Glutaredoxins and iron-sulfur protein biogenesis at the interface of redox biology and iron metabolism

Abstract: The physiological roles of the intracellular iron and redox regulatory systems are intimately linked. Iron is an essential trace element for most organisms, yet elevated cellular iron levels are a potent generator and amplifier of reactive oxygen species and redox stress. Proteins binding iron or iron-sulfur (Fe/S) clusters, are particularly sensitive to oxidative damage and require protection from the cellular oxidative stress protection systems. In addition, key components of these systems, most prominently glutathione and monothiol glutaredoxins are involved in the biogenesis of cellular Fe/S proteins. In this review, we address the biochemical role of glutathione and glutaredoxins in cellular Fe/S protein assembly in eukaryotic cells. We also summarize the recent developments in the role of cytosolic glutaredoxins in iron metabolism, in particular the regulation of fungal iron homeostasis. Finally, we discuss recent insights into the interplay of the cellular thiol redox balance and oxygen with that of Fe/S protein biogenesis in eukaryotes.

Keywords: CIA machinery; glutathione; ISC machinery; thioredoxin and glutaredoxin systems; transcriptional regulation.

Introduction

Iron is a key metal in biology. It functions as an essential co-factor in central cellular processes such as photosynthesis, respiration, metabolite conversion, DNA synthesis and repair, ribosome biogenesis, vertebrate oxygen transport, and the sensing of environmental cues. Iron ions are associated with proteins in mononuclear or dinuclear form, most commonly at the side chains of cysteine, histidine, aspartate, or glutamate residues. Occasionally, inorganic ligands such as molecular oxygen, CN\(^{-}\), or CO are additionally involved in iron coordination. Moreover, iron is bound to proteins after being incorporated into heme cofactors or into iron-sulfur (Fe/S) clusters. The latter moieties are particularly sensitive to oxidation by oxygen and/ or reactive oxygen species (ROS) and require protection from the cellular oxidative stress protection systems particularly under stress conditions (Figure 1). The delicate sensitivity of Fe/S clusters is prominently utilized by bacteria that employ transcription factors with sensitized Fe/S centers for orchestrating their adaptation to ambient oxygen and/or oxygen radical levels (Crack et al. 2014, 2018).

In all organisms, the maturation of Fe/S proteins essentially relies on complex proteinaceous biogenesis machineries. These systems, too, are particularly susceptible to changes in the redox status of the cell. Most non-plastidial eukaryotes utilize the mitochondrial iron-sulfur cluster assembly (ISC) system for the de novo synthesis of Fe/S clusters of cellular Fe/S apoproteins (Lill 2009; Lill and Freibert 2020). The formation of cytosolic and nuclear Fe/S proteins further
requires the cytosolic iron-sulfur protein assembly (CIA) system (Figure 1B) (Giofi-Baffoni et al. 2018; Maio et al. 2020; Paul and Lill 2015). Many of the up to 18 ISC and 11 known CIA components are essential for viability, reflecting the importance of Fe/S proteins for various aspects of cellular physiology. Photosynthetic and few other eukaryotes further possess the sulfur mobilization (SUF) machinery for the formation of Fe/S proteins in plastids that operates independently of the other systems (Connorton et al. 2017; Przybyla-Toscano et al. 2018). Both the ISC and the SUF systems are of bacterial origin, and were inherited by eukaryotes in primary and secondary endosymbiotic events, respectively. Bacteria harboring both machineries (e.g., *Escherichia coli*) preferentially utilize the SUF system under conditions of iron limitation or oxidative stress (Garcia et al. 2019). The fact that plants and algae harbor the SUF system in their plastids likely indicates that this system is more compatible with the numerous side effects of oxygen-evolving photosynthesis. Because all these biosynthesis systems are more or less sensitive to oxidative damage, non-green eukaryotes that depend on the more sensitive mitochondrial ISC system particularly rely on the cellular redox control and oxidative stress protection systems to sustain the maturation of cellular Fe/S proteins (Figure 1) (Gomez et al. 2014; Missirlis et al. 2003; Strain et al. 1998). Moreover, in non-green eukaryotes defects in the mitochondrial ISC system induce cellular iron overload, and a (post-)transcriptional deregulation of cellular iron homeostasis that is similar to that of an iron-deprived cell (Camaschella et al. 2007; Misslinger et al. 2018)). Excess intracellular iron usually accumulates within mitochondria causing oxidative damage which likely further aggravates existing defects in mitochondrial functions, again requiring responses by the cellular redox control and oxidative stress protection systems (Gomez et al. 2014; Hadzhieva et al. 2014; Herrero et al. 2008).

The members of the thioredoxin (Trx) and glutaredoxin (Grx) families make up an extensive array of proteins, which execute central roles in redox biology, including the antioxidant response to oxygen and ROS. Trxs and Grxs are found in nearly all known organisms, where they are located...
in various cellular organelles (Figure 1A) (Jacquot and Zaffagnini 2019; Lu and Holmgren 2014). The yeast Saccharomyces cerevisiae encodes three thioredoxins, Trx1, Trx2, and Trx3, and four class I dithiol glutaredoxins, Grx1, Grx2, Grx8 and Mgp12 (Herrero et al. 2010). These proteins facilitate the reduction of other proteins by cysteine thiol-disulfide exchange. They harbor an active site with a dithiol CxxC motif that is essential for function. Trxs further act as electron donors to, e.g., peroxidases thereby linking cellular redox control with oxidative stress response (Figure 1). The related dithiol Grxs share many of the functions of Trxs, but are reduced by the tripeptide glutathione (GSH) which functions as a central reservoir for upholding the cellular thiol redox balance (Deponte 2017; Hanschmann et al. 2013; Jacquot and Zaffagnini 2019; Lillig and Berndt 2013; Lillig et al. 2008; Toledano and Huang 2017). GSH furthermore cooperates with, e.g., glutathione peroxidases (Gpx) and glutathione S-transferases and thus contributes significantly to the cellular response to ROS. Oxidized glutathione (GSSG) and Trxs are reduced by cognate NADPH-dependent reductases (Figure 1A). Simple eukaryotes such as S. cerevisiae tolerate the deletion of either their glutaredoxin reductases (Glr) or thioredoxin reductases (Trr), indicating that these proteins have partially overlapping functions. Yet, the simultaneous deletion of all genes encoding Trrs and Glrs, just like deletion of all Trx- and Grx-encoding genes is lethal for S. cerevisiae (Herrero et al. 2010; Herrero et al. 2008).

Several members of the cellular thiol redox systems play central roles in the biosynthesis of Fe/S proteins. Of these, GSH is an indispensable compound for the maturation of cytosolic and nuclear Fe/S proteins (Figures 1 and 2) (Kumar et al. 2011; Li and Cowan 2015; Lill et al. 2015; Schaedler et al. 2014, 2015; Sipos et al.; Srinivasan et al. 2014; Wachnowsky et al. 2018). Moreover, the mitochondrial glutaredoxin Grx5 and the cytosolic Grx3 serve as intermediate Fe/S cluster trafficking proteins in the mitochondrial ISC and the cytosolic CIA systems (Figures 1 and 2) (Berndt and Lillig 2017; Braymer and Lill 2017; Jia et al. 2020; Lill and Freibert 2020; Rouhier et al. 2010). These proteins belong to the class II Grxs with a conserved monothiol CGFS active-site motive and are, in contrast to most dithiol Grxs, not particularly active in catalyzing dithiol-disulfide redox reactions (Berndt and Lillig 2017; Lillig et al. 2008). Additionally, S. cerevisiae contains the two cis-Golgi-localized monothiol Grx6 and Grx7 with yet ill-defined cellular roles (Abdalla et al. 2016; Izquierdo et al. 2008; Mesecke et al. 2008) (Figure 1A).

As outlined above, the metabolism of iron and the process of Fe/S protein biogenesis are intimately entangled with the cellular redox control and oxidative stress protection systems. In this review, we summarize the recent developments at the interface of these important cellular pathways in eukaryotes. We first focus on the role of GSH and Grxs as important components in cellular Fe/S protein maturation. Second, we review the recent developments of the roles of cytosolic Grxs in iron metabolism and the regulation of fungal iron homeostasis. Finally, we will address new insights into the interplay between iron and cellular redox control with a focus on how oxygen, ROS, and thiol redox systems impact Fe/S protein biogenesis and Fe/S proteins.

Role of glutathione and mitochondrial glutaredoxins in cellular Fe/S protein maturation

Overview of cellular Fe/S protein assembly pathways

The following is a brief description of eukaryotic Fe/S protein biogenesis. For in depth descriptions, we refer the reader to recent reviews (Ciofi-Baffoni et al. 2018; Dutkiewicz and Nowak 2018; Lill et al. 2015; Lill and Freibert 2020; Maio and Rouault 2015, 2020; Wachnowsky et al. 2018). One of the essential functions of the mitochondrion is the production of Fe/S clusters for the incorporation into Fe/S apoproteins by the mitochondrial ISC system. Maturation begins with the de novo synthesis of [2Fe-2S] clusters at the six-component protein complex of the core ISC system (Braymer and Lill 2017) (Figure 2). This complex comprises a cysteine-bound persulfide (CysS-SH)-generating cysteine desulfurase complex (Nfs1-Isd11-Acp1), a [2Fe-2S] scaffold protein (Isu1) for cluster assembly, reduced ferredoxin, and the Friedreich’s Ataxia-causing protein frataxin (Yfh1) which acts as an allosteric effector (Boniecki et al. 2017; Fox et al. 2019). The three substrates, Fe²⁺, persulfide, and electrons are used to assemble a [2Fe-2S] cluster on the scaffold protein Isu1 (Boniecki et al. 2017; Gervason 2019 #2464; Webert et al. 2014) (Figures 1 and 2). Holo-Isu1 binds its cluster with decent stability and hence is not an efficient Fe/S cluster donor without assistance. In vivo, Fe/S cluster release is assisted by the dedicated Hsp70 chaperone and Hsp40 co-chaperone system (Ssq1 and Jac1 in S. cerevisiae) which directly binds to Isu1 to transfer its bound [2Fe-2S] clusters to the monothiol Grx5. This ISC factor serves as a key Fe/S cluster trafficking protein (Uzarska et al. 2013).
The maturation of cytosolic Fe/S proteins by the CIA pathway involves 11 known proteins (Figure 2) (for a detailed description of the CIA system see (Cioffi-Bafoni et al. 2018; Lill 2020; Maio et al. 2020; Paul and Lill 2015). The CIA factors Cfd1-Nbp35 are proposed to form the central scaffold complex of the CIA system on which the clusters for cytosolic [4Fe-4S] proteins are formed. Fe/S cluster formation on Cfd1-Nbp35 requires the compound X-S (see above) that is produced by the mitochondrial ISC system. Further, electrons are required that are provided by the cytosolic NADPH-Tah18-Dre2-dependent electron transfer chain. Tah18-Dre2 and Cfd1-Nbp35 of the early CIA system work in concert to generate [4Fe-4S] clusters, which are then passed to the CIA factor Nar1. Finally, the CIA targeting complex (CTC) formed by three late-acting CIA components, Cia1, Cia2, and Mms19, facilitates the transfer of [4Fe-4S] clusters into cytosolic and nuclear [4Fe-4S] proteins. The maturation of the ABC protein Rli1 involved in ribosome assembly and recycling further requires the deca-GX3 proteins Yae1-Lto1 that recruit Rli1 to the CTC for [4Fe-4S] cluster insertion. The CIA system is also dependent on cytosolic Grx3/Grx4 that deliver [2Fe-2S] clusters to the CIA factor Dre2 (see below).

**Role of Atm1 and GSH in the maturation of cytosolic and nuclear Fe/S proteins**

The mitochondrial ISC and cytosolic CIA machineries are functionally connected via the mitochondrial ABC transporter Atm1 that exports the sulfur- and possibly iron-containing compound X-S that is produced by the mitochondrial ISC system (Kispal et al. 1999; Pandey et al. 2019). Atm1 has long been known to be involved in cellular iron homeostasis in yeast by performing a specific role in cytosolic Fe/S protein biogenesis (Kispal et al. 1997; Miao et al. 2009). Further support for a crucial role of Atm1 in exporting a substrate for the CIA pathway comes from studies of copper (Cu) stress in the yeast Cryptococcus neoformans. In this situation, Atm1 is overexpressed which may account for the disruption of [4Fe-4S] clusters on cytosolic proteins by Cu (Garcia-Santamarina et al. 2017). Homologues of Atm1 in other eukaryotes are well conserved in function (Bernard et al., 2009; Cavadini et al. 2007; Garcia-Santamarina et al. 2017; Kushnir et al. 2001; Pondarre et al. 2006; Wang et al. 2019; Zuo et al. 2017), including the human pathogenic protozoan parasite Leishmania major (Martinez-Garcia et al. 2016). The essentiality of Atm1 is also evident by its presence in the...
highly reduced mitosomes of the microsporidian species *Encephalitozoon cuniculi* and *Trachipleistophora hominis*, where Fe/S protein biogenesis is proposed to be the sole essential biochemical function of these mitochondria-derived organelles (Braymer et al. 2020; Freibert et al. 2017).

Depletion of GSH induces a similar phenotype as the lowering of Atm1 levels, suggesting that GSH is playing its essential role in both iron regulation and cytosolic Fe/S protein biogenesis, either in the Atm1-assisted export process or as a cofactor of Grx3/Grx4 (Figures 1 and 2) (Kumar et al. 2011; Sipos et al. 2002). There is compelling evidence that the export of the X-S species is dependent on the core ISC system including monothiol Grx5, as well as GSH, which is likely a component of X-S (Figure 2, see review by (Lill et al. 2015)). *In vitro* transport assays have indicated the transport of various substrates by Atm1 including oxidized glutathione persulfide (GSSSG) or GSSG (Schaedler et al. 2014), and a tetra-GSH-bound [2Fe-2S] cluster ([2Fe-2S](GS)₄) (Li and Cowan 2015; Pearson et al. 2020; Qi et al. 2014). Structural and biochemical studies have further observed the binding of GSH and [2Fe-2S](GS)₄ to Atm1, respectively (Pearson et al. 2020; Srinivasan et al. 2014), yet *in vivo* experiments are still awaited to clarify the speciation of the transported species. To this regard, recent reports in yeast suggest that Atm1 is responsible for the export of two different types of sulfur-containing species, i.e. (Fe/S)ₙ for cytosolic Fe/S cluster biogenesis and Sₙ for tRNA thiolidinylation (Pandey et al. 2018; Pandey et al. 2019). While both pathways depend on the mitochondrial desulfurase Nfs1 and the scaffold Isu1, export of Sₙ is observed to be independent of the Hsp70 chaperone complex. These findings, along with the literature on Atm1 as a supplier of X-S for the CIA system support the hypothesis that both cytosolic Fe/S clusters and thiol modifications of tRNA are dependent on mitochondria-produced sulfur substrates (Figure 2) and not from a cytosolic desulfurase activity, as alternatively proposed for human cells (Maio et al. 2020). It is also of interest that the human homologue of Atm1, ABCB7, has been observed to be in complex with ferrochelatase, the last enzyme in heme synthesis (Maio et al. 2019; Taktani et al. 2003). However, a direct connection between Atm1 and heme trafficking remains undefined. The identity and specificity of the transported physiological Atm1 substrate(s) therefore remains to be determined. Their elucidation will have profound implications for iron homeostasis, Fe/S protein biogenesis, tRNA modification, and thiol redox balance.

**Function of the mitochondrial [2Fe-2S] cluster-binding monothiol glutaredoxin 5**

Most eukaryotes harbor the well-conserved mitochondrial monothiol Grx5 (Grx5 in *S. cerevisiae*; GLRX5 in human). This protein is a member of the class of single domain class II Grxs that are found in all kingdoms of life and contain an invariant CGFS active-site sequence motif (Berndt and Lillig 2017; Lillig et al. 2008). Its structure displays the typical Trx fold consisting of five α-helices and four β-sheets. Monothiol Grxs, including Grx5 bind a [2Fe-2S] co-factor that bridges two monomers. The cluster is coordinated symmetrically by the two active-site cysteine residues and the sulfhydryl group of two GSH molecules that are non-covalently bound to Grx5 in a specific GSH-binding pocket (Iwema et al. 2009; Johansson et al. 2011). The [2Fe-2S] cluster on monothiol Grxs is intrinsically labile and its rapid transfer to Fe/S apo-proteins *in vitro* is well documented (Banci et al. 2014; Bandyopadhyay et al. 2008; Berndt and Lillig 2017; Brancaccio et al. 2014; Iwema et al. 2009; Jia et al. 2020; Mapolelo et al. 2013; Moseler et al. 2015; Nasta et al. 2020; Rouhier et al. 2010). Consistent with a general role of monothiol Grxs in Fe/S cluster trafficking, *S. cerevisiae* Grx5 can be replaced by diverse monothiol Grxs with mobile Fe/S clusters from different species *in vivo*, including those with variations in the classical CGFS active site motif (Mapolelo et al. 2013; Molina et al. 2004; Moseler et al. 2015). Yet, most *in vitro* transfer assays involving monothiol Grxs as Fe/S cluster donors reported in the literature were carried out in the presence of the artificial thiol-specific reductants DTT (Banci et al. 2014; Brancaccio et al. 2014; Jia et al. 2020; Sen and Cowan 2017; Wachnowsky et al. 2016). DTT is a cytotoxic chemical that is known to support non-physiological Fe/S cluster-related reactions (Bridwell-Rabb et al. 2014; Gervason et al. 2019; Webert et al. 2014) raising the question in how far the conclusions drawn from these studies are relevant for the situation *in vivo*. *In vitro* studies in which artificial thiol-specific reductants have been avoided in favor of natural ones are so far rare and include the Fe/S cluster transfer from bacterial IscU to Grx5 by the Hsp70 chaperone system (Shakamuri et al. 2012), from cytosolic Grx4 to the transcription factor Aft2 (Poor et al. 2014), and the recent study on human GLRX5 (Weiler et al. 2020). In contrast to reactions involving DTT, *in vitro* Fe/S cluster transfer reactions that avoid this chemical in favor of a natural thiol-specific reducing system such as dithiol Grx/GSH show several features that are in line with *in vivo* observations indicating that such assay systems more faithfully reflect the physiological situation. First, the rate of Fe/S cluster
transfer from GLRX5 to apo-FDX1 is several orders of magnitude higher than those from other mitochondrial Fe/S cluster-binding ISC factors including ISCU2, ISCA1, ISCA2, and NFU1 (Weiler et al. 2020). This kinetic advantage of GLRX5 as a [2Fe-2S] cluster donor makes a similar role of the other ISC factors unlikely. This conclusion is in full agreement with in vivo findings, which confined the role of yeast and human ISCA1, ISCA2, and NFU1 proteins to the maturation of mitochondrial [4Fe-4S] proteins (Lill and Freibert 2020; Muhlenhoff 2011 #52; Sheftel et al. 2012). Second, Fe/S cluster transfer from human GLRX5 to human FDX1, a physiological client protein, is much faster than that to an apo-ferredoxin from a different species. This likely reflects an optimized interaction between physiological ISC donor and apoprotein acceptor pairs in a given organism (Weiler et al. 2020).

Grx5 deficiency in S. cerevisiae, zebrafish, or human diminishes the activities of all mitochondrial and extramitochondrial Fe/S proteins and impairs heme biosynthesis (Camaschella et al. 2007; Muhlenhoff et al. 2003; Rodriguez-Manzaneque et al. 2002; Wingert et al. 2005; Ye et al. 2010). In yeast, Grx5 deficiency is further associated with a massive deregulation of the cellular iron uptake systems, which is a hallmark of all components of the core ISC assembly and export machineries. Moreover, yeast grx5Δ cells lose mitochondrial DNA, a phenotype they share with the late ISC factors Isa1, Isa2, and Iba57 that are essential for the formation of mitochondrial [4Fe-4S] proteins (Molina et al., 2004). The sum of these phenotypes indicates that Grx5 occupies a central position in the assembly line of the mitochondrial ISC system (Figures 1 and 2). Despite this central role and in contrast to several other core ISC components, Grx5 is not essential in S. cerevisiae, indicating that its function can be bypassed. Yet, taken the strong phenotype of grx5Δ cells, this bypass is inefficient. In metazoans where respiration is essential unlike in S. cerevisiae, GLRX5 deletion is lethal, and mutations in GLRX5 are associated with severe mitochondrial disorders in humans (Ast et al. 2019; Baker et al. 2014; Camaschella et al. 2007; Wingert et al. 2005; Ye et al. 2010).

In S. cerevisiae, Fe/S cluster formation on Grx5 in vivo depends on all components of the core ISC machinery, including the scaffold protein Isu1 and the Hsp70/Hsp40 chaperones Ssq1/Jac1 (Figure 2) (Uzarska et al. 2013). Moreover, depletion of yeast Grx5 results in the accumulation of Fe/S clusters on Isu1, a phenotype shared with cells depleted for the Hsp70 chaperone Ssq1 and its co-chaperone Jac1 (HSPA9 and HSC20 in human) (Muhlenhoff et al. 2003). This indicates that Grx5 cooperates with this dedicated chaperone system in order to facilitate Fe/S cluster transfer from Isu1 to recipient apoproteins (Dutkiewicz and Nowak 2018; Kampinga et al. 2010; Uzarska et al. 2013; Vickery and Cupp-Vickery 2007). In S. cerevisiae, Isu1 binds to the specific peptide-binding domain of the Hsp70 chaperone Ssq1 in an ATP-dependent manner. Grx5 binding to a yet unidentified part of Ssq1 occurs independently of nucleotides. ATP hydrolysis by Ssq1, which is stimulated by both Isu1 and Jac1 but not Grx5, stabilizes the Ssq1-Grx5 interaction and weakens Fe/S cluster binding on Isu1. Simultaneous binding of holol Isu1 and Grx5 on Ssq1 likely facilitates Fe/S cluster transfer from the scaffold protein to monothiol Grx5 (Uzarska et al. 2013). This Hsp70-mediated Fe/S cluster transfer is consistent with the observation that bacterial Hsp70 HscA and Hsp40 HscB strongly stimulate Fe/S cluster transfer from IscU to Grx5 in vitro (Bonomi et al. 2011; Shakamuri et al. 2012). An analogous in vitro transfer system has not been reported yet for the eukaryotic system.

Taken together, Grx5 occupies an important position in the ISC assembly line, connecting the early part of the ISC system that is dedicated to the de novo synthesis of [2Fe-2S] clusters on the scaffold protein Isu1 with the later parts of the ISC system that are involved in (1) the transfer of [2Fe-2S] clusters to recipient apoproteins, (2) the formation and export of X-S required for the maturation of cytosolic/nuclear Fe/S proteins, and (3) the conversion of [2Fe-2S] into [4Fe-4S] clusters and their insertion into target apoproteins (Berndt and Lillig 2017; Braymer and Lill 2017; Lill and Freibert 2020; Rouhier et al. 2010). Cluster transfer from Grx5 to model [2Fe-2S] apoproteins can occur spontaneously in vitro, yet whether additional targeting factors, i.e. Bol1/Bol3, are needed for certain client [2Fe-2S] proteins remains open (Figure 2). The maturation of [4Fe-4S] proteins essentially involves the late-acting ISC proteins Isa1-Isa2-Iba57 for the synthesis and Nfu1 for subsequent targeting of [4Fe-4S] clusters to client proteins (Branaccio et al. 2014; Muhlenhoff et al. 2011) (Figure 1). This late reaction of the ISC system involves the transfer of [2Fe-2S] clusters from Grx5 to Isa1-Isa2-Iba57, where they are converted to [4Fe-4S] clusters by reductive fusion. Electrons are provided by NADPH via ferredoxin reductase Arh1 and the mitochondrial ferredoxin Yah1 (Banci et al. 2014; Branaccio et al. 2014; Weiler et al. 2020).

**Cellular roles of cytosolic multi-domain monothiol glutaredoxins**

The cytosolic and nuclear multi-domain Grxs belong to a subgroup of class II Grxs and possess a tandem
Role of cytosolic glutaredoxins in iron delivery

In all species analyzed, the cytosolic and nuclear multidomain Grxs play a central role in cellular iron sensing. In addition, low levels of S. cerevisiae cytosolic Grx3/Grx4 result in multiple defects of cytosolic iron metabolism decreasing iron loading of several classes of iron-dependent proteins and the import of iron into mitochondria (Muhlenhoff et al. 2010). At the same time, cells display cytosolic iron overload and a massive increase of cellular GSH suggesting that yeast cytosolic Grxs are essential for intracellular trafficking of iron to make it bioavailable for the assembly of iron-containing proteins. In zebrafish embryos, cytosolic Grxs are required for the maturation of hemoglobin (Haunhorst et al. 2013). In human cell culture, too, depletion of cytosolic GLRX3 impairs the activity of cellular Fe/S proteins and causes a deregulated cellular iron uptake as a result of the reduced levels of Fe/S cluster binding to cytosolic iron-regulatory protein 1 (IRP1). Yet, in comparison to S. cerevisiae, these iron-related defects are mild (Frey et al. 2016; Haunhorst et al. 2013).

Cytosolic Grxs interact with several components of the cytosolic CIA system and play an important role in the maturation of cytosolic [4Fe-4S] proteins (Figure 2). In S. cerevisiae the paralogous Grx3/Grx4 physically interact with the early CIA factor Dre2 (human CIAPIN1) (Zhang et al. 2014) and are required for Fe/S cluster assembly on Dre2 (Muhlenhoff et al. 2010; Zhang et al. 2014). Similar complex formation between cytosolic Grx3 and Dre2 has been detected in Arabidopsis thaliana and A. fumigatus (Inigo et al. 2016; Misslinger et al. 2019) as well as in human cells and in mice (Banci et al. 2015; Frey et al. 2016; Saito et al. 2011). This interaction essentially requires both the Fe/S cluster on Grx3 and the Trx domain (Banci et al. 2015). The complex of mammalian GLRX3 and the mammalian Dre2 homolog CIAPIN1 (anamorsin) also includes NDOR1 (yeast Tah18), the diflavin reductase that forms an electron transfer chain with Dre2/CIAPIN1 required for the generation of [4Fe-4S] clusters on the CIA factors CFD1 and NBP35 (Figure 2) (Frey et al. 2016; Netz et al. 2010; Paul and Lill 2015). Depletion of GLRX3 and the Grx-interacting protein BOLA2 reduces iron incorporation into CIAPIN1 in cultivated human cells in vivo, strongly suggesting that Fe/S clusters coordinated by GLRX3 are directly transferred to CIAPIN1 in cells (Frey et al. 2016). Transfer of [2Fe-2S] clusters from GLRX3 to CIAPIN1 via heterodimeric complex formation was also demonstrated in vitro. Yet, since this in vitro transfer was carried out in the presence of DTT, the physiological relevance of this finding requires further confirmation (Banci et al. 2015; Netz et al. 2016; Zhang et al. 2017). Cytosolic monothiol Grxs were reported to interact with several other CIA factors including those of the CIA targeting complex, Cia1 (CIAO1 in human), Mms19 (MMS19), and Cia2 (CIAO2B) (Frey et al. 2016; Inigo et al. 2016; Misslinger et al. 2019). The biochemical meaning of these interactions is as yet unclear.

The involvement of cytosolic Grxs in the maturation of iron-containing (non Fe/S) proteins is best studied for ribonucleotide reductase (RNR, Figure 2). RNR is an iron-, oxygen-, and thiol redox-dependent enzyme that is essential for DNA replication and repair, as it catalyzes the conversion of nucleoside 5′-diphosphates (NDPs) to the corresponding deoxynucleotides (Cotruvo and Stubbe 2011). Eukaryotic RNR is a multimeric protein complex composed of a large α subunit (yeast Rnr1, Rnr3) that binds substrates and allosteric effectors and a small β subunit (yeast Rnr2) that contains a diferric-tyrosyl radical (Fe₂ Tyriday ) site, which is used to initiate ribonucleotide reduction. S. cerevisiae RNR contains an unusual co-factor-less second β subunit (β', Rnr4) that plays a critical role in facilitating iron insertion into the β subunit (Sommerhalter et al. 2004). In vitro assembly of the metal cofactor requires reducing equivalents, β', Fe²⁺, and O₂. The iron source has been proposed to be the [2Fe-2S] cluster of Grx3/Grx4, whereas the electrons originate from the electron transfer chain NADPH-Tah18-Dre2 (Figure 2) (Li et al. 2017; Muhlenhoff et al. 2010; Zhang et al. 2014; Zhang et al. 2011). Since Grx3/Grx4 and Dre2 are Fe/S proteins,
Iron sensing involving fungal multi-domain monothiol glutaredoxins

Due to the low solubility of ferric iron in water, iron availability is scarce under aerobic conditions. Conversely, high intracellular iron levels are both a source and an amplifier of reactive oxygen species and thus are toxic. To acquire appropriate levels of intracellular iron and at the same time avoid iron-overloading, cells have developed sophisticated systems for assuring a balanced cellular iron homeostasis (Gupta and Outten 2020; Lane et al. 2015; Misslinger et al. 2020; Philpott and Jadhav 2019). At the cellular level, this balance is maintained through a strict coupling of cellular iron uptake at the plasma membrane to intracellular iron demands and a balanced distribution of iron between the cellular compartments involved in iron-utilization and storage. Fungi respond to low ambient iron levels by inducing cellular iron and iron-siderophore uptake systems at the plasma membrane (Gupta and Outten 2020; Labbe et al. 2013; Misslinger et al. 2018; Muhlenhoff et al. 2015; Wang et al. 2019). This strategy to increase iron supply is paralleled by the mobilization of iron from the vacuole, the major iron-storage organelle in fungi, and the down-regulation of iron-consuming pathways in order to make iron available for the most essential tasks. The involvement of cytosolic monothiol Grxs in the post-translational adaptation to iron limitation in fungi is the best understood aspect of monothiol Grx function. In all fungi studied, depletion of cytosolic Grxs results in profound deregulation of the responsible transcription factors (Gupta and Outten 2020; Labbe et al. 2013; Misslinger et al. 2018; Muhlenhoff et al. 2015; Wang et al. 2019). The CGFS active-site cysteine residue which is essential for binding Fe/S clusters is absolutely required for the iron adaptation response, suggesting that the Fe/S cluster on cytosolic and nuclear monothiol Grxs functions as an iron sensor in fungi (Kim et al. 2011; Misslinger et al. 2019; Muhlenhoff et al. 2010). The following sections outline this sensing function in various fungi.

Php4- and Fep1-dependent iron regulation in *S. pombe*

Most fungi orchestrate iron-responsive gene expression by the interplay of two conserved transcriptional repressors, a strategy that is widely employed in fungi (Figure 3A). This mode of regulation has been studied most intensively in the fission yeast *S. pombe* (Gupta and Outten 2020; Labbe et al. 2013). In this organism, the GATA-type transcription factor Fep1 represses genes involved in iron uptake under normal iron conditions (Brault et al. 2015; Jbel et al. 2009; Kim et al. 2016; Kim et al. 2011; Labbe et al. 2013). The repression of genes involved in iron-utilizing pathways is mediated by the transcriptional co-repressor Php4 that binds to the CCAAT-binding complex (Php2/3/5) under iron-limiting conditions. Both repressors are under mutual transcriptional control. Upon iron sufficiency, transcription of the *php4* gene is repressed by Fep1, while Php4 represses *fep1* under iron limitation (Brault et al. 2015; Labbe et al. 2013; Mercier et al. 2008). The mechanism of iron sensing by both transcription factors involves cytosolic Grx4, as *S. pombe* lacking Grx4 displays constitutively low levels of genes involved in cellular iron uptake (Jacques et al. 2014; Jbel et al. 2011; Kim et al. 2016; Kim et al. 2011). At the same time, genes of iron-consuming pathways remain constitutively low (Encinar del Dedo et al. 2015; Mercier and Labbe 2009; Vachon et al. 2012). In consequence, *S. pombe* cells lacking Grx4 or that harbor Grx4 variants in which the active site CGFS cysteine residue is replaced, are unable to properly respond to neither iron starvation nor iron sufficiency.

Php4, the negative regulatory subunit of the CCAAT-binding complex undergoes nuclear-cytoplasmic shuttling, being imported in the nucleus under low iron conditions and exported to the cytoplasm by the exportin Crm1 in response to iron sufficiency (Khan et al. 2014; Mercier and Labbe 2009; Vachon et al. 2012). In the nucleus, it interacts with monothiol Grx4. The role of Grx4 in iron sensing of Php4 is best explained by Fe/S cluster transfer from holo-Grx4 to Php4 which inactivates Php4’s repressor function upon iron sufficiency and initiates its export from the cytosol (Figure 3A). Upon iron deprivation, Fe/S clusters on Grx4 and Php4 are lost resulting in the accumulation of Php4 in the nucleus and the repression of genes involved in iron-consuming pathways (Dlouhy et al. 2017; Encinar del Dedo et al. 2015; Jacques et al. 2014).
Consequently, Php4 remains firmly bound to the CCAAT-binding complex in cells lacking Grx4. This model of regulation is supported by ample biochemical evidence. When co-expressed in E. coli, S. pombe Php4 and Grx4 form a stable complex that harbors a bridging [2Fe-2S] cluster that was verified by EPR spectroscopy and elemental analysis (Dlouhy et al. 2017). The spectroscopic characteristics are distinct from the [2Fe-2S] cluster on Grx4, indicating that the [2Fe-2S] cluster is shared between Php4 and Grx4. The active-site cysteine on Grx4 and two conserved, catalytically important residues on Php4 are specifically required for Fe/S cluster binding and Php4--Grx4 complex formation. These residues are likely involved in iron-mediated inactivation of Php4 (Dlouhy et al. 2017).

In contrast to Php4, the GATA-type transcription factor Fep1 localizes constitutively to the nucleus. Its repressor function is regulated via physical complex formation with holo-Grx4 and the Grx-interacting protein Bol2 protein (Figure 3A). Similar to Grx4, deletion of Bol2 results in the constitutive repression of iron uptake genes (Jacques et al. 2014; Jbel et al. 2011). Yet, Bol2 is not involved in regulating S. pombe Php4 (Dlouhy et al. 2017). Fep1 (and also Php4) interact with Grx4 via its N-terminal Trx- and C-terminal Grx domains (Jbel et al. 2011; Vachon et al. 2012). Binding to the Trx domain is constitutive, while association with the Grx-domain is weaker, iron-dependent, and requires the conserved Grx-active site cysteine residue (Encinar del Dedo et al. 2015; Kim et al. 2011; Mercier and Labbe 2009; Vachon et al. 2012). In vitro, the N-terminal domains of Fep1 from S. pombe and Pichia pastoris form [2Fe–2S]$^{2+}$ cluster-binding homodimers (Cutone et al. 2016; Kim et al. 2016; Patti et al. 2018). Iron-responsive fungal GATA factors typically possess an N-terminal DNA-binding domain that harbors a Cys-X5-Cys-X8-Cys-X2-Cys motif located between two zinc fingers (Cutone et al. 2016; Patti et al. 2018). The four conserved cysteine residues serve as Fe/S cluster ligands in vitro, modulate Fep1’s affinity for DNA and are essential for the repressor function of Fep1 under iron deficiency. Yet, Fep1 lacking these four cysteine residues is still dimeric and binds a [2Fe–2S]$^{2+}$ cluster ligated to two cysteine residues of the first zinc finger. This cluster is spectroscopically distinct from that of the wild-type protein and is also slowly formed on wild-type holo-Fep1 upon exposure to air. It was suggested that Fep1 might be both an iron and an oxygen sensor. S. pombe Fep1 was also isolated as a Fe-only protein (Encinar del Dedo et al. 2015), similar to the Fep1 homologs SRE from Neurospora crassa (Harrison and Marzluf 2002) and Sre1 from Histoplasma capsulatum (Chao et al. 2008). Iron binds to four conserved central cysteines that modulate the affinity of Fep1 for DNA, i.e. to the same site used for Fe/S cluster binding in the other studies. Ferric iron induces binding of H. capsulatum Sre1 to target promoters in vitro, indicating that iron alone is sufficient for its iron-responsive regulation. In vitro, S. pombe Fep1 transfers iron to apo-Grx4-Bol2, suggesting that the inactivation of Fep1 upon iron limitation possibly involves reverse metal transfer from Fep1 to Grx4-Fra2 (Encinar del Dedo et al. 2015). Taken together, Fe and/or Fe/S cluster

---

**Figure 3:** Monothiol glutaredoxins as regulators of fungal iron homeostasis.

(A) Interplay of the two iron-responsive transcriptional repressors in fission yeast S. pombe. Right: Fep1 represses iron uptake genes under iron-replete conditions, while the co-repressor Php4 (left) binds to the CCAAT-binding complex (Php2/3/5) under iron-limiting conditions, in order to repress genes involved in iron utilization. In addition, Php4 blocks fep1 gene expression during iron limitation, while Fep1 mutually represses the php4 gene under iron sufficiency. (B) Role of cytosolic and nuclear Grx3/Grx4 in the regulation of the iron-responsive transcriptional activators Aft1-Aft2 in budding yeast S. cerevisiae. Aft1-Aft2 bind to target promoters under iron-limiting conditions and activate the expression of iron uptake genes, including Aft1 and CTH2. Cth2 binds to mRNAs of genes involved in iron-consuming pathways inducing their degradation. Some iron-responsive transcription factors in (A) and (B) form physical complexes with cysteine and nuclear Grx3 and Grx4 and likely are regulated on the post-translational level via the binding of iron or Fe/S clusters that are provided via Grx3 and Grx4 under iron-replete conditions.

---

**A: Monothiol glutaredoxins and transcriptional (co)repressors**

- Php4 interacts with CCAAT via iron utilization.
- Grx4 interacts with Php4 via iron uptake.
- Fep1 interacts with GATA via iron deficiency.

**B: Monothiol glutaredoxins and transcriptional activators**

- Aft1/2 interacts with FeSE via iron uptake.
- CTH2 interacts with iron utilization.
- CCAAT interacts with iron utilization.

---
binding to Fepl proteins explains how Grx4/Bol2 induces binding of Fepl to target promoters under iron sufficiency (Figure 3A).

Iron regulation in fungal pathogens

The role of cytosolic Grxs as iron sensors has also been extensively studied in the fungal pathogens *A. fumigatus*, *C. neoformans*, and *Fusarium graminearum* (Gupta and Outten 2020; Misslinger et al. 2020; Misslinger et al. 2018; Trnka et al. 2020; Wang et al. 2019). The strategies for adaptation to iron starvation of these fungi are variations of those utilized by *S. pombe* and are important aspects of virulence (Figure 3A). *A. fumigatus* orchestrates iron-responsive gene expression by the repressors SreA (the ortholog of Fep1) and HapX (the ortholog of Php4). Similar to *S. pombe*, HapX represses transcription of sreA during iron limitation and, in turn, hapX transcription is repressed by SreA under iron sufficiency (Schrettl and Haas 2011). Yet, in contrast to *S. pombe*, *A. fumigatus* HapX is also essential for adaptation to toxic iron excess (Gassler et al. 2014). The monothiol glutaredoxin GrxD from *A. fumigatus* simultaneously activates the HapX iron-starvation function and disables the repressor function of SreA at the post-translational level (Misslinger et al. 2019). Lack of GrxD results in intracellular iron deficiency as both SreA and HapX require physical interaction with GrxD to sense iron levels. Yet, in *Aspergilli* cytosolic Bol2 proteins are missing (Misslinger et al. 2019). GrxD binds to HapX irrespectively of the cellular iron status and, at least under iron sufficiency and iron excess, also to SreA. The N-terminal thioreredoxin domain of GrxD is essential for interaction with SreA in vivo, yet dispensable for GrxD-HapX complex formation. Furthermore, the conserved active-site cysteine residue of GrxD is essential for the adaptation to iron starvation, but is not involved in complex formation with HapX. Remarkably, SreA represses the expression of GrxD under iron-replete conditions and by this regulates the levels of its own inhibitor (Misslinger et al. 2019). *A. fumigatus* GrxD and HapX form iron-binding hetero-tetramers upon co-expression in *E. coli* (Misslinger et al. 2019). The UV–Vis absorption spectrum of this complex indicates a bound [2Fe-2S] cluster, and recombinant HapX alone displays a similar spectrum. A more detailed biochemical analysis was not carried out yet. Thus, HapX is able to coordinate an Fe/S cluster without GrxD. In vivo, this Fe/S cluster is likely inserted via GrxD upon iron sufficiency. The cluster-ligating residues were not identified, yet HapX contains four cysteine-rich repeats that are reminiscent of the [2Fe-2S] cluster binding sites at the activator domain of *S. cerevisiae* Yap5 (Misslinger et al. 2019; Rietzschel et al. 2015). Similar to *S. pombe* and *S. cerevisiae*, iron sensing in *A. fumigatus* requires an input of the mitochondrial, but not cytosolic Fe/S protein biogenesis machinery, which is consistent with the ISC-dependent and CIA-independent formation of Fe/S clusters on cytosolic Grxs, HapX and SreA (Misslinger et al. 2018; Mühlenhoff et al. 2010).

In *C. neoformans* the GATA-type regulator Cir1, the ortholog of *S. pombe*’s Fep1, is an important virulence factor that regulates iron uptake functions and the elaboration of virulence (Jung and Kronstad 2011). Cells lacking Grx4 display a deregulated iron homeostasis similar to cells lacking Cir1 (Attarian et al. 2018). Moreover, Grx4 deletion has negative effects on the formation of major virulence-related phenotypes and grx4 mutants are unable to cause disease in mice. Regulation of Cir1 involves complex formation with Grx4 in the nucleus under the low-iron condition. Under iron-replete condition, complex formation is lost and Grx4 localizes to the cytoplasm while Cir1 remains in the nucleus as an active repressor.

Aft1/Aft2- and Yap5-dependent iron regulation in *S. cerevisiae*

The regulation of cellular iron homeostasis has been intensively studied in budding yeast *S. cerevisiae* (Brault et al. 2015; Gupta and Outten 2020; Labbe et al. 2013). Its strategy of adaptation to iron limitation is unusual in that it employs transcriptional activators, Aft1 and Aft2, rather than repressors and is restricted to a small clade of yeasts (Gupta and Outten 2020) (Figure 3B). In contrast to other fungi, the CCAAT-binding complex (Hap) of *S. cerevisiae* is not iron-responsive and functions as global regulator of respiratory gene expression (Guarente 1995). In *S. cerevisiae*, the downsizing of iron-consuming pathways is regulated by the RNA-binding proteins Cth1/Cth2 that bind to mRNAs of iron-consuming genes upon iron-limitation thereby inducing their degradation (Martinez-Pastor et al. 2013). Aft1 and its paralog Aft2, two Zn-finger DNA binding proteins of the WRKY-GCM1 family (Babu et al. 2006) bind to target promoters under iron-limiting conditions and activate the expression of the yeast iron regulon, a set of ~40 genes mostly involved in iron uptake and intracellular iron distribution (Courel et al. 2005). Aft1 also induces the transcription of its own gene and this auto-amplification circuit is essential for a robust response of *S. cerevisiae* to iron-limitation (U. Mühlenhoff, unpublished). Similar to other fungal iron responsive transcription factors, Aft1 and Aft2 require cytosolic Grx3/Grx4 function for iron sensing. Upon iron
sufficiency, they physically interact with Grx3/Grx4 and the Grx-interacting protein Bol2 (formerly termed Fra2) in the nucleus, which together promote dissociation of Aft1/Aft2 from target promoters and their subsequent nuclear export which is escorted by the specialized exportin Msn5 (Kumanovics et al. 2008; Ueta et al. 2012). Aft1 binds to Grx3/Grx4 at the C-terminal α-helical segment of the Grx domain that is opposite to the Grx active site, and this association is essential for Aft1 regulation (Hoffmann et al. 2011). This C-terminal region is also the binding interface of Grx3/Grx4 with Bol2 (Chi et al. 2018; Li and Outten 2019; Poor et al. 2014). The structure of the N-terminal domain of Aft2 bound to DNA has been solved (Poor et al. 2014). This functional domain contains two cysteine residues in a conserved Cys-Asp-Cys motif that are essential for deactivation of Aft1 and Aft2 under iron sufficiency. In vitro, Aft2 receives a [2Fe-2S] cluster from Grx3-Bol2 (Li and Outten 2019; Poor et al. 2014). The Fe/S cluster bridges two Aft2 monomers in a homodimer and is coordinated by the two conserved cysteine residues. This Fe/S cluster-induced dimerization reduces the affinity of Aft2 to target promoters resulting in the deactivation of Aft2. Moreover, physical complex formation of Aft2 with Bol2 likely plays an important role in the deactivation of Aft2 (Chi et al. 2018; Li and Outten 2019; Poor et al. 2014). By analogy, the regulatory mechanism of inactivation of Aft1 is most likely similar.

Physical Fe/S cluster binding using 55Fe radiolabeling in vivo has directly been demonstrated for the high-iron sensing regulator Yap5 from S. cerevisiae (Rietzschel et al. 2015). It is important to note that for all other transcription factors discussed above, Fe/S cluster binding was so far documented only by in vitro analyses of truncated proteins expressed in E. coli. Interestingly, the [2Fe-2S] clusters on Yap5 are inserted in a Grx-independent fashion, as observed for HapX stated above. Their maturation also does not involve the CIA system, which strongly suggests that a subset of cytosolic/nuclear [2Fe-2S] proteins receive their cluster with the help of mitochondria, yet without further involvement of other known cytosolic Fe/S protein assembly factors including Grx3/Grx4 (Misslinger et al. 2019).

Taken together, the current literature uniformly suggests that fungal iron-responsive transcription factors are iron binding or, more likely, Fe/S cluster-containing proteins that are regulated on the post-translational level by cofactor occupancy (Figure 3). In many cases, the Fe/S cluster is provided by cytosolic monothiol Grxs, likely during physical complex formation between the partners, in some cases requiring the assistance by Bol2. The Fe/S cluster on Grxs is acquired from the mitochondrial core ISC system in a CIA-independent manner, explaining why fungal iron uptake regulation is controlled by the status of the mitochondrial, but not the cytosolic Fe/S protein assembly machinery.

Redox-related functions of multi-domain monothiol glutaredoxins?

There is only limited evidence that cytosolic multi-domain monothiol Grxs execute roles independent of their Fe/S cluster-associated functions in iron metabolism. Yeast monothiol Grx3 possesses little thiol-reductase activity in vitro, amounting to ~1% of that of dithiol Grxs in the standard bis-(2-hydroxyethyl) disulfide (HEDS) assay (Chi et al. 2018). Therefore, this redox activity at best plays only a minor role in general thiol redox-related reactions. Grx3’s thiol-reductase activity could still be important for a specific subset of proteins, yet, for structural reasons, this seems unlikely, as it was shown that a distinct loop structure adjacent to the active site forces the GSH thiol in monothiol Grxs into an position that is unfavorable for thiol redox activity (Liedgens et al. 2020; Trnka et al. 2020). Multi-domain monothiol Grxs possess glutathione S-transferase (GST) activity at a catalytic velocity similar to that of dithiol Grxs and 25% of that of canonical GSTs in standard assays (Chi et al. 2018). This indicates that these Grxs might be involved in the detoxification of xenobiotic compounds via conjugation to GSH. The GST activity is executed by the Grx-domain with assistance by the active-site cysteine of the Trx domain likely via a transient disulfide bond exchange with the Grx active-site cysteine. As a curiosity, yeast grx4/ade2Δ cells do not form the typical red pigment of ade2Δ cells, an end product of GSH-mediated detoxification in yeast (Jainarayanan et al. 2020). This phenotype is not found for any other member of the Trx superfamily pointing towards a specific glutathione S-transferase function of Grx4 (Jainarayanan et al. 2020). Further, S. cerevisiae Grx3/Grx4 S-glutathionylate the deacetylase Sir2 and thereby regulate its deacetylase activity (Vall-Llaura et al. 2016). Sir2 is essentially involved in transcriptional silencing at cryptic mating type loci, at telomerases, and some ribosomal rDNA loci, and is highly conserved from archaea to humans. Its mammalian counterpart also deacetylates substrates other than histones.

There are several studies that describe interactions of cytosolic multi-domain monothiol Grxs with kinases. S. cerevisiae Grx4 itself is phosphorylated by an atypical protein kinase Bud32, yet the role of the Grx4 phosphorylation remains unclear (Peggion et al. 2008). Further, S. cerevisiae Grx3/Grx4 form a physical complex with the stress-activated serine/threonine MAP kinase Slt2 that
plays a regulatory role in oxidative stress response (Pujo- Carrion and Torre-Ruiz 2017). Slt2 forms Fe/S cluster- bridged complexes with Grx3/Grx4 involving the Grx active-site cysteine, GSH, and specific cysteine residues on Slt2. The latter are also required for Slt2 dimerization, and mutations in these cysteine ligands display a severe impairment of Slt2 phosphorylation and Slt2 activation upon oxidative stress. Grx3/Grx4 are required for Slt2 phosphorylation under oxidative conditions and participate in the positive regulation of Slt2. In mammals, GLRX3 was initially described as protein kinase C9-binding protein, PICOT, yet the relevance of this interaction remains unclear. PICOT plays a role in the development of stress- induced cardiac hypertrophy in mouse and rat models (Jeong et al. 2006; Kato et al. 2008). A characterization of iron-related functions of heart tissue from GLRX3-deficient mice was not carried out. Thus, whether this function is related to its role in iron metabolism or to other functions in cellular stress response is not yet resolved (Donelson et al. 2019; Pham et al. 2015).

The interplay between Fe/S clusters and cellular redox balance

The proper synthesis, trafficking, and functioning of redox-active Fe/S clusters is important to cellular redox balance at many levels. Even though the redox capabilities of Fe/S clusters make them so useful for biological reactions, these cofactors are prone to oxidative degradation by oxygen and ROS (Figure 1). Dissecting of how redox species affect Fe/S clusters and their biogenesis and vice versa has been important in understanding how Fe/S proteins interfere with cellular redox pathways. As a case in point, the first study on a yeast strain lacking monothiol Grx5 (grx5Δ) attributed an observed increase in oxidative damage to the lack of thiol reducing capacity (Rodriguez-Manzaneque et al. 1999). Subsequent studies clarified that under aerobic conditions oxygen and/or ROS react with the accumulated mitochondrial iron in grx5Δ cells to create oxidative stress conditions (Muhlenhoff et al. 2003; Rodriguez-Manzaneque et al. 2002). Hence, a deregulation of iron homeostasis and not a lack of reducing equivalents delivered to Grx5 by the Grx/GSH system cause the oxidative stress phenotype. This iron-overload phenotype is generally observed for cells with a defective mitochondrial core ISC system and for the disruption of Atm1 function, and is relevant to human mitochondrial disorders (Gomez et al. 2014; Miao et al. 2009; Muhlenhoff et al. 2015; Stehling et al. 2014). Sections 12 the next sections briefly discusses examples of new work that broadens the existing knowledge of the known cellular redox pathways and redox species that influence Fe/S proteins in both apo- and holoforms as well as components of the Fe/S protein biogenesis machineries.

The impact of oxygen on Fe/S proteins and their utilization

As stated above, some Fe/S proteins are susceptible to oxidative damage by molecular oxygen and ROS derived from oxygen (Figure 1) (Imlay 2008). Highly susceptible Fe/S clusters can be found close to protein surfaces or involve [4Fe-4S] clusters with exposed non-protein-coordinated iron ions, e.g., in aconitate and isopropylmalate isomerase (Leu1) of the [4Fe-4S] dehydratase family (Figure 4) (Imlay 2006; Imlay et al. 2019). Therefore, the antioxidant proteins catalase and superoxide dismutase play important protective roles to maintain Fe/S proteins and/or their biogenesis (Figure 1) (Gomez et al. 2014; Missirlis et al. 2003; Strain et al. 1998). While the oxidative sensitivity of Fe/S clusters is detrimental in most Fe/S proteins to varying degrees, nature has also taken advantage of this feature. Many bacteria orchestrate their adaptation to ambient oxygen, iron, and/or oxygen radical levels by employing transcription factors with sensitive Fe/S centers for various regulatory tasks (Crack and Le Brun 2018; Mettert and Kiley 2015). In some α-proteobacteria, the global regulator RirA has been proposed to uniquely sense both oxygen and iron at the same [4Fe-4S] cluster (Pellicer Martinez et al. 2019). The release of free iron under low iron conditions from an uncoordinated site of the [4Fe-4S]2+ cluster is proposed to drive the iron-sensing mechanism, which may have important implications for many other [4Fe-4S] clusters that are not fully protein sidechain- coordinated (e.g., dehydratases, radical SAM enzymes). The resulting [3Fe-4S]0 species is then responsible for O2 sensing. Other prominent examples of Fe/S-binding transcription factors are the oxygen-responsive transcriptional regulator FNR, the SoxR transcriptional activator that recognizes superoxide, and the Rrf2 type transcriptional regulator NsrR that regulates the response to NO stress (Kobayashi et al. 2014; Mettert and Kiley 2018; Volbeda et al. 2017). In eukaryotes, S. cerevisiae’s Aft1 and Yap5 contain reactive [2Fe-2S] clusters that elicit a functional response upon oxidative degradation or under iron limitation (Castells-Roca et al. 2011; Rietzschel et al. 2015).

Metazoans contain multiple levels of iron- and oxygensensing via Fe/S clusters bound to iron regulatory proteins (IRPs), in addition to the hydroxylation of hypoxia-
inducible factors (HIFs) by non-heme iron prolyl hydroxylases (Taabazuing et al. 2014; Wang et al. 2020). IRP1 and IRP2 are members of the aconitase family of proteins, where only the former is able to bind a [4Fe-4S] cluster (for detailed reviews see (Anderson et al. 2012; Kuhn 2015)). The CIA system is responsible for inserting the [4Fe-4S] cluster into IRP1 (Paul and Lill 2015). When cellular iron levels are low, IRP1 switches from an active cytosolic aconitase to a cluster-less protein that binds iron-responsive elements (IREs), i.e. stem-loop structures in the mRNAs of iron metabolism-related proteins, thereby dictating the low iron response (Anderson et al. 2012; Kuhn 2015). Oxygen tensions in mammalian tissues have been shown to regulate iron homeostasis via IRP1, and ROS have been observed to deactivate IRP1 via oxidation of the [4Fe-4S] cluster (Mueller 2005). IRP2 regulation has recently been shown to be oxygen-responsive via a [2Fe-2S] cluster, which bridges IRP2 and the E3 ubiquitin ligase adaptor protein FBXL5 (Wang et al. 2020). Oxidation of the [2Fe-2S] cluster leads to ubiquitination and degradation of IRP2, and hence signals that the cell contains adequate iron levels under elevated oxygen levels.

The impact of oxygen on Fe/S protein biogenesis

Oxygen can also have a significant impact on the synthesis and trafficking of Fe/S clusters. This fact is well known in many (cyano-)bacteria and plastids, which utilize the more oxidative stress-resistant SUF system for Fe/S protein maturation as compared to the ISC system (Garcia et al. 2019). In eukaryotes that depend on the ISC and CIA systems, the question remains, which steps in the assembly line are most susceptible to redox perturbations induced by oxygen or redox stress? In S. cerevisiae, the ISC-factor Jac1 is not essential under anaerobic conditions, and cellular defects stemming from a lack of frataxin (Yfh1), Atm1, and Arh1 observed aerobically are recovered under anaerobic conditions (Ast et al. 2019; Bulteau et al. 2007; Snoek and Steensma 2006). Anoxic conditions rescue the [4Fe-4S] protein deficiency phenotype of cells lacking the late-acting Fe/S protein biogenesis component Nfu1, suggesting that Nfu1 is predominately important under normal oxygen levels (Melber et al. 2016). This either indicates that these five proteins are among the most oxygen-sensitive ISC components or that these factors can most easily be bypassed (Figure 4). In the CIA system, the lethal phenotype of Nar1 depletion is partially relieved under anaerobic conditions (Figure 4) (Fujii et al. 2009). Further, Lto1 and Yae1, two accessory CIA factors that facilitate [4Fe-4S] cluster insertion into Rli1 are not essential anymore under anaerobic conditions, indicating that these factors can most easily be bypassed (Figure 4). In yeast, the question remains, which steps in the assembly line are most susceptible to redox perturbations induced by oxygen or redox stress? In S. cerevisiae, the ISC-factor Jac1 is not essential under anaerobic conditions, and cellular defects stemming from a lack of frataxin (Yfh1), Atm1, and Arh1 observed aerobically are recovered under anaerobic conditions (Ast et al. 2019; Bulteau et al. 2007; Snoek and Steensma 2006). Anoxic conditions rescue the [4Fe-4S] protein deficiency phenotype of cells lacking the late-acting Fe/S protein biogenesis component Nfu1, suggesting that Nfu1 is predominately important under normal oxygen levels (Melber et al. 2016). This either indicates that these five proteins are among the most oxygen-sensitive ISC components or that these factors can most easily be bypassed (Figure 4). In the CIA system, the lethal phenotype of Nar1 depletion is partially relieved under anaerobic conditions (Figure 4) (Fujii et al. 2009). Further, Lto1 and Yae1, two accessory CIA factors that facilitate [4Fe-4S] cluster insertion into Rli1 are not essential anymore under anaerobic conditions, indicating that these factors can most easily be bypassed (Figure 4). In yeast, the question remains, which steps in the assembly line are most susceptible to redox perturbations induced by oxygen or redox stress? In S. cerevisiae, the ISC-factor Jac1 is not essential under anaerobic conditions, and cellular defects stemming from a lack of frataxin (Yfh1), Atm1, and Arh1 observed aerobically are recovered under anaerobic conditions (Ast et al. 2019; Bulteau et al. 2007; Snoek and Steensma 2006). Anoxic conditions rescue the [4Fe-4S] protein deficiency phenotype of cells lacking the late-acting Fe/S protein biogenesis component Nfu1, suggesting that Nfu1 is predominately important under normal oxygen levels (Melber et al. 2016). This either indicates that these five proteins are among the most oxygen-sensitive ISC components or that these factors can most easily be bypassed (Figure 4). In the CIA system, the lethal phenotype of Nar1 depletion is partially relieved under anaerobic conditions (Figure 4) (Fujii et al. 2009). Further, Lto1 and Yae1, two accessory CIA factors that facilitate [4Fe-4S] cluster insertion into Rli1 are not essential anymore under anaerobic conditions, indicating that these factors can most easily be bypassed (Figure 4). In yeast, the question remains, which steps in the assembly line are most susceptible to redox perturbations induced by oxygen or redox stress? In S. cerevisiae, the ISC-factor Jac1 is not essential under anaerobic conditions, and cellular defects stemming from a lack of frataxin (Yfh1), Atm1, and Arh1 observed aerobically are recovered under anaerobic conditions (Ast et al. 2019; Bulteau et al. 2007; Snoek and Steensma 2006). Anoxic conditions rescue the [4Fe-4S] protein deficiency phenotype of cells lacking the late-acting Fe/S protein biogenesis component Nfu1, suggesting that Nfu1 is predominately important under normal oxygen levels (Melber et al. 2016). This either indicates that these five proteins are among the most oxygen-sensitive ISC components or that these factors can most easily be bypassed (Figure 4). In the CIA system, the lethal phenotype of Nar1 depletion is partially relieved under anaerobic conditions (Figure 4) (Fujii et al. 2009). Further, Lto1 and Yae1, two accessory CIA factors that facilitate [4Fe-4S] cluster insertion into Rli1 are not essential anymore under anaerobic conditions, indicating that these factors can most easily be bypassed (Figure 4).
mitochondrial [4Fe-4S] targeting factor IND1 have been categorized as selectively essential, indicating their dispensability under anaerobic conditions (Figure 4). Taken together, the non-essentiality of several ISC and CIA factors under low oxygen and/or anaerobic conditions can be largely explained by a higher stability of Fe/S clusters in the absence of oxidants, the reduced demand for many Fe/S proteins (e.g., those involved in respiration), and the increased availability of iron, which collectively reduce the requirement for de novo Fe/S cluster synthesis. On the other hand, although the ISC system is severely impaired in the absence of frataxin, Jaj1, or Grx5, it apparently retains a remnant functionality that is sufficient to produce enough Fe/S clusters to secure survival under these conditions (Figure 4). This reasoning parallels findings in bacteria, where the simplified NIF system (with only cysteine desulphurase NifS and the Fe/S scaffold NifU) can take the place of the ISC and SUF systems under anaerobic conditions (Tokumoto et al. 2004).

The protective role of thiol redox systems on Fe/S proteins and their biogenesis

In addition to the Fe/S cofactor, the cysteine thiols that ligate these clusters are susceptible to redox modifications. The binding of Fe/S clusters requires that the cysteine residues are in the reduced, thiol or thiolate state. Curiously, multiple studies have identified highly reactive cysteines in many Fe/S proteins, including cluster-binding residues (Backus et al. 2016; Bak et al. 2017; Brandes et al. 2011; Leichert et al. 2008; Weerapan et al. 2010). These reactive cysteines could be sites of relevant sulfhydryl modifications, e.g., persulfidation (–S-S–H), glutathionylation (–S-SG), oxidation (–S-S–), nitrosylation (–S-NO), which remain to be further explored. With regards to Fe/S proteins, the U-type Fe/S cluster scaffolds Isu1 and bacterial NifU and SufB are Fe/S proteins that have been verified to bind functionally relevant persulfides at Fe/S cluster-ligating cysteines (Lill and Freibert 2020; Parent et al. 2015; Przybyla-Toscano et al. 2018). In yeast, this chemistry is orchestrated by the Nfs1-Isd11-Acp1-Isu1-Yah1-Yfh1 ISC complex for the de novo synthesis of [2Fe-2S] clusters and is dependent on cysteine desulfurase activity (Figure 4). Most other Fe/S proteins would likely be functionally hindered by thiol modifications on cysteine residues, as the Fe/S cluster-binding site would be inaccessible. However, direct evidence for sulfhydryl derivatization of apoproteins has been obtained in yeast cells where the ISC machinery is impaired. Polysulfide adducts on a mitochondria-targeted Fe/S reporter protein distinctly increase upon depletion of early-acting ISC proteins, but not when late-acting ISC factors were diminished (Christ et al. 2016). Cytosolic Fe/S apoproteins do not show such modifications. Consistent with this observation, the reduction of target apoproteins by the dithiol-Grx/GSH or the Trx system is a prerequisite of Fe/S cluster insertion in vitro (Weiler et al. 2020).

If such per- and polysulfide modifications were physiologically relevant, they could function in protecting Fe/S cluster-binding sites in the apo state under stress conditions. Removal of these sulfur adducts may then be carried out by the Trx/Grx systems (Figure 1), which has been observed in human cells (Doka et al. 2016; Wedmann et al. 2016). Yeast cells lacking both the mitochondrial Trx and Grx systems by deleting the respective reductases (trr2Δ and ghrΔ, Figure 1) are viable (Trotter and Grant 2005) and the core ISC machinery remains functional as evidenced by normal iron homeostasis, a functional CIA machinery, and the normal function of various mitochondrial Fe/S proteins (Braymer et al. 2019; Song et al. 2006). A subset of mitochondrial Fe/S target proteins (e.g., succinate dehydrogenase and biotin synthase), however, indeed become inactivated indicating that certain Fe/S proteins are particularly sensitive to thiol redox perturbations (Figure 4). These observations agree with experiments in humans cell lysates showing that succinate dehydrogenase has several reactive, cluster-coordinating cysteine residues (Bak et al. 2017). Surprisingly, the cytosol is more sensitive and the depletion of cellular Grx/Trx oxidoreductases (TRR1, trr2Δ, and ghrΔ, Figure 1) prevents all cytosolic Fe/S cluster target proteins from being matured due to a defect starting at the CIA component Nar1 (Braymer et al. 2019). Therefore, Nar1 is not only implicated in oxygen sensitivity but is likely coupled to thiol redox balance (Figure 4). Taken together, the maintenance of Fe/S proteins and their biogenesis overlap with thiol redox balance. Further detailed studies are required to identify the exact Nar1 modifications and how they influence protein function. Similarly, studies in bacterial systems have suggested that thiol-reducing equivalents are required for Fe/S cluster biogenesis (Ding et al. 2005; Nomata et al. 2015; Zheng et al. 2019).

Lastly, thiol redox balance and Fe/S cluster biogenesis also appear to intersect in higher eukaryotes. In human cells, depletion of the mitochondrial dithiol glutaredoxin GLRX2 decreases activities of complex I and aconitate (Johansson et al. 2007; Lee et al. 2009; Lillig et al. 2005). GLRX2 is proposed to be a redox sensor in the cell via its [2Fe-2S] cluster, which is destabilized and disassembled upon oxidative stress or exposure to nitric
oxide, promoting either the dithiol-dependent oxidoreductase activity of GLRX2, or nitric oxide detoxification to combat oxidative stress (Berndt et al. 2007; Lepka et al. 2017; Lillig et al. 2005; Scalcon et al. 2019). Importantly, this cellular function is distinct from that of monothiol Grxs that are involved in Fe/S cluster trafficking. Various Trxs have also been characterized in vitro as Fe/S cluster-binding proteins. Whether they may act as additional cellular redox sensors awaits experimental verification in vivo (Bisio et al. 2016; Zannini et al. 2019; Zheng et al. 2019). In summary, a number of proteins in the glutaredoxin-thioredoxin family can bind Fe/S clusters and in this respect, the monothiol Grxs are the best functionally characterized members.

Outlook

In recent years many aspects of the intimate and multilayered connection between cellular iron metabolism and components of the cellular thiol redox systems have been studied. The precise roles of Grxs and GSH in the maturation of Fe/S proteins and the regulation of cellular iron homeostasis have been characterized in detail. Yet, many important questions remain. In particular, the mechanistic details of the function of cytosolic glutaredoxins in various aspects of cellular iron metabolism remain to be elucidated and may be distinct in different organisms. Moreover, we expect that further connections between the cellular thiol redox systems and cellular Fe/S protein maturation will be elucidated in the future.

Acknowledgments: We thank our colleagues at the Collaborative research Center 987 for their support and the members of our group for their great work and helpful discussions.

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: We acknowledge generous financial support: R.L. from Deutsche Forschungsgemeinschaft (DFG) (SFB 987, Koseleck grant LI 415/6, LI 415/5, SPP 1710, and SPP 1927), COST Action FeSBioNet (Contract CA15133), and Volkswagen Foundation (‘Life’ program); U.M. from DFG (SFB 987); J.J.B. from the European Union (Marie-Curie Fellowship 659325) and DFG (SPP 1927).

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Abdalla, M., Dai, Y.N., Chi, C.B., Cheng, W., Cao, D.D., Zhou, K., Ali, W., Chen, Y., and Zhou, C.Z. (2016). Crystal structure of yeast monothiol glutaredoxin Grx6 in complex with a glutathione-coordinated [2Fe-2S] cluster. Acta Crystallogr F Struct Biol Commun 72: 732–737.

Alhebshi, A., Sideri, T.C., Holland, S.L., and Avery, S.V. (2012). The essential iron-sulfur protein Rli1 is an important target accounting for inhibition of cell growth by reactive oxygen species. Mol. Biol. Cell 23: 3582–3590.

Alves, R., Vilaprinyo, E., Sorribas, A., and Herrero, E. (2009). Evolution based on domain combinations: the case of glutaredoxins. BMC Evol. Biol. 9: 66.

Anderson, C.P., Shen, M., Eisenstein, R.S., and Leibold, E.A. (2012). Mammalian iron metabolism and its control by iron regulatory proteins. Biochim. Biophys. Acta 1823: 1468–1483.

Ast, T., Meisel, J.D., Patra, S., Wang, H., Grange, R.M.H., Kim, S.H., Calvo, S.E., Orefice, L.L., Nagashima, F., Ichinose, F., et al. (2019). Hypoxia rescues frataxin loss by restoring iron sulfur cluster biogenesis. Cell 177: 1507–1521 e1516.

Attarian, R., Hu, G., Sanchez-Leon, E., Caza, M., Croll, D., Do, E., Bach, H., Missall, T., Lodge, J., Jung, W.H., et al. (2018). The monothiol glutaredoxin Grx4 regulates iron homeostasis and virulence in Cryptococcus neoformans. mBio 9, https://doi.org/10.1128/mbio.02377-18.

Babu, M.M., Iyer, L.M., Balaji, S., and Aravind, L. (2006). The natural history of the WRKY-GCM1 zinc fingers and the relationship between transcription factors and transposons. Nucleic Acids Res. 34: 6505–6520.

Backus, K.M., Correia, B.E., Lum, K.M., Forli, S., Horning, B.D., Gonzalez-Paez, G.E., Chatterjee, S., Lanning, B.R., Teljaro, J.R., Olson, A.J., et al. (2016). Proteome-wide covariant ligand discovery in native biological systems. Nature 534: 570–574.

Bak, D.W., Pizzagalli, M.D., and Weerapana, E. (2017). Identifying functional cysteine residues in the mitochondria. ACS Chem. Biol. 12: 947–957.

Baker, P.R., 2nd, Friederich, M.W., Swanson, M.A., Shaikh, T., Bhattacharya, K., Scharer, G.H., Aicher, J., Creadon-Swindell, G., Geiger, E., MacLean, K.N., et al. (2016). Variant nonketotic hyperglycemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. Brain 137: 366–379.

Banci, L., Branccacci, D., Ciofi-Baffoni, S., Del Conte, R., Gadeppalli, R., Mikolaiczky, M., Neri, S., Piccioli, M., and Winkelmann, J. (2014). [2Fe-2S] cluster transfer in iron-sulfur protein biogenesis. Proc. Natl. Acad. Sci. U.S.A. 111: 6203–6208.

Banci, L., Ciofi-Baffoni, S., Gajda, K., Muzzioli, R., Peruzzini, R., and Winkelmann, J. (2015). N-terminal domains mediate [2Fe-2S] cluster transfer from glutaredoxin-3 to anamorsin. Nat. Chem. Biol. 11: 772–777.

Bandyopadhyay, S., Gama, F., Molina-Navarro, M.M., Gualberto, J.M., Claxton, R., Naik, S.G., Huyhn, B.H., Herrero, E., Jacquot, J.P., Johnson, M.K., et al. (2008). Chloroplast monothiol glutaredoxins as scaffold proteins for the assembly and delivery of [2Fe-2S] clusters. EMBO J. 27: 1122–1133.

Bernard, B.G., Cheng, Y., Zhao, Y., and Balk, J. (2009). An allelic mutant series of ATM3 reveals its key role in the biogenesis of cytosolic iron-sulfur proteins in Arabidopsis. Plant Physiol. 151: 590–602.
Berndt, C., Hudemann, C., Hanchschmann, E.M., Axelsson, R., Holmgren, A., and Lillig, C.H. (2007). How does iron-sulfur cluster coordination regulate the activity of human glutaredoxin 2? Antioxid. Redox Signal. 9: 151–157.

Berndt, C. and Lillig, C.H. (2017). Glutathione, glutaredoxins, and iron. Antioxid. Redox Signal. 27: 1235–1251.

Bisio, H., Bonilla, M., Manta, B., Grana, M., Salzman, V., Aguilar, P.S., Gladyshev, V.N., Comini, M.A., and Salinas, G. (2016). A new class of thioredoxin-related protein able to bind iron-sulfur clusters. Antioxid. Redox Signal. 24: 205–216.

Boniecki, M.T., Freibert, S.A., Muhlenhoff, U., Lill, R., and Cygler, M. (2017). Structure and functional dynamics of the mitochondrial Fe/S cluster synthesis complex. Nat. Commun. 8: 1287.

Bonomi, F., Iametti, S., Morleo, A., Ta, D., and Vickery, L.E. (2011). Using quantitative redox proteomics to dissect the yeast redoxome. J. Biol. Chem. 286: 41893–41899.

Borner, S., Domenach, A., Carmona, M., Garcia-Santamarina, S., Boni, M.C., Ayte, J., and Hidalgo, E. (2017). Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery. J. Am. Chem. Soc. 136: 16240–16250.

Brandes, N., Reichmann, D., Tienson, H., Leichert, L.I., and Jakob, U. (2009). Mechanistic aspects of Fe/S cluster assembly in the mitochondrial Fe/S cluster synthesis complex. Nat. Rev. Biochem. 8: 733–743.

Brandt, A., Mourer, T., and Labbe, S. (2015). Molecular basis of the regulation of iron homeostasis in fission and filamentous yeasts. IUBMB Life 67: 801–815.

Braymer, J.M., Wajcman, A., Mitalipova, M., and Lillig, C.H. (2007). The role of iron-sulfur clusters in the folding of proteins and their potential involvement in human disease. Antioxid. Redox Signal. 9: 151–157.

Buckle, W.R., Tissue, D., and Devine, D.J. (2017). The oxidative stress response in yeast cells involves changes in the stability of Aft1 regulon mRNAs. Mol. Microbiol. 113: 232–248.

Cavalli, P., Biasiotti, G., Poli, M., Levi, S., Verardi, R., Zanella, I., Derosas, M., Ingrassia, R., Corrado, M., and Arosio, P. (2007). RNA silencing of the mitochondrial ABCB7 transporter in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload. Blood 109: 3552–3559.

Chao, L.Y., Marletta, M.A., and Rine, J. (2008). Sre1, an iron-modulated GATA DNA-binding protein of iron-uptake genes in the fungal pathogen Histoplasma capsulatum. Biochemistry 47: 7274–7283.

Cheng, N.H., Zhang, W., Chen, W.Q., Jin, J., Cui, X., Butte, N.F., Chan, L., and Hirschi, K.D. (2011). A mammalian monothiol glutaredoxin, Grx3, is critical for cell cycle progression during embryogenesis. FEBS J. 278: 2525–2539.

Chi, C.B., Tang, Y., Zhang, J., Dai, Y.N., Abdalla, M., Chen, Y., and Zhou, C.Z. (2018). Structural and biochemical insights into the multiple functions of yeast Grx3. J. Mol. Biol. 430: 1235–1248.

Christ, S., Leichert, L.I., Willms, A., Lill, R., and Muhlenhoff, U. (2016). Effects of mitochondrial iron-sulfur cluster assembly induce cysteine S-polythiolation on iron-sulfur apoproteins. Antioxid. Redox Signal. 25: 28–40.

Cioffi-Baffoni, S., Nasta, V., and Bacci, L. (2018). Protein networks in the maturation of mammalian iron-sulfur proteins. Metallol 10: 49–72.

Connorton, J.M., Balk, J., and Rodriguez-Celma, J. (2017). Iron homeostasis in plants—a brief overview. Metallomics 9: 813–823.

Cotruvo, J.A. and Stroope, K. (2011). Class I ribonucleotide reductases: metallocofactor assembly and repair in vitro and in vivo. Annu. Rev. Biochem. 80: 733–767.

Courel, M., Lallet, S., Camadro, J.M., and Blaiseau, P.L. (2005). Direct activation of genes involved in intracellular iron use by the yeast iron-responsive transcription factor Aft2 without its paralog Aft1. Mol. Cell Biol. 25: 6760–6771.

Crack, J.C., Green, J., Thomson, A.J., and LeBrun, N.E. (2014). Iron-sulfur clusters as biological sensors: the chemistry of reactions with molecular oxygen and nitric oxide. Acc. Chem. Res. 47: 3196–3205.

Crack, J.C. and Le Brun, N.E. (2018). Redox-sensing iron-sulfur cluster regulators. Antioxid. Redox Signal. 29: 1809–1829.

Cutone, A., Howes, B.D., Miele, A.E., Miele, R., Giorgi, A., Battistoni, A., Smulevich, G., Musci, G., and di Patti, M.C. (2016). Pichia pastoris Fep1 is a [2Fe-2S] protein with a Zn finger that displays an unusual oxygen-dependent role in cluster binding. Sci. Rep. 6: 31872.

Deponte, M. (2017). The incomplete glutathione puzzle: just guessing at numbers and figures? Antioxid. Redox Signal. 27: 1130–1161.

Ding, H., Harrison, K., and Lu, J. (2005). Thioredoxin reductase system mediates iron binding in IscA and iron delivery for the iron-sulfur cluster assembly in IscO. J. Biol. Chem. 280: 30432–30437.

Dlouhy, A.C., Beaudoin, J., Labbe, S., and OUtten, C.E. (2017). Schizosaccharomyces pombe Grx4 regulates the transcriptional repressor Pph4 via [2Fe-2S] cluster binding. Metallomics 9: 1096–1105.
Doka, E., Pader, I., Biró, A., Johansson, K., Cheng, Q., Ballago, K., Prigge, J.R., Pastor-Flores, D., Dick, T.P., Schmidt, E.E., et al. (2016). A novel persulfide detection method reveals protein persulfide- and polysulfide-reducing functions of thioredoxin and glutathione systems. Science advances 2: e1500968.

Donelson, J., Wang, Q., Monroe, T.O., Jiang, X., Zhou, J., Yu, H., Mo, Q., Sun, Q., Marini, J.C., Wang, X., et al. (2019). Cardiac-specific ablation of glutaredoxin 3 leads to cardiac hypertrophy and heart failure. Phys. Rep. 7: e14071.

Dutkiewicz, R. and Nowak, M. (2018). Molecular chaperones involved in mitochondrial iron-sulfur protein biogenesis. J. Biol. Inorg. Chem. 23: 569–579.

Encinar del Dedo, J., Gabrielli, N., Carmona, M., Ayte, J., and Hidalgo, E. (2015). A cascade of iron-containing proteins governs the genetic iron starvation response to promote iron uptake and inhibit iron storage in fission yeast. PLoS Genet. 11: e1005106.

Fox, N.G., Yu, X., Feng, X., Bailey, H.J., Martelli, A., Nabhan, J.F., Strain-Dutkiewicz, R. and Nowak, M. (2018). Transcriptional coactivators in yeast and beyond. Trends Biochem. Sci. 20: 517–521.

Gupta, M. and Outten, C.E. (2020). Iron-sulfur cluster signaling: the common thread in fungal iron regulation. Curr. Opin. Chem. Biol. 55: 189–201.

Hadzhieva, M., Kirches, E., and Mawrin, C. (2014). Review: iron metabolism and the role of iron in neurodegenerative disorders. Neuropathol. Appl. Neurobiol. 40: 240–257.

Hanschmann, E.M., Godoy, J.R., Berndt, C., Hudemann, C., and Lillig, C.H. (2013). Thioredoxins, glutaredoxins, and peroxiredoxins: molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. Antioxid. Redox Signal. 19: 1539–1605.

Harrison, K.A. and Marzluf, G.A. (2002). Characterization of DNA binding and the cysteine rich region of SRE, a GATA factor in Neurospora crassa involved in siderophore synthesis. Biochemistry 41: 15288–15295.

Haunhorst, P., Berndt, C., Eltner, S., Godoy, J.R., and Lillig, C.H. (2010). Characterization of the human monothiol glutaredoxin 3 (PICOT) as iron-sulfur protein. Biochem. Biophys. Res. Commun. 394: 372–376.

Haunhorst, P., Hanschmann, E.M., Brautigam, L., Stehling, O., Hoffmann, B., Muhlenhoff, U., Lill, R., Berndt, C., and Lillig, C.H. (2013). Crucial function of vertebrate glutaredoxin 3 (PICOT) in iron homeostasis and hemoglobin maturation. Mol. Biol. Cell 24: 1895–1903.

Herrero, E., Belli, G., and Casa, C. (2010). Structural and functional diversity of glutaredoxins in yeast. Curr. Protein Pept. Sci. 11: 659–668.

Herrero, E., Ros, J., Belli, G., and Cabisco, E. (2008). Redox control and oxidative stress in yeast cells. Biochim. Biophys. Acta 1780: 1217–1235.

Hoffmann, B., Uzarska, M.A., Berndt, C., Godoy, J.R., Haunhorst, P., Lillig, C.H., Lill, R., and Muhlenhoff, U. (2011). The multidomain thioredoxin-monothiol glutaredoxins represent a distinct functional group. Antioxid. Redox Signal. 15: 19–30.

Imlay, J.A. (2008). Cellular defenses against superoxide and hydrogen peroxide. Annu. Rev. Biochem. 77: 755–776.

Imlay, J.A. (2006). Iron-sulfur clusters and the problem with oxygen. Mol. Microbiol. 59: 1073–1082.

Imlay, J.A., Sethu, R., and Rohaun, S.K. (2019). Evolutionary adaptations that enable enzymes to tolerate oxidative stress. Free Radic. Biol. Med. 140: 4–13.

Inigo, S., Durand, A.N., Ritter, A., Le Gall, S., Termathe, M., Klassen, R., Tohge, T., De Coninck, B., Van Leeuwen, J., De Clercq, R., et al. (2016). Glutaredoxin GRXS17 associates with the cytosolic iron-sulfur cluster assembly pathway. Plant Physiol. 172: 858–873.

Iwema, T., Picciocchi, A., Traore, D.A., Ferrer, J.L., Chauvat, F., and Jacquemet, L. (2009). Structural basis for delivery of the intact [Fe2S2] cluster by monothiol glutaredoxin. Biochemistry 48: 6041–6043.

Izquierdo, A., Casas, C., Muhlenhoff, U., Lillig, C.H., and Herrero, E. (2008). Saccharomyces cerevisiae Grx6 and Grx7 are monothiol glutaredoxins associated with the early secretory pathway. Eukaryot. Cell 7: 1415–1426.

Jacques, J.F., Mercier, A., Brault, A., Mourer, T., and Labbe, S. (2014). Fra2 is a co-regulator of Pep1 inhibition in response to iron starvation. PloS One 9: e98959.

Jacquot, J.P. and Zaffagnini, M. (2019). Thioredoxin and glutaredoxin systems antioxidants Special Issue, vol 8. Basel: Antioxidants.
Jain, I.H., Calvo, S.E., Markhard, A.L., Skinner, O.S., To, T.L., Ast, T., and Mootha, V.K. (2020). Genetic screen for cell fitness in high or low oxygen highlights mitochondrial and lipid metabolism. Cell 181: 716–727 e711.

Jainarayanan, A.K., Yadav, S., and Bachhawat, A.K. (2020). Yeast glutaredoxin, Grx4, functions as a glutathione S-transferase required for red ade pigment formation in Saccharomyces cerevisiae. J. Biosci. 45, https://doi.org/10.1007/s12038-020-0015-z.

Jbel, M., Mercier, A., and Labbe, S. (2011). Grx4 monothiol glutaredoxin is required for iron limitation-dependent inhibition of Fep1. Eukaryot. Cell 10: 629–645.

Jbel, M., Mercier, A., Pelletier, B., Beaudoin, J., and Labbe, S. (2009). Iron activates in vivo DNA binding of Schizosaccharomyces pombe transcription factor Fep1 through its amino-terminal region. Eukaryot. Cell 8: 649–664.

Jeong, D., Cha, H., Kim, E., Kang, M., Yang, D.K., Kim, J.M., Yoon, P.O., Oh, J.G., Bernecker, O.Y., Sakata, S., et al. (2006). PICOT inhibits cardiac hypertrophy and enhances ventricular function and cardiomyocyte contractility. Circ. Res. 99: 307–314.

Jia, M., Sen, S., Wachnowsky, C., Fidai, I., Cowan, J.A., and Wysocki, V.H. (2020). Characterization of [2Fe-2S]-cluster-bridged protein complexes and reaction intermediates by use of native mass spectrometric methods. Angew Chem. Int. Ed. Engl. 59: 6724–6728.

Johansson, C., Kavanagh, K.L., Gileadi, O., and Oppermann, U. (2007). Reversible sequestration of active site cysteines in a 2Fe-2S-bridged dimer provides a mechanism for glutaredoxin 2 regulation in human mitochondria. J. Biol. Chem. 282: 3077–3082.

Johansson, C., Roos, A.K., Montano, S.J., Sengupta, R., Filippakopoulos, P., Guo, K., von Delft, F., Holmgren, A., Oppermann, U., and Kavanagh, K.L. (2011). The crystal structure of human GLRXS: iron-sulfur cluster co-ordination, tetrameric assembly and monomer activity. Biochem. J. 433: 303–311.

Jung, W.H. and Cronstad, J.W. (2011). Iron influences the abundance of the iron regulatory protein Cir1 in the fungal pathogen Cryptococcus neoformans. FEBS Lett. 585: 3342–3347.

Kampinga, H.H. and Craig, E.A. (2010). The HSP70 chaperone machinery: I proteins as drivers of functional specificity. Nat. Rev. Mol. Cell Biol. 11: 579–592.

Kato, N., Motohashi, S., Okada, T., Ozawa, T., and Mashima, K. (2008). Characterization of the nuclear import mechanism of the nuclear import mechanism of Fep1. Eukaryot. Cell 10: 629–645.

Kobayashi, K., Fujikawa, M., and Kozawa, T. (2014). Oxidative stress sensing by the iron-sulfur cluster in the transcription factor. SsxxR. J Inorg Biochem. 133: 87–91.

Kuhn, L.C. (2015). Iron regulatory proteins and their role in controlling iron metabolism. Metall 7: 232–243.

Kumanovics, A., Chen, O.S., Li, L., Bagley, D., Adkins, E.M., Lin, H., Dingra, N.N., Outten, C.E., Keller, G., Winge, D., et al. (2008). Identification of Fra1 and Fra2 as genes involved in regulating the yeast iron regulon in response to decreased mitochondrial iron-sulfur cluster synthesis. J. Biol. Chem. 283: 10276–10286.

Kumar, C., Igbaria, A., D’Autreux, B., Planson, A.G., Junot, C., Godat, E., Bachhawat, A.K., Delaunay-Moisans, A., and Toledano, M.B. (2011). Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol-redox control. EMBO J. 30: 2044–2056.

Kushnir, S., Babychuk, E., Storozhenko, S., Davey, M.W., Pappenbrock, J., De Rycke, R., Engler, G., Stephan, U.W., Lange, H., Kispal, G., et al. (2003). A mutation of the mitochondrial ABC transporter Sta1 leads to dwarfism and chlorosis in the Arabidopsis mutant star1. Plant Cell 13: 89–100.

Labbe, S., Khan, M.G., and Jacques, J.F. (2013). Iron uptake and regulation in Schizosaccharomyces pombe. Curr. Opin. Microbiol. 16: 669–676.

Lane, D.J., Merlot, A.M., Huang, M.L., Bae, D.H., Jansson, P.J., Sahni, S., Kalinowski, D.S., and Richardson, D.R. (2015). Cellular iron uptake, trafficking and metabolism: key molecules and mechanisms and their roles in disease. Biochim. Biophys. Acta 1853: 1130–1144.

Lee, D.W., Kaur, D., Chinta, S.J., Rajagopalan, S., and Andersen, J.K. (2009). A disruption in iron-sulfur center biogenesis via inhibition of mitochondrial dithiol glutaredoxin 2 may contribute to mitochondrial and cellular iron dysregulation in mammalian glutathione-depleted dopaminergic cells: implications for Parkinson’s disease. Antioxid. Redox Signal. 11: 2083–2094.

Leichert, L.I., Gehrke, F., Gudiseva, H.V., Blackwell, T., Ilbert, M., Walker, A.K., Strahler, J.R., Andrews, P.C., and Jakob, U. (2008). Quantifying changes in the thiol redox proteome upon oxidative stress in vivo. Proc. Natl. Acad. Sci. U.S.A. 105: 8197–8202.

Lepka, K., Volbracht, K., Hill, E., Schneider, R., Rios, N., Hildebrandt, T., Ingwersen, J., Prozorovski, T., Lillig, C.H., van Horssen, J., et al. (2017). Iron-sulfur glutaredoxin 2 protects oligodendrocytes against damage induced by nitric oxide release from activated microglia. Glia 65: 1521–1534.

Li, H., Mapolelo, D.T., Randeniya, S., Johnson, M.K., and Outten, C.E. (2012). Human glutaredoxin 3 forms [2Fe-2S]-bridged complexes with human B0A2. Biochemistry 51: 1687–1696.

Li, H. and Outten, C.E. (2019). The conserved CDC motif in the yeast iron regulator Atf2 mediates iron-sulfur cluster exchange and protein-protein interactions with Grx3 and B0Z2. J. Biol. Inorg. Chem. 24: 809–815.

Li, H., Stumpflig, M., Zhang, C., An, X., Stubbe, J., Lill, R., and Huang, M. (2017). The diferric-tyrosyl radical cluster of ribonucleotide reductase and cytosolic iron-sulfur clusters have distinct and similar biogenesis requirements. J. Biol. Chem. 292: 11445–11451.

Li, J. and Cowan, J.A. (2015). Glutathione-coordinated [2Fe-2S] cluster: a viable physiological substrate for mitochondrial ABCB7 transport. Chem. Commun. 51: 2253–2255.
Nasta, V., Suraci, D., Gourdoupis, S., Ciofi-Baffoni, S., and Banci, L. (2020). A pathway for assembling [4Fe-4S][2+] clusters in mitochondrial iron-sulfur protein biogenesis. FEBS J. 287: 2312–2327.

Netz, D.J., Genau, H.M., Weiler, B.D., Bill, E., Pierik, A.J., and Lill, R. (2016). The conserved protein Dre2 uses essential [2Fe-2S] and [4Fe-4S] clusters for its function in cytosolic iron-sulfur protein assembly. Biochem. J. 473: 2073–2085.

Netz, D.J., Stumpf, M., Dore, C., Muhlenhoff, U., Pierik, A.J., and Lill, R. (2010). Tah18 exchanges transferrins to Dre2 in cytosolic iron-sulfur protein biogenesis. Nat. Chem. Biol. 6: 758–765.

Nomata, J., Maeda, M., Isu, A., Inoue, K., and Hisabori, T. (2015). The conserved protein Dre2 uses essential [2Fe-2S] and [4Fe-4S] clusters for its function in cytosolic iron-sulfur protein assembly. FEBS J. 287: 758–764.

Pandey, A., Pain, J., Dziuba, N., Pandey, A.K., Dancis, A., Lindahl, P.A., Pearson, S.A., Wachnowsky, C., and Cowan, J.A. (2020). The basic leucine zipper stress response regulator Yap5 senses high-iron conditions by coordination of [2Fe-2S] clusters. Mol. Cell Biol. 35: 370–378.

Pandey, A.K., Pain, J., Dancis, A., and Pain, D. (2019). Mitochondria export iron-sulfur and iron-sulfur intermediates to the cytoplasm for the activity of iron/sulfur enzymes. Trends Biochem. Sci. U.S.A. 111: 4043–4048.

Parent, A., Elduque, X., Cornu, D., Belot, L., Le Caer, J.P., Grandas, A., Peggion, C., Lopreiato, R., Casanova, E., Ruzzene, M., Facchin, S., Pham, K., Pal, R., Qu, Y., Liu, X., Yu, H., Shiao, S.L., Wang, X., O’Brien Smith, E., Cui, X., Rodnie, G.O., and Cheng, N. (2015). Nuclear glutaredoxin 3 is critical for protection against oxidative stress-induced cell death. Free Radic. Biol. Med. 85: 197–206.

Pellicer Martinez, M.T., Crack, J.C., Stewart, M.Y., Bradley, I.M., Svistunenko, D.A., Johnston, A.W., Cheesman, M.R., Todd, J.D., and Le Brun, N.E. (2019). Mechanisms of iron- and O2-sensing by the [4Fe-4S] cluster of the global iron regulator RirA. Cell Chem Biol 25: 738–748.

Peggion, C., Lopreiato, R., Casanova, E., Ruzzene, M., Facchin, S., Pham, K., Pal, R., Qu, Y., Liu, X., Yu, H., Shiao, S.L., Wang, X., O’Brien Smith, E., Cui, X., Rodnie, G.O., and Cheng, N. (2015). Nuclear glutaredoxin 3 is critical for protection against oxidative stress-induced cell death. Free Radic. Biol. Med. 85: 197–206.

Pham, K., Pal, R., Qu, Y., Liu, X., Yu, H., Shiao, S.L., Wang, X., O’Brien Smith, E., Cui, X., Rodnie, G.O., and Cheng, N. (2015). Nuclear glutaredoxin 3 is critical for protection against oxidative stress-induced cell death. Free Radic. Biol. Med. 85: 197–206.

Pondarre, C., Antichos, B.B., Campagna, D.R., Clarke, S.L., Greer, E.L., Deck, K.M., McDonald, A., Han, A.P., Medlock, A., Kutok, J.L., et al. (2006). The mitochondrial ATP-binding cassette transporter Abcb7 is essential in mice and participates in cytosolic iron-sulfur cluster biogenesis. Hum. Mol. Genet. 15: 953–964.

Pow, C.B., Wegner, S.V., Li, H., Dlouhy, A.C., Schuermann, J.P., Sanishvili, R., Hinshaw, J.R., Riggs-Gelasco, P.J., Utten, C.E., and He, C. (2014). Nuclear glutaredoxin 3 function in storing and transporting [Fe2S2] clusters in plastids. J. Biol. Inorg. Chem. 23: 545–566.

Pujol-Carrion, N. and Torre-Ruiz, M.A. (2017). Physical interaction between the PAPK Slit2 of the PKC1-MAPK pathway and Gx4 glutaredoxins is required for the oxidative stress response in budding yeast. Free Radic. Biol. Med. 103: 107–120.

Qi, W., Li, J., and Cowan, J.A. (2016). A structural model for glutathione-complexed iron-sulfur cluster as a substrate for ABCB7-type transporters. Chem. Commun. 50: 3795–3798.

Rietzschel, N., Pierik, A.J., Sartori, G. (2008). Dimers of glutaredoxin 2 as mitochondrial redox sensors for cytosolic metal cofactor assembly. J. Biochem. (Tokyo) 158: 253–261.

Rouhier, N., Couturier, J., and Rouver, I. (2018). The basic leucine zipper stress response regulator Yap5 senses high-iron conditions by coordination of [2Fe-2S] clusters. Mol. Cell Biol. 35: 370–378.

Rodriguez-Manzaneque, M.T., Ros, I., Cabiscol, E., Sorribas, A., and Herrero, E. (1999). Gx5 glutaredoxin plays a central role in protection against protein oxidative damage in Saccharomyces cerevisiae. Mol. Cell Biol. 19: 8180–8190.

Rodriguez-Manzaneque, M.T., Tamarit, J., Belli, G., Ros, J., and Herrero, E. (2002). Gx5 is a mitochondrial glutaredoxin required for the activity of iron/sulfur enzymes. Mol. Cell Biol. 13: 1109–1121.

Rouquier, N., Couturier, J., and Jacquot, J.P. (2010). Glutaredoxins: roles in iron homeostasis. Trends Biochem. Sci. 35: 43–52.

Saito, Y., Shibayama, H., Tanaka, H., Tanimura, A., Matsumura, I., and Nakamura, Y. (2011). PICOT is a molecule which binds to anamorins. Biochem. Biophys. Res. Commun. 408: 329–333.

Scalcon, V., Tonolo, F., Folda, A., Bindoli, A., and Rigobello, M.P. (2019). Dimer of glutaredoxin 2 as mitochondrial redox sensors in senile-iron-induced oxidative stress. Metallomics 11: 1241–1251.

Schäfer, T.A., Faust, B., Shintre, C.A., Carpenter, E.P., Srivinavan, V., van Veen, H.W., and Balk, J. (2015). Structures and functions of mitochondrial ABC transporters. Biochem. Soc. Trans. 43: 943–951.

Schäfer, T.A., Thornton, J.D., Kruse, I., Schwarzlander, M., Meyer, A.J., van Veen, H.W., and Balk, J. (2014). A conserved mitochondrial ATP-binding cassette transporter exports glutathione polysulfide for cytosolic metal cofactor assembly. J. Biol. Chem. 289: 23264–23274.

Schrenk, M. and Has, H. (2011). Iron homeostasis–Achilles’ heel of Aspergillus fumigatus? Curr. Opin. Microbiol. 14: 400–405.

Seh, S. and Cowan, J.A. (2017). Role of protein-glutathione contacts in defining glutaredoxin-3 [2Fe-2S] cluster chirality, ligand exchange and transfer chemistry. J. Biol. Inorg. Chem. 22: 1075–1087.

Shakamuri, P., Zhang, B., and Johnson, M.K. (2012). Monothiol glutaredoxins function in storing and transporting [Fe2S2] clusters in plastids. J. Biol. Inorg. Chem. 23: 112–117.
clusters assembled on IscU scaffold proteins. J. Am. Chem. Soc. 134: 15215–15216.

Sheftel, A.D., Stiehl, O., Pierik, A.J., Netz, D.J., Kerscher, S., Elsasser, H.P., Wittig, I., Balk, J., Brandt, U., and Lill, R. (2009). Human indo1, an iron-sulfur cluster assembly factor for respiratory complex I. Mol. Biol. Cell 20: 6059–6073.

Sheftel, A.D., Wilbrecht, C., Stiehl, O., Niggemeyer, B., Elsasser, H.P., Muhlenhoff, U., and Lill, R. (2012). The human mitochondrial ISCA1, ISCA2, and IBA57 proteins are required for [4Fe-4S] protein maturation. Mol. Cell Biol. 32: 1157–1166.

Sipos, K., Lange, H., Fekete, Z., Ullmann, P., Lill, R., and Kispal, G. (2002). Maturation of cytosolic iron-sulfur proteins requires glutathione. J. Biol. Chem. 277: 26944–26949.

Snoek, I.S. and Steenstra, N.Y. (2006). Why does Klyveromyces lactis not grow under anaerobic conditions? Comparison of essential anaerobic genes of Saccharomyces cerevisiae with the Klyveromyces lactis genome. FEMS Yeast Res. 6: 393–403.

Sommerhalter, M., Voegtl, W., Perlstein, D.L., Ge, J., Stubbe, J., and Rosenzweig, A.C. (2004). Structures of the yeast ribonucleotide reductase Rnr2 and Rnr4 homodimers. Biochemistry 43: 7736–7742.

Song, J.Y., Cha, J., Lee, J., and Roe, J.H. (2006). Glutathione reductase and a mitochondrial thioreredoxin play overlapping roles in maintaining iron-sulfur enzymes in fission yeast. Eukaryot. Cell 5: 1857–1865.

Srinivasan, V., Pierik, A.J., and Lill, R. (2014). Crystal structures of nucleotide-free and glutathione-bound mitochondrial ABC transporter Atm1. Science 343: 1137–1140.

Stiehl, O., Wilbrecht, C., and Lill, R. (2014). Mitochondrial iron-sulfur protein biogenesis and human disease. Biochimie 100: 61–77.

Strain, J., Lorenz, C.R., Bode, J., Garland, S., Smolen, G.A., Ta, D.T., Vickery, L.E., and Culotta, V.C. (1998). Suppressors of superoxide dismutase (SOD1) deficiency in Saccharomyces cerevisiae. Identification of proteins predicted to mediate iron-sulfur cluster assembly. J. Biol. Chem. 273: 31138–31144.

Taabazuing, C.Y., Hangasky, J.A., and Knapp, M.J. (2014). Oxygen sensing strategies in mammals and bacteria. J. Inorg. Biochem. 133: 63–72.

Taketani, S., Kakimoto, K., Ueta, H., Masaki, R., and Furukawa, T. (2003). Involvement of ABC7 in the biosynthesis of heme in erythroid cells: interaction of ABC7 with ferrochelatase. Blood 101: 3274–3280.

Tokumoto, U., Kitamura, S., Fukuyama, K., and Takahashi, Y. (2004). Interchangeability and distinct properties of bacterial Fe-S cluster assembly systems: functional replacement of the isc and suf operons in Escherichia coli with the nifSU-like operon from Helicobacter pylori. J. Biochem. (Tokyo) 136: 199–209.

Toledano, M.B. and Huang, M.E. (2017). The unfinished puzzle of glutathione physiological functions, an old molecule that still retains many enigmas. Antioxid. Redox Signal. 27: 1127–1129.

Trnka, D., Engelke, A.D., Gellert, M., Moseler, A., Hossain, M.F., Lindenberg, T.T., Pedrotelli, L., Odermatt, B., de Souza, J.V., Bronowska, A.K., et al. (2020). Molecular basis for the distinct functions of redox-active and FeS-transferring glutaredoxins. Nat. Commun. 11: 3445.

Trotter, E.W. and Grant, C.M. (2005). Overlapping roles of the cytoplasmic and mitochondrial redox regulatory systems in the yeast Saccharomyces cerevisiae. Eukaryot. Cell 4: 392–400.

Ueta, R., Fujimura, N., Iwai, K., and Yamaguchi-Iwai, Y. (2012). Iron-induced dissociation of the Aft1p transcriptional regulator from target gene promoters is an initial event in iron-dependent gene suppression. Mol. Cell Biol. 32: 4998–5008.

Uzarska, M.A., Dutkiewicz, R., Freibert, S.A., Lill, R., and Muhlenhoff, U. (2013). The mitochondrial Hsp70 chaperone Ssq1 facilitates Fe/S cluster transfer from Isu1 to Grx3 by complex formation. Mol. Biol. Cell 24: 1830–1841.

Vachon, P., Mercier, A., Jouel, M., and Labbe, S. (2012). The monothiol glutaredoxin Grx4 exerts an iron-dependent inhibitory effect on Php4 function. Eukaryot. Cell 11: 806–819.

Vall-Llaurà, N., Reverter-Branchat, G., Vived, C., Weertman, N., Rodriguez-Colman, M.J., and Cabic, S. (2016). Reversible glutathionylation of Sir2 by monothiol glutaredoxins Grx3/4 regulates stress resistance. Free Radic. Biol. Med. 96: 45–56.

Vickery, L.E. and Cupp-Vickery, J.R. (2007). Molecular chaperones HscA/Ssq1 and HscB/Jac1 and their roles in iron-sulfur protein maturation. Crit. Rev. Biochem. Mol. Biol. 42: 95–111.

Volbeda, A., Dodd, E.L., Darnault, C., Crack, J.C., Renoux, O., Hutchings, M.I., Le Brun, N.E., and Fontecilla-Camps, J.C. (2017). Crystal structures of the NO sensor NsrR reveal how its iron-sulfur cluster modulates DNA binding. Nat. Commun. 8: 15052.

Wachnowska, C., Fidai, I., and Cowan, J.A. (2018). Iron-sulfur cluster biosynthesis and trafficking – impact on human disease conditions. Metallomics 10: 9–29.

Wachnowska, C., Fidai, I., and Cowan, J.A. (2016). Iron-sulfur cluster exchange reactions mediated by the human Nfu protein. J. Biol. Inorg. Chem. 21: 825–836.

Wang, H., Shi, H., Rajan, M., Canarie, E.R., Hong, S., Simoneschi, D., Pagano, M., Bush, M.F., Stoll, S., Leibold, E.A., and Zheng, N. (2020). FBXL5 regulates IRP2 stability in iron homeostasis via an oxygen-responsive [2Fe2S] cluster. Mol. Cell 78: 31–41 e35.

Wang, Z., Ma, T., Huang, Y., Wang, J., Chen, Y., Kistler, H.C., Ma, Z., and Yin, Y. (2019). A fungal ABC transporter FgAtm1 regulates iron homeostasis via the transcription factor cascade FgAreA-HapX. PLoS Pathog. 15: e1007791.

Weber, H., Freibert, S.A., Gallo, A., Heidenreich, T., Linne, U., Amlacher, S., Hurt, E., Muhlenhoff, U., Banci, L., and Lill, R. (2014). Functional reconstitution of mitochondrial Fe/S cluster synthesis on Isu1 reveals the involvement of ferrodoxin. Nat. Commun. 5: 5013.

Wedmann, R., Onderka, C., Wei, S., Szijarto, I.A., Miljkovic, J.L., Mitrovic, A., Lange, M., Savitsky, S., Yadav, P.K., Torregrossa, R., et al. (2016). Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. Chem. Sci. 7: 3416–3426.

Weerapan, E., Wang, C., Simon, G.M., Richter, F., Khare, S., Dillon, M.B., Bachovchin, D.A., Mowen, K., Baker, D., and Cravatt, B.F. (2010). Quantitative reactivity profiling predicts functional cysteines in proteomes. Nature 468: 790–795.

Weller, B.D., Bruck, M.C., Kothe, I., Bill, E., Lill, R., and Muhlenhoff, U. (2020). Mitochondrial [4Fe-4S] protein assembly involves reductive [2Fe-2S] cluster fusion on ISC41/ISC42 by electron flow from ferrodoxin in FDX2. Proc. Natl. Acad. Sci. U.S.A., https://doi.org/10.1073/pnas.2003982117.

Wingert, R.A., Galloway, J.L., Barut, B., Footh, H., Fraenkel, P., Axe, J.L., Weber, G.J., Dooley, K., Davidson, A.J., Schmid, B., et al. (2005). Deficiency of glutaredoxin 5 reveals Fe-S clusters are required for vertebrate haem synthesis. Nature 436: 1035–1039.
Ye, H., Jeong, S.Y., Ghosh, M.C., Kovtunovych, G., Silvestri, L., Ortill, D., Uchida, N., Tisdale, J., Camaschella, C., and Rouault, T.A. (2010). Glutaredoxin 5 deficiency causes sideroblastic anemia by specifically impairing heme biosynthesis and depleting cytosolic iron in human erythroblasts. J. Clin. Invest. 120: 1749–1761.

Zannini, F., Moseler, A., Bchini, R., Dhalleine, T., Meyer, A.J., Rouhier, N., and Couturier, J. (2019). The thioredoxin-mediated recycling of Arabidopsis thaliana GRXS16 relies on a conserved C-terminal cysteine. Biochim. Biophys. Acta Gen. Subj. 1863: 426–436.

Zhang, Y., Li, H., Zhang, C., An, X., Liu, L., Stubbe, J., and Huang, M. (2014). Conserved electron donor complex Dre2-Tah18 is required for ribonucleotide reductase metallocofactor assembly and DNA synthesis. Proc. Natl. Acad. Sci. U.S.A. 111: E1695–1704.

Zhang, Y., Liu, L., Wu, X., An, X., Stubbe, J., and Huang, M. (2011). Investigation of in vivo diferric tyrosyl radical formation in Saccharomyces cerevisiae Rnr2 protein: requirement of Rnr4 and contribution of Grx3/4 AND Dre2 proteins. J. Biol. Chem. 286: 41499–41509.

Zhang, Y., Yang, C., Dancis, A., and Nakamaru-Ogiso, E. (2017). EPR studies of wild type and mutant Dre2 identify essential [2Fe–2S] and [4Fe–4S] clusters and their cysteine ligands. J. Biochem. 161: 67–78.

Zheng, C., Guo, S., Tennant, W.G., Pradhan, P.K., Black, K.A., and Dos Santos, P.C. (2019). The thioredoxin system reduces protein persulfdide intermediates formed during the synthesis of thio-cofactors in Bacillus subtilis. Biochemistry 58: 1892–1904.

Zuo, J., Wu, Z., Li, Y., Shen, Z., Feng, X., Zhang, M., and Ye, H. (2017). Mitochondrial ABC transporter ATM3 is essential for cytosolic iron-sulfur cluster assembly. Plant Physiol. 173: 2096–2109.