or Grx4. A strain lacking both Grx3 and Grx4 is also unviable [52,53], demonstrating that the function of the cytoplasmic monothiol glutaredoxin is as important as that of their mitochondrial counterpart. Thus, monothiol glutaredoxins probably can, but only partially, substitute for each other in their respective [Fe–S] assembly function.

4.4. Genetic interplay between the thioredoxin and glutathione pathways

The existence of a functional overlap between the two branches of the thiol redox system is difficult to establish, because, unlike *E. coli*, ribonucleotide reduction is not the only unique function that makes the system essential. Further yeast does not have as *E. coli* an anaerobic RNR that bypasses the thiol redox system. As mentioned above, the GSH pathway can, albeit inefficiently, substitute for the thioredoxin pathway in RNR reduction, explaining the unviable phenotype of the quadruple mutant lacking both thioredoxins and both dithiol glutaredoxins [34]. However, the GSH pathway has also an exclusive essential function in [Fe–S] assembly unrelated to thiol redox homeostasis. Furthermore, as detailed below and unlike *E. coli*, the thioredoxin pathway has a prominent role in peroxide catabolism that is not properly compensated by the GSH pathway, probably explaining that in strains with an inactivated thioredoxin pathway, the GSH redox state becomes significantly oxidized [36,43], which might impinge on its essential function. In fact, for these reasons all mutants with inactivation of both pathways are unviable probably due to collapse of multiple essential defects in RNR reduction, [Fe–S] assembly, and disulfide stress (Table 2). The essential requirement of *GLR1* in a strain lacking thioredoxin (Δ*trx1*Δ*trx2*) [36] is the unique situation in which the lethal defect can be ascribed to disulfide stress, because of the growth rescue of the strain under anaerobiosis. This might indicate that in the absence of thioredoxin, reduced GSH can and becomes critically required to compensate for the defect of thioredoxin in peroxide catabolism, and conversely that in the absence of glutathione reductase, thioredoxin is needed for direct or indirect reduction of GSH.

4.5. The yeast thioredoxin pathway has a prominent role in peroxide catabolism

Cells are permanently exposed to reactive oxygen species that are produced during respiration or that originate from exogenous sources. Both superoxide dismutases and catalases, which scavenge the superoxide anion and H_2O_2, respectively, have an autocatalytic mechanism, whereas thiol- and selenothiol-peroxidases require a reducing power to scavenge peroxides provided by either the thioredoxin or the GSH pathways. Which of the GSH or the thioredoxin pathways assist the scavenging of peroxides in yeast?

Yeast has two thiol-peroxidases family enzymes (for a review see [54]). The peroxiredoxins (Prxs) consists of five isoenzymes (Tsa1, Tsa2, Ahp1, nTpx, and mTpx) that are strictly dependent upon thioredoxin for reduction of peroxides [30]. The GPx-like Gpx1, Gpx2, and Gpx3 enzymes are also strictly thioredoxin-dependent despite their name [55,56]. Peroxides oxidize the methionine residue to the methionine sulfoxyde form that is catalytically reduced by methionine sulfoxyde reductase (MSR), which forms a catalytic disulfide that is reduced by thioredoxin [57,58]. Thus, the two *S. cerevisiae* MSRs, MsrA also known as Mxr1 and MrsB, must also be considered as peroxide-reducing enzymes that are strictly thioredoxin-dependent. Likewise, the Yap1 transcriptional regulator of the yeast peroxide response, which is activated by peroxides by oxidation and deactivated by reduction by thioredoxin [37,38], must also be considered. It thus appears that, based on biochemical activities, the thioredoxin pathway has a prominent role in peroxide metabolism in yeast, as it exclusively assists the major yeast peroxide-metabolism pathways. What about the GSH pathway?

GSH cannot react with peroxides in vivo because of its very low reactivity towards these compounds [59] and do not participate in peroxide scavenging by GSH-dependent GPx enzymes due to lack of these enzymes in yeast. Nevertheless, GSH may indirectly participate to this metabolism by forming *S*-glutathionylated adducts with protein-sulfenic acids formed by oxidation of thiols with peroxides, thereby protecting them from irreversible oxidation to the sulfinic or sulfonic acid forms. This activity might be significant given the abundance of this redox-active tripeptide in the cell, in the mM range, as suggests the increase in the GSSG levels and in the glutathionylation of gluceraldehyde 3-phosphate dehydrogenase and of other protein thiols in cells exposed to H_2O_2 [60,61]. The glutaredoxins Grx1 and Grx2 have been reported to act as peroxide-reducing enzymes [62], by a GSH-*S*-transferase mechanism involving GSH, glutathione reductase and the Ycf1 vacuolar ATP-dependent GS-X pump. However, the modest catalytic efficiency ($K_{cat}/K_m = 2–6 \times 10^4\ M^{-1}\ S^{-1}$) together with a high

K_m for the substrate in the mM range [62] suggest that these enzymes act as minor peroxidases and only when the intracellular peroxide concentration is very high.

Tolerance phenotypes correlate with biochemical data also pointing to a predominant role of the thioredoxin pathway in H_2O_2 metabolism. Although inactivation of the thioredoxin [36,63,64] or GSH [44,45,47,65] pathways lead to peroxide sensitivity, mutants of the former pathway are much more severe phenotypes [64]. In fact, whereas the thioredoxin pathway, by being actively involved in H_2O_2 catabolism, is critically required for growth in the presence of H_2O_2, the GSH pathway seems important for survival upon an acute H_2O_2 challenge [47], in keeping with its suggested redox buffer function.

The prominent role of the thioredoxin pathway in H_2O_2 metabolism is also suggested by a proteomic analysis of oxidized protein thiols [66]. This study showed that the *S. cerevisiae* cytoplasm contains as much as 200 proteins carrying one or more oxidized cysteine residue. Surprisingly, the oxidized protein thiols proteome of strains lacking either the thioredoxin or the GSH pathways were strikingly different. Thioredoxin pathway mutants had both a specific increase in the oxidation of many proteins and the appearance of newly oxidized proteins. Many of these oxidized proteins were H_2O_2-metabolizing enzymes such as Tsa1, Tsa2, Ahp1, Ntpx, Gpx2, and Mxr1, suggesting an important role of the thioredoxin pathway in H_2O_2 metabolism. Interestingly, all new oxidized spots corresponded to proteins expected to be oxidized according to their function, ruling out random disulfide bond formation that could have potentially resulted from inactivation of the thioredoxin pathway. In contrast, inactivation of the GSH pathway led to a general decrease of protein oxidation that was not modified by exogenous H_2O_2, and which was interpreted as reflecting the irreversible oxidation of protein thiols, which might have occurred due to the lack of GSH, in keeping with its general redox buffer function.

4.6. Is the essential function of the yeast GSH pathway related to iron metabolism?

In contrast to prokaryotes, GSH is essential for growth in *S. cerevisiae* and also in all eukaryotes where its requirement has been looked at. Identifying the essential biological process (es) in which this redox-active tripeptide takes part is a major biological question but also a long-standing puzzle.

One answer to this question may lie in the cellular [Fe–S] clusters biogenesis machinery and iron homeostasis. Chronologically, the first hint to the function of GSH in iron metabolism was the observation that GSH depletion elicits a specific defect in the maturation of cytoplasmic [Fe–S] proteins and to iron cellular accumulation of [67], and reciprocally cells with a defect in cytoplasmic Fe–S protein maturation accumulate GSH [68]. The second hint came from the phenotypes of the Δ*grx5* that associates defective mitochondrial [Fe–S] proteins assembly, cellular iron accumulation and the inability to respire [69]. Suppression of the Δ*grx5* phenotypes by overexpression of two genes involved in [Fe–S] cluster-protein biogenesis indicated that Grx5 operates as part of this machinery. The actual role of Grx5 is not yet elucidated but could possibly be to facilitate the transfer of clusters preassembled on the Isu1/2 scaffold proteins onto acceptor proteins [70]. A third, and probably the major piece of this jigsaw puzzle is a recent discovery tying together monothiol glutaredoxins and GSH into the same molecular process in [Fe–S] metabolism [71–74]. Dithiol mammalian mitochondrial Grx2 [72,73] and poplar cytoplasmic glutaredoxin C1 [71] holenzymes exist as dimers bridged by a [2Fe–2S] cluster that is ligated by the catalytic cysteines of the two glutaredoxins of the dimer and unexpectedly by the cysteines of two GSH molecules. A glycine residue located right after the first catalytic Cys residue is crucial for poplar Grx-C1 to assemble a [Fe–S] cluster. The presence of a conserved glycine residue in the CGFS motif of yeast monothiol glutaredoxins together with the lack of a role of the Grx-C1 second active-site Cys residue strongly suggest that Grx3, Grx4 and Grx5 are all able to incorporate [Fe–S] clusters [74]. The last important recent discovery is the role of both GSH and the redundant nucleocytoplasmic monothiol glutaredoxins Grx3 and Grx4 in iron sensing by the Aft1 transcriptional regulator of iron homeostasis [52,53,75]. Aft1 indirectly senses iron through the cellular [Fe–S] biogenesis status [75–78], and is thus activated when [Fe–S] biogenesis decreases as a result of either cellular iron depletion or a crippled [Fe–S] biogenesis machinery. Lack of either GSH or both Grx3 and Grx4 lead to a constitutive activation of Aft1 under iron repletion conditions, indicating that they are probably needed to signal to Aft1 the [Fe–S] biosynthetic status [52,53].

How can these data be drawn into a unifying model? It is first important to consider some basics of the biosynthesis of [Fe–S] proteins. The mitochondrion is required for maturation of both mitochondrial and cytoplasmic [Fe–S] proteins (for a review, see [79]). Maturation of cytoplasmic of [Fe–S] proteins further requires the mitochondrial export of an as yet unidentified component and the recently discovered cytoplasmic [Fe–S] assembly system termed CIA [80–86]. Further the mitochondrial export of the elusive component requires the mitochondrial inner membrane ATP-binding cassette (ABC) transporter Atm1, the ERO1-related mitochondrial intermembrane space FAD-sulfydryl oxidase Erv1p, and GSH. The elusive component is probably translocated through ATM, but the function of both Erv1 and GSH in this process is unknown. Interestingly, Erv1 has recently been shown to function together with Mia40 in a thiol redox relay that constitutes a specific IMS import machinery [87].

A speculative model of this pathway is proposed (Fig. 3), which is based on the existence of the suspected GSH-ligated [Fe–S] cluster of yeast monothiol glutaredoxins that might serve as [Fe–S] shuttles within and outside mitochondria. It thus supposes that all functions of GSH and monothiol Grxs in iron metabolism are shared through a unique function. In mitochondria, a GRx5-GSH [Fe–S] cluster would transfer [Fe–S] cluster from Isu1/2 scaffold proteins onto acceptor proteins, and from mitochondria onto GSH-Grx3 or GSH-Grx4 in the cytoplasm, thus constituting, at least part of both the missing exported component of the CIA machinery, and the signal alerting to Aft1 the mitochondrial [Fe–S] biogenesis status. The lack of defective mitochondrial [Fe–S] biogenesis in GSH depleted cells is against an [Fe–S] assembly function of GSH in mitochondria [75], but in the cells used in these assays, traces of mitochondrial GSH might have remained in amounts sufficient to perform this essential function. The combined role of GSH, Grx3, and Grx4 is strongly suggested by the importance of the GSH-binding pocket of glutaredoxins in the ability of the later to signal to Aft1 [52]. The functions of GSH and monothiol Grx suggested here might underlie their essential requirement for yeast viability because [Fe–S] biogenesis is essential for life [79].

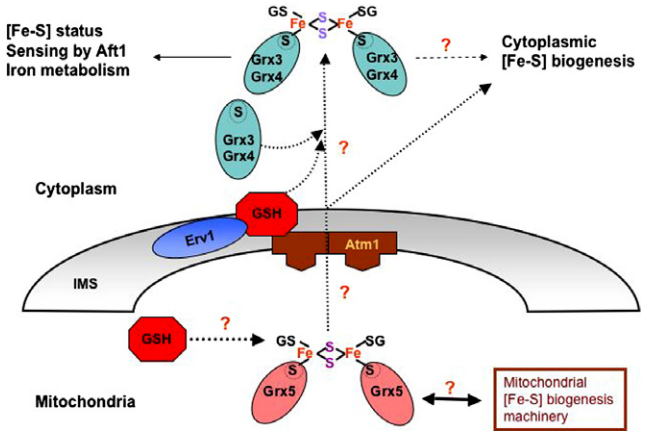

Fig. 3. A speculative model of the function of glutathione and monothiol glutaredoxin in iron–sulfur assembly in yeast. The model is based on the hypothetical existence of GSH-ligated [Fe–S] cluster of yeast monothiol glutaredoxins that might serve as [Fe–S] shuttles within and outside mitochondria. [Fe–S] clusters are synthesized by the mitochondrial [Fe–S] biosynthetic machinery and transferred to acceptor proteins. Mitochondria export an unknown component emanating from the [Fe–S] biogenesis machinery, which requires the mitochondrial inner membrane transporter, Atm1, the mitochondrial intermembrane space (IMS) protein Erv1, GSH and an intact [Fe–S] machinery. This unknown component, which is needed both for [Fe–S] status sensing by Aft1 and for cytoplasmic [Fe–S] assembly, might involve at least in part the hypothetical Grx-GSH-ligated [Fe–S] complexes. In mitochondria, a GRx5-GSH [Fe–S] cluster could also be involved in the transfer of [Fe–S] cluster from Isu1/2 scaffold proteins onto acceptor proteins. For references and further explanations, see text.

5. Comparison of the *E. coli* and yeast thiol redox systems

In *E. coli* the thioredoxin and GSH pathways are functionally redundant and therefore not essential individually (Fig. 1). They also operate in a virtually identical manner, using electrons from NADPH to reduce by a thiol-disulfide exchange mechanism catalytic disulfide bonds, some of which are essential as in RNR and other are not as in PAPS reductase, and to prevent the occurrence of unwanted disulfide bonds. Redundancy is presumably due to the fact that each pathway can substitute for the other for important or essential functions, which raises the question of why does *E. coli* carry two so closely related systems. Notwithstanding, substrate-specificity exists within and between these branches (see Fig. 1); specificity is established through the use of sets of terminal thioltransferases, the thioredoxins and glutaredoxins, which differ from each other by their abundance, redox potential and affinities towards substrates. Remarkably, the *E. coli* thiol redox system is only essential under aerobiosis, and this is uniquely due to its need for RNR reduction and DNA synthesis. It is dispensable under anaerobiosis provided an exogenous source of organic sulfate, and this dispensability is not due to relieve of the low levels oxidative stress of aerobic growth, but to a switch to anaerobic RNR that uses an alternate reducing power source. Disulfide stress, or the accumulation of unwanted disulfide bonds, is fully compatible with life but might impinge to some degrees on the essential role of the thiol redox system under extreme conditions.

Although consisting of a GSH and thioredoxin pathways and fulfilling many of the same functions, the *S. cerevisiae* thiol redox system is clearly different from that of *E. coli*. The two branches of this system are both critically required under both aerobiosis and anaerobiosis and are not functionally redundant, each having preferred or exclusive targets some of which are critical for life. Thus, the *S. cerevisiae* thioredoxin is the preferred reductant of RNR and PAPS reductase and has a prominent role in H_2O_2 metabolism with regard to the GSH pathway. The GSH pathway provides the redox buffering function of GSH, the biological importance of which is not yet clearly established in yeast. More importantly this pathway has been abducted to operate an essential function in [Fe–S] metabolism totally distinct from thiol redox control, thus providing what could be a [Fe–S] shuttle constituted by monothiol glutaredoxins and GSH. Whether a similar function of GSH and glutaredoxin exist in prokaryotes is possible in view of the functional complementation of the yeast Δ*grx5* strain by the *E. coli* monothiol Grx4 [88], a protein of yet no known function that carries the CGSF of poplar Grx-C1 [8].

6. Conclusions

The thiol redox system appears as a highly efficient proton-shuttle system that helps cell to domesticate the oxidizing power of O_2, but because of its intrinsic reactivity it can also become a double-edge sword under some circumstances, leading to unwanted redox reactions. Its role in vivo has been established mainly by the effect of inactivating one or more of its components. However, genetics is necessarily fraught with caveats. Unviability, which is often seen in the genetics of thiol redox control pathways lead to insoluble biological cul-de-sacs, as in the case of the biochemical functions of the GSH pathway. The caveats are also in the interpretation of data, because removing a thiol redox component of one of the two pathways will necessarily perturb the overall thiol redox control equilibrium of the cell. However, such perturbations also suggests the interdependence between the GSH and thioredoxin pathways, especially in yeast in which these pathways are non-redundant. The dynamic interplay between redox systems has not been clearly established because of the cited experimental difficulties, but might be important especially under oxidative stress, conditions under which a very important load is applied on the system. A dynamic interplay necessarily exists as suggested by the presence of an oxidized GSH redox ratio in mutants of the thioredoxin pathway [43]. The activation the unfolded protein response (UPR) of the ER in these same mutants [89] also suggest an interplay between cytoplasmic thiol redox control and the process of oxidative protein folding in the ER. Note also that GSH has an important function in regulating the thiol redox balance in the lumen of the ER and hence the functionality of the Ero1-dependent oxidative protein folding pathway that has not been considered here. In this cell compartment, GSH acts as a load against oxidation by Ero1 [90], and as recently shown is also required for feedback redox regulation of Ero1 activity [91]. The effects of inactivating the thioredoxin pathway on the activation of the UPR could thus be mediated by GSH, the absolute levels of which are significantly increased in these mutants and that is present in both compartments unlike thioredoxin.

The yeast thiol redox system is thus much more complex than its prokaryotic counterpart, and probably represents a very good model of this system in higher eukaryotes. The yeast model should thus be useful to begin to rationalize the lethal phenotypes associated with the mouse knock-outs of thioredoxin 1 [92], thioredoxin 2 [93], mitochondrial and cytoplasmic thioredoxin reductases [94,95] and γ-glutamyl synthase [96], and the defect of monothiol glutaredoxin 5 in human and the zebra fish that leads to defective heme synthesis, anemia and iron overload [97,98].

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