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FORUM REVIEW ARTICLE

Low-Molecular-Mass Antioxidants in Parasites

R. Luise Krauth-Siegel and Alejandro E. Leroux

Abstract

Significance: Parasitic infections continue to be a major problem for global human health. Vaccines are practically not available and chemotherapy is highly unsatisfactory. One approach toward a novel antiparasitic drug development is to unravel pathways that may be suited as future targets. Parasitic organisms show a remarkable diversity with respect to the nature and functions of their main low-molecular-mass antioxidants and many of them developed pathways that do not have a counterpart in their mammalian hosts. ***Recent Advances:*** Work of the last years disclosed the individual antioxidants employed by parasites and their distinct pathways. *Entamoeba*, *Trichomonas*, and *Giardia* directly use cysteine as main low-molecular-mass thiol but have divergent cysteine metabolisms. Malarial parasites rely exclusively on cysteine uptake and generate glutathione (GSH) as main free thiol as do metazoan parasites. Trypanosomes and *Leishmania* have a unique trypanothione-based thiol metabolism but employ individual mechanisms for their cysteine supply. In addition, some trypanosomatids synthesize ovothiol A and/or ascorbate. Various essential parasite enzymes such as trypanothione synthetase and trypanothione reductase in Trypanosomatids and the *Schistosoma* thioredoxin GSH reductase are currently intensively explored as drug target molecules. ***Critical Issues:*** Essentiality is a prerequisite but not a sufficient property of an enzyme to become a suited drug target. The availability of an appropriate *in vivo* screening system and many other factors are equally important. ***Future Directions:*** The current organism-wide RNA-interference and proteome analyses are supposed to reveal many more interesting candidates for future drug development approaches directed against the parasite antioxidant defense systems. *Antioxid. Redox Signal*. 17, 583–607.

Cysteine Supply in Different Parasitic Protozoa

For all living organisms, cysteine is vitally important as an amino acid for protein biosynthesis, as a precursor for glutathione (GSH) or coenzyme A, and as a source of sulfide for the synthesis of iron–sulfur complexes. Cysteine forms the basic building block of all thiol antioxidants. It either acts as direct antioxidant or can serve as precursor molecule for the synthesis of GSH, trypanothione, or ovothiol (Table 1). Parasites have developed a variety of mechanisms to fulfill their cysteine needs. Some organisms rely exclusively on uptake from their mammalian host; others can generate cysteine by *de novo* biosynthesis from serine and/or by the so-called reverse *trans*-sulfuration (RTS) pathway from methionine (Fig. 1). Mammals possess only the latter mechanism.

Plasmodia *rely exclusively on cysteine uptake from the host*

Plasmodium falciparum, the causative agent of tropical malaria, multiplies in erythrocytes of its human host, thereby ingesting and degrading the host cell hemoglobin. However, hemoglobin degradation appears insufficient for its metabolic needs since the protein is a poor source of Met, Cys, Glu, and Gln and completely lacks Ile (78). In comparison to normal red blood cells, erythrocytes infected with *P. falciparum* have a dramatically increased permeability for Met, which is mainly attributable to new permeability pathways induced by the parasite in the host cell membrane (48). A wide range of neutral amino acids, including Cys, significantly compete with Met uptake providing a putative mechanism to supply the parasite with Cys. No genes for enzymes of the two cysteine biosynthetic pathways have been annotated in the genome of *P. falciparum* in accordance with earlier studies showing that different malarial parasites rely on exogenous cysteine supply (for reviews see refs. 175, 225).

Trypanosomatids obtain cysteine by synthesis and/or uptake

Trypanosomatids are a family of parasitic protozoa within the order Kinetoplastida (Table 1). The medically important trypanosomatids have digenetic life cycles that alternate between the human host and an insect vector.

Pathogenic trypanosomatids differ in their mechanisms for cysteine supply. *Leishmania major* possesses enzymes for both *de novo* biosynthesis catalyzed by the concerted action of

Biochemie-Zentrum der Universität Heidelberg, Heidelberg, Germany.

Table 1. Medically Important Human Parasites, the Diseases Caused, and the Main Low-Molecular-Mass Thiol in These Organisms

	Disease	*Main thiol antioxidant*
Protozoan parasites		
Microaerophilic protozoa		Cysteine
Entamoeba histolytica	Amoebic colitis	
Trichomonas vaginalis	Trichomoniais	
Giardia lambia	Giardiasis	
Apicomplexa		GSH
Plasmodium falciparum	Malaria tropica	
Kinetoplastida		
Trypanosoma brucei gambiense	African sleeping sickness	Trypanothione
Trypanosoma brucei rhodesiense	African sleeping sickness	
Trypanosoma cruzi	Chagas' disease (American trypanosomiasis)	
Leishmania donovani	Visceral leishmaniasis (kala azar)	
Metazoan parasites		
Filaria (round worms)		GSH
Wucheria bancrofti	Lymphatic filariasis	
Brugia malayi	Lymphatic filariasis	
Onchocerca volvulus	Onchocerciasis (river blindness)	
Platyhelminths (flat worms)		GSH
Schistosoma mansoni	Schistosomiasis	
Taenia crassiceps	Cysticercosis	
Echinococcus granulosus	Hydatic disease	

GSH, glutathione.

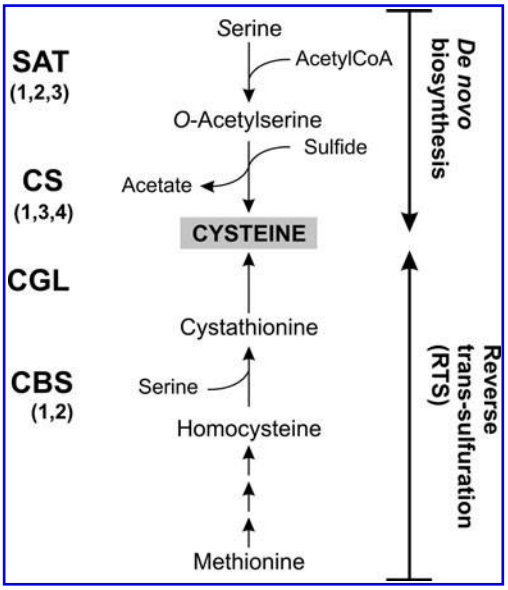
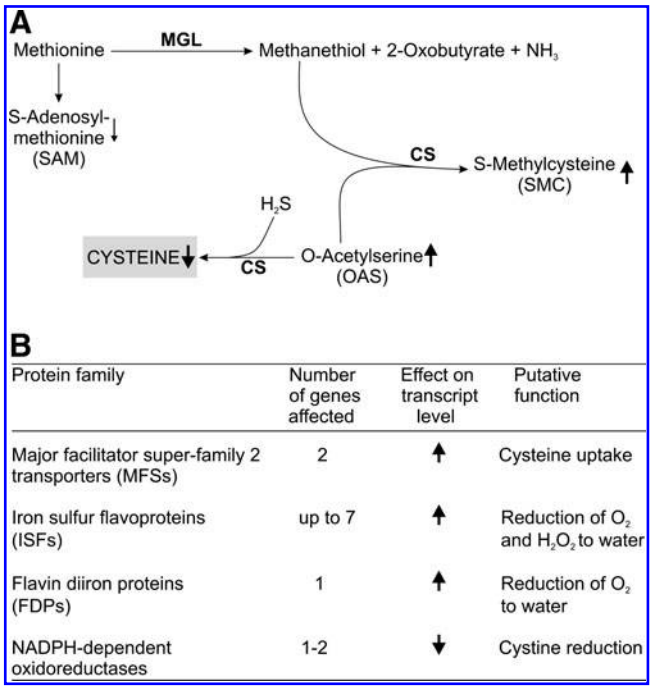


FIG. 1. Simplified scheme for cysteine biosynthesis in different parasitic protists. The following enzymes from (1) *Leishmania major* (277), (2) *Trypanosoma cruzi* (177), (3) *Entamoeba histolytica* (106, 176), and (4) *Trichomonas vaginalis* (267) have been experimentally characterized. *Trypanosoma brucei* contains putative genes for CBS and CGL, but cysteine is an essential nutrient (67, 277). Other protozoa such as *Plasmodium falciparum* and *Giardia intestinalis* (154) lack both pathways and rely exclusively on cysteine uptake. SAT, serine acetyl-CoA transferase; CS, cysteine synthase; CGL, cystathionine-γ-lyase; CBS, cystathionine-β-synthase.

serine acetyl-CoA transferase (SAT) and cysteine synthase (CS) and by the RTS pathway composed of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CGL) (Fig. 1) (277). *L. major* CBS has both CBS and CS activity. The source of sulfide for Cys biosynthesis is not clear since *Leishmania* lack genes for sulfur assimilation. This sulfate reduction occurs in *Entamoeba* as well as plants and bacteria. It has been proposed that the sulfide required for CS may be derived from 3-mercaptopyruvate by the action of mercaptopyruvate sulfurtransferase (MST; see Fig. 3 for the reaction) (276, 277). Cysteine transport into *Leishmania* can occur, but the rate is some 200-fold lower when compared to *Trypanosoma brucei*. Together with the high K_m-value of 230 μM, this renders uptake unlikely as a main mechanism to supply the parasite with cysteine. Serine and methionine, but not cysteine, are essential for growth of promastigote *Leishmania* which underlines the importance of cysteine synthesis for this parasite (277).

Trypanosoma cruzi can also produce cysteine by *de novo* biosynthesis as well as by RTS from Met (177). Indeed, this parasite was the first protist shown to possess both pathways. The activity of CBS is about eight times higher in the insect epimastigote form than in the infectious amastigote and trypomastigote forms of the parasite, suggesting a stage-specific preference of the two synthetic pathways (177). Recently, an interactome analysis of tryparedoxin (Tpx) 1 (see below) in *T. cruzi* revealed CGL as an interacting protein (201). Thus, CGL is expressed in the parasite and may be redox regulated. *T. cruzi* epimastigotes possess a high-affinity and high-specificity transport system for L-cysteine (36). The maximum rate is, however, as low as that reported for *Leishmania* and the physiological role of cysteine uptake remains to be verified.

T. brucei encodes putative genes for CBS and CGL (277). However, cysteine is an essential nutrient (67), which suggests that the RTS pathway is inadequate to satisfy the cysteine requirements of African trypanosomes.



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Protein family	Number of genes affected	Effect on transcript level	Putative function
Major facilitator super-family 2 transporters (MFSs)	2	↑	Cysteine uptake
Iron sulfur flavoproteins (ISFs)	up to 7	↑	Reduction of O_2 and H_2O_2 to water
Flavin diiron proteins (FDPs)	1	↑	Reduction of O_2 to water
NADPH-dependent oxidoreductases	1-2	↓	Cystine reduction

FIG. 2. Effects of cysteine depletion on the thiol metabolism and the transcription of thiol redox proteins in *E. histolytica*. **(A)** Cultivation of trophozoites in cysteine-depleted medium results in nearly undetectable levels of intracellular cysteine and cystine. The condition does not lead to an increased cysteine biosynthesis but to a strong increase in the levels of OAS and SMC and a decrease of SAM. **(B)** Cysteine depletion induces the transcription of proteins that are putatively involved in the redox homeostasis and antioxidant defense of the parasite as well as a lowering of transcription level of cystine-reducing enzymes (104, 105, 112). MGL, methionine-γ-lyase; SAM, S-adenosylmethionine; OAS, O-acetylserine; SMC, S-methylcysteine.

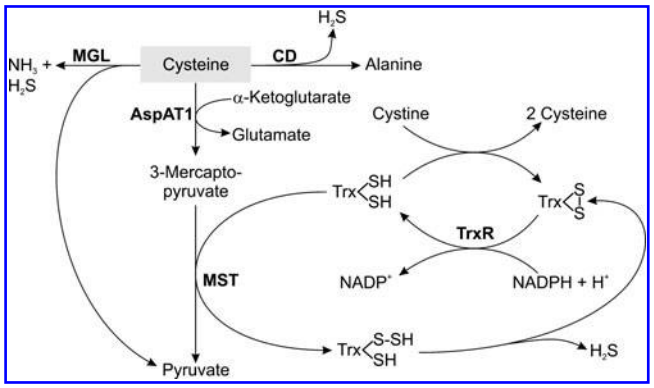


FIG. 3. Cysteine metabolism in *T. vaginalis*. Cysteine can be catabolized by three different pathways. The *trans*-sulfurations by MGL and CD generate pyruvate and alanine, respectively. The third pathway represents the concerted action of Asp-AT and MST. The latter reaction transfers a sulfur onto an acceptor molecule such as Trx, which results in the formation of a persulfide intermediate that spontaneously generates sulfide. The disulfide form of Trx is then reduced by NADPH and TrxR. *T. vaginalis* Trx spontaneously reduces cystine and is thus able to restore the cysteine pool of the parasite. CD, cysteine desulfurase; Asp-AT1, aspartate aminotransaminase; MST, mercaptopyruvate sulfur transferase; Trx, thioredoxin; TrxR, thioredoxin reductase.

Microaerophilic protozoa developed individual pathways for their cysteine supply

Entamoeba histolytica, *Trichomonas vaginalis*, and *Giardia lamblia* (Table 1) share a variety of biological and biochemical peculiarities. These microaerophilic protozoa lack typical mitochondria, have an anaerobic carbohydrate metabolism, and use cysteine as the principal low-molecular-mass thiol (see next section) (71; for reviews see refs. 175, 244). However, the parasites employ divergent pathways for their cysteine supply. All three organisms lack the genes for enzymes of both the forward and RTS pathways and thus are unable to catalyze *trans*-sulfurations between Met and Cys (Fig. 1). Instead, *E. histolytica* is capable of sulfur-assimilatory *de novo* cysteine biosynthesis (146, 176). Three isoforms of SAT occur in *E. histolytica*, which show remarkable differences in their sensitivity to inhibition by cysteine (106). Overexpression of CS confers *E. histolytica* with a reduced sensitivity toward exogenous hydrogen peroxide, which is probably attributable to changes in membrane sensitivity (176), and suggests that cysteine biosynthesis plays a role in the oxidative stress defense. A recent metabolome analysis, however, indicates that the biosynthesis is not sufficient but cysteine uptake is essential (105).

De novo cysteine biosynthesis occurs also in *T. vaginalis*. Six genes encoding CSs have been identified, but SAT genes appear to be missing. Recombinant *T. vaginalis* CS1 accepts O-acetylserine (OAS), the common substrate of these enzymes, but also O-phosphoserine. This has led to the suggestion that O-phosphoserine, despite its high apparent K_m-value, may be the physiological substrate of CS in *T. vaginalis* (267). The cellular level of CS is greatly reduced when the cells are grown in the presence of exogenous cysteine, suggesting that *T. vaginalis* can also salvage cysteine from its host (267).

Giardia duodenalis seems to rely exclusively on uptake for cysteine supply. Genes for both cysteine *de novo* synthesis and the RTS pathway are missing as it is the case in *P. falciparum* (175). This is in accordance with previous studies showing that the enzymatic activities are missing in homogenates of *G. duodenalis* and that exogenous cysteine is indispensable for both growth and protection against oxidative stress of the parasite (154).

The Microaerophilic Protozoan Parasites Use Cysteine as Key Antioxidant

Cultivation of *E. histolytica* requires high concentrations of cysteine in the medium that cannot be replaced by other thiols. Cysteine is required for growth, attachment, survival, and protection from oxidative stress (86). Recently, the cysteine metabolism of *E. histolytica* has been studied by metabolome and transcriptome approaches (104, 105). Cultivation of axenic trophozoites in the absence of cysteine results in nearly undetectable intracellular levels of cysteine and cystine. This strongly suggests that the endogenous biosynthesis is not sufficient but that the parasite greatly depends on cysteine uptake (105). Cysteine-depleted cells are highly sensitive toward paraquat and the intracellular levels of reactive oxygen species (ROS) increase three- to fourfold in accordance with cysteine being an important antioxidant. The metabolome analysis further revealed a dramatic accumulation of OAS

and S-methylcysteine (SMC) and a decrease of S-adenosylmethionine (SAM) upon cysteine-depletion (Fig. 2A). SMC occurs in several legumes where it is considered to serve as a sulfur storage form (105). Surprisingly, depletion of cysteine does not result in an increased *de novo* synthesis from OAS. Instead, OAS is converted into SMC. The apparent failure to synthesize cysteine is not attributable to a lack of sulfide. It supports the conclusion that *E. histolytica* obtains cysteine primarily by uptake.

Cysteine deprivation affects the expression of a large number of genes (104), but the transcripts of most genes of the cysteine metabolism and of putative ROS- and reactive nitrogen species-detoxifying proteins are not significantly modulated in accordance with a previous study (257). This suggests that *E. histolytica* mainly relies on post-transcriptional mechanisms to adapt to oxidative stress. The most important changes caused by all three types of stresses are the induction of several major facilitator superfamily (MFS) transporters and iron–sulfur flavoproteins (ISFs) (104) (Fig. 2B). The *E. histolytica* genome encodes about 24 different genes for MFS proteins (104). It is tempting to speculate that under cysteine deprivation, the parasite may induce expression of cysteine or cystine transporters. ISFs are widely distributed in anaerobic prokaryotes where they may function in combating oxidative stress by reducing molecular oxygen and hydrogen peroxide (55). *E. histolytica* and *T. vaginalis* are the only known eukaryotes possessing ISF genes. Thus, an important *in vivo* role of cysteine is probably the regulation of different antioxidant enzymes. However, cysteine may also act as a direct antioxidant. This is corroborated by the fact that *E. histolytica* expresses an NADPH:flavin oxidoreductase and two other NADPH-dependent oxidoreductases, which all catalyze the reduction of cystine (32, 112). The expression of the latter enzymes is regulated by the availability of extracellular cysteine (112).

T. vaginalis possesses a full thioredoxin system, consisting of thioredoxin (Trx), thioredoxin reductase (TrxR), and Trx peroxidase, which probably represents a major antioxidant defense line (54, 139). The parasite does not produce GSH and lacks GSH metabolizing enzymes. Cysteine is present in the parasite at a concentration of about 600 μM and represents >70% of the total cellular thiols. Cysteine homeostasis in *T. vaginalis* is achieved by uptake and/or *de novo* synthesis as described above as well as by the catabolism of cysteine (Fig. 3). Cysteine can be degraded by the desulfurases methionine-γ-lyase (MGL) and cysteine desulfurase. The third mechanism is the concerted action of aspartate aminotransaminase (Asp-AT) and MST (266). *T. vaginalis* Asp-AT1 catalyzes the transamination of Asp and to a lesser extent of Cys. The observation that high levels of extracellular cysteine cause a three- to fourfold increase in MST activity suggests a role of this pathway in the redox homeostasis and antioxidant defense of *T. vaginalis*.

For most organisms, high concentrations of free cysteine are toxic and the cellular levels are maintained at 100–200 μM. In the presence of transition metal ions, cysteine rapidly undergoes autoxidation and the resulting hydroxyl radicals are highly toxic for cells and in particular for neurons (263). The use of cysteine as redox buffer in *T. vaginalis* may be an adaptation to its microaerophilic lifestyle. *T. vaginalis* is exposed to low levels of oxygen and does not produce ROS by aerobic respiration, which may increase its tolerance to comparably high cellular cysteine levels. It has even been suggested that the pronounced susceptibility of cysteine, compared with GSH, to autoxidation may serve to scavenge oxygen and thus protect the highly sensitive hydrogenosomal enzymes of the parasite from oxygen (266). The catabolism of cysteine is linked to the Trx system. Trx can act as sulfur acceptor in the reaction catalyzed by MST resulting in a cysteine persulfide. In the subsequent spontaneous reaction, sulfide is released and Trx is oxidized (Fig. 3). The dithiol form of Trx is then regenerated by TrxR. Persulfides are the source of sulfur for the biosynthesis of cofactors such as iron–sulfur clusters and lipoic acid or the sulfuration of tRNAs (167). The catabolism of cysteine *via* Asp-AT and MST may be a major source of sulfide also in these parasites. Another link between the cysteine and Trx systems is given by the fact that the parasite Trx spontaneously reduces cystine, suggesting that the Trx system is responsible for maintaining cysteine in its reduced state (54).

G. lamblia also contains cysteine as main low-molecular-mass thiol and GSH has been reported to be absent (30). The genome, however, encodes putative genes for the GSH biosynthetic enzymes γ-glutamylcysteine synthetase (GSH1) and glutathione synthetase (GSH2) (163). Recently, *G. lamblia* has been shown to possess a monothiol glutaredoxin (1-C-Grx) (202). These small redox proteins are characterized by a CGFS active site motif. They can dimerize by coordinating a [2Fe2S] cluster *via* the active site cysteines of each monomer together with the cysteines of two bound GSH molecules. 1-C-Grxs are involved in (probably the final step of) mitochondrial iron–sulfur cluster biosynthesis (143). The *G. lamblia* 1-C-Grx is localized in the mitosomes, highly reduced mitochondria-type organelles that harbor iron–sulfur cluster biosynthesis as only known function. The recombinant parasite 1-C-Grx has been shown to form a homodimeric iron–sulfur cluster complex that can be stabilized by GSH (202). It is therefore conceivable that *G. lamblia* contains at least low levels of GSH that may specifically be used in iron–sulfur cluster biogenesis.

G. lamblia possesses a flavin diiron protein (FDP) that efficiently reduces molecular oxygen to water (60). The primary function of the enzyme has been proposed to be the scavenging of oxygen, allowing the parasite to survive in the human intestine. A putative regulation of *G. lamblia* FDP expression by cysteine availability as it has been found in *E. histolytica* (see Fig. 2B) has not yet been studied. *G. lamblia* has an active Trx/TrxR system and encodes a gene for a putative Trx peroxidase. It is therefore likely that the parasite has a Trx-dependent peroxidase system as it is the case in *T. vaginalis* (54). *G. lamblia* TrxR has cystine reductase activity (31), which offers the possibility that the cysteine/cystine couple also acts directly in the redox homeostasis and antioxidant defense of the parasite.

Malarial Parasites Have a GSH Metabolism That Can Be Fueled by the Trx System

The antioxidant defense of *P. falciparum* is based on both a full GSH system as well as a Trx system (17, 59, 120, 169).

GSH synthesis and reduction in Plasmodia

P. falciparum is able to synthesize GSH (Fig. 4). The genes for GSH1 and GSH2 encode proteins with large insertions compared with the enzymes from other organisms, a phenomenon widely observed for plasmodial proteins (152, 160). Transcription of GSH1 is highest in the trophozoite state

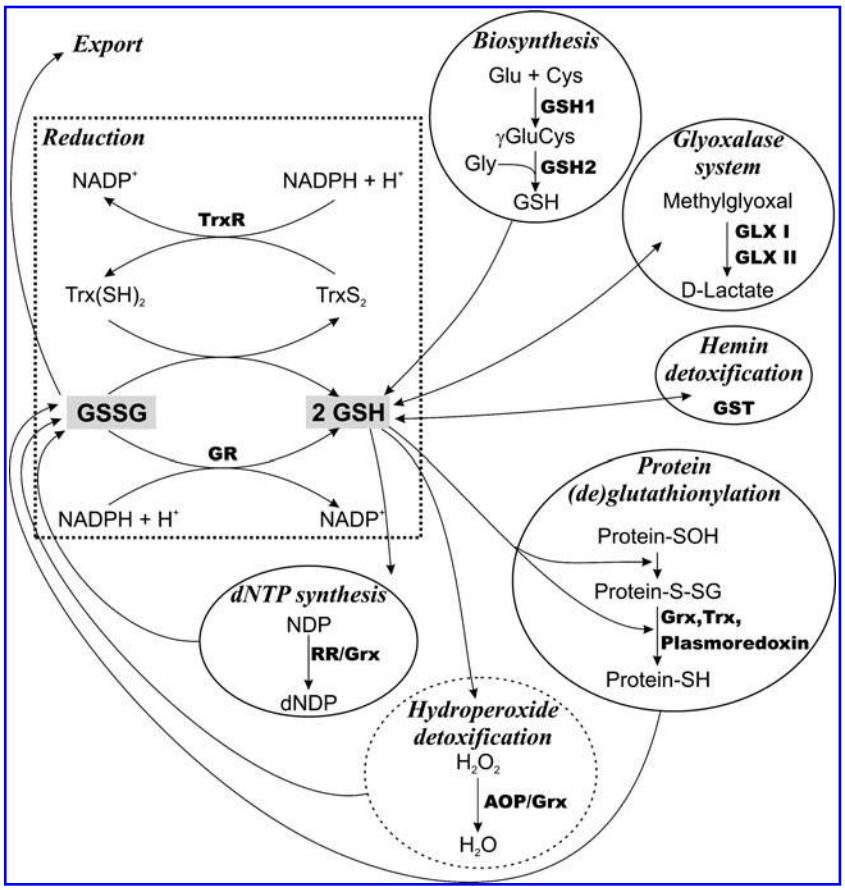


FIG. 4. Pathways involving GSH in *P. falciparum*. In the malarial parasite, GSH is synthesized by GSH1 and GSH2 and kept reduced by GR as it is the case in most organisms. GSSG can also be reduced spontaneously by the parasite Trx closely linking both thiol redox systems. GSH acts as cofactor in the detoxification of methylglyoxal catalyzed by GLX I and II. It is involved in the detoxification of hemin by GST. GSH may also contribute to hydroperoxide reduction. The AOP, a parasite oxidoreductase, has been shown to catalyze the reaction and to prefer Grx as reducing agent. Together with Grx, GSH can deliver electrons for the synthesis of dNDPs by RR. Another important role of GSH may be the regulation of protein functions by reversible binding to specific cysteine residues (for details see the text). AOP, antioxidant protein; dNDPs, deoxyribonucleotides; GLX, glyoxalase; GR, glutathione reductase; Grx, glutaredoxin; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione-S-transferase; RR, ribonucleotide reductase.

where hemoglobin degradation, and thus probably also the formation of ROS, is maximal (152). Recombinant *P. falciparum* GSH2 displays kinetic parameters comparable with the enzymes from other sources (160).

The total GSH content in erythrocytes infected with trophozoite *P. falciparum* is only halve that in noninfected red blood cells. This is probably mainly due to loss of glutathione disulfide (GSSG) from the infected erythrocytes, which occurs at a rate about 50-fold higher than in the case of uninfected cells (13, 153, 169). Decreased GSH levels were also reported for erythrocytes from patients infected with *Plasmodium vivax* (21). In other studies, the levels of GSH in intact *P. falciparum*–infected erythrocytes remained unchanged (for a review see ref. 17). In contrast, mice erythrocytes infected with *Plasmodium berghei* or *Plasmodium vinckei* have more than twice the GSH content found in uninfected erythrocytes (68, 232). This indicates that data obtained in rodent malaria models do not necessarily reflect the situation in the human pathogens (see below).

Treatment of *P. falciparum*–infected red blood cells with sub-lethal concentrations of buthionine sulfoximine (BSO), a specific inhibitor of GSH1, results in about 50% loss of the total GSH within 2.5 h. In contrast, an identical treatment of uninfected erythrocytes lowers the levels only slightly, 50% of GSH being lost after 4–5 days. This indicates that infection of erythrocytes by *P. falciparum* results in a strongly increased consumption of GSH (153). BSO inhibits the proliferation of cultured *P. falciparum*, suggesting that GSH biosynthesis is essential for parasite survival. However, the plasmodicidal effect of BSO is not due to specific inhibition of the parasite enzyme but to the altered metabolic needs for GSH in the infected cell (153).

By targeted gene disruption, GSH1-deficient mutants of the rodent malaria parasite *P. berghei* have been generated (256). These mutants are able to infect mice but show significantly reduced proliferation compared with wild-type parasites. Interestingly, the most dramatic effect is observed in the insect vector. Infection of Anopheles with GSH1-deficient *P. berghei* results in a reduced number of oocysts with aberrant morphology that do not produce sporozoites, thus interrupting transmission to the mammalian host.

P. falciparum glutathione reductase (GR) has first been isolated from infected erythrocytes (129). The gene has been cloned and overexpressed (74) and the crystal structure of the recombinant protein has been solved (215). Parasite and host cell GRs are studied as putative targets for antimalarial drug development (for reviews see refs. 111, 125). A recent thorough analysis of the compartmentation of the thiol redox proteins in *P. falciparum* revealed a dual subcellular localization of GR (120). Alternative translation initiation results in either the cytosolic or an apicoplast form of the enzyme. Together with several other redox proteins in this organelle, the data suggest the presence of an independent GSH-based antioxidant metabolism in this nonphotosynthetic plastid organelle in addition to the common cytosolic system.

Disruption of the GR gene in *P. berghei* only slightly affects proliferation of the asexual blood-stage parasites in cultured as well as in infected mice (33, 190). However, any attempt to generate mutants that lack both GR and GSH1 was not successful. This is evidence that the blood-stage parasites require

either *de novo* GSH biosynthesis or the ability to regenerate GSH from GSSG. As observed for the GSH1-deficient parasites (256), GR-deficient *P. berghei* show the most severe phenotype in the insect stages. The lack of GR totally prevents formation of infective sporozoites. Taken together, in the case of the rodent malaria species either GSH biosynthesis or GSH reduction seems to be sufficient for parasite proliferation in the mammalian host. However, it is not known if this is also true for the human pathogens provided the difference in the GSH levels upon erythrocyte infection by individual malarial parasites described above. In addition, the *P. falciparum* Trx/TrxR couple has been shown to efficiently reduce GSSG and thus may replace GR activity especially at low substrate and enzyme concentrations (Fig. 4) (117).

GSH-dependent pathways in malarial parasites

Studies on the specific role(s) of GSH in *P. falciparum* revealed a number of GSH-dependent pathways (Fig. 4) (17, 119, 253). Upon infection, the glucose consumption of the red blood cell increases up to 75-fold (226). Both the host cell and the parasite rely on glycolysis for energy production. This is inevitably linked to the spontaneous formation of methylglyoxal, which is detoxified by conversion into D-lactate. The nearly ubiquitous glyoxalase (GLX) system, composed of GLX I and GLX II, uses GSH as a regenerated cosubstrate, not as reducing agent (for a recent review see ref. 283). Both the erythrocyte and the parasite harbor a functional GLX system (for a recent review see ref. 253).

P. falciparum has a single glutathione-S-transferase (GST) that, despite structural similarity to μ class enzymes, cannot be assigned to any known GST class (79). In addition to the common reactions catalyzed by these enzymes, in the presence of GSH the parasite GST can bind hemin (ferriprotoporphyrin IX) and thus contribute to the protection of *Plasmodia* toward this heme-derived toxic product (101, 142). The *P. falciparum* GST undergoes a dimer to tetramer transition that is accompanied by a loss of the ability to sequester hemin. GSH or GSSG prevents the tetramerization, suggesting that the protection of the parasite from hemin toxicity is independent of the redox status of the cell (141).

P. falciparum possesses a classical glutaredoxin (Grx) that catalyzes the GSH-dependent reduction of hydroxyethyl disulfide and can serve as electron donor for the synthesis of deoxyribonucleotides catalyzed by ribonucleotide reductase (RR) (203). A recent interactome analysis identified 17 target candidates for the *P. falciparum* Grx (235), but the specific physiological role of the protein remains to be elucidated since 10 of these interactions were also observed with Trx. A reaction usually catalyzed by Grxs is protein (de)glutathionylation. Formation of protein-GSH mixed disulfides is a reversible reaction allowing the positive or negative regulation of protein functions. In addition, the mechanism can protect thiol groups from over-oxidation and serve as a cellular storage form of GSH (reviewed in ref. 58). Nearly 500 proteins undergoing glutathionylation have been identified in *P. falciparum* suggesting a wide regulatory role of GSH in the parasite. Not only Grx but also Trx and the *Plasmodium*-specific plasmoredoxin can catalyze protein deglutathionylation *in vitro* (119).

Classical selenocysteine-containing GSH peroxidases as well as catalase are missing in *P. falciparum*. A total of five putative peroxidases have been described (for reviews see refs. 59, 173). The most important enzyme for hydrogen peroxide removal may be a 2-Cys-peroxiredoxin (2-Cys-Prx), which uses Trx as electron source (3, 118, 132). However, knockout parasites show only growth retardation when cultured in the presence of paraquat but proliferate normally in the absence of exogenous stressors (122). This may suggest that the 2-Cys-Prx is not essential for the detoxification of endogenously produced ROS at least under culture conditions (59). The nonselenium GSH peroxidase of *P. falciparum* also prefers Trx instead of GSH as reducing substrate. However, the low rate of hydrogen peroxide reduction in the presence of either thiol system points to a biological role distinct from antioxidant defense (238). The so-called antioxidant protein (AOP) of *P. falciparum* shows high sequence similarity to human Prx V and thus is an atypical 2-Cys-Prx. *In vitro*, AOP prefers Grx over Trx and accepts lipid-derived hydroperoxides as substrates although the overall peroxidase activity is low (173). The recent finding that the parasite Grx is located in the cytosol whereas AOP resides in the apicoplast (120) necessitates further studies of a possible *in vivo* interaction of both proteins. Overall, GSH seems to play only a minor role for hydroperoxide detoxification in malarial parasites (Fig. 4).

For yeast cells GSH has recently been shown to play an essential role in the cytosolic iron–sulfur cluster assembly (135). In *Plasmodia*, iron–sulfur cluster biogenesis has not yet been studied in detail. Several reactions in the plastid of the parasite require iron–sulfur clusters and an active assembly pathway has been suggested to be present in the organelle (134), suggesting another role of GSH in these parasites.

Trypanosomatid Parasites Have a Unique Trypanothione-Based Antioxidant Metabolism

Trypanosomatids show a large number of biochemical, morphological, and genetic peculiarities. The thiol and polyamine pathways are directly linked in these parasites, transforming their unique redox metabolism in one of the most distinctive features. The genome sequencing projects of *T. brucei* (19), *T. cruzi* (70), and *L. major* (110) revealed that trypanosomatids lack genes for GR and TrxR. While in most eukaryotic organisms the GSH/GR and Trx/TrxR systems maintain the intracellular thiol redox homeostasis, trypanosomatids possess a redox metabolism that is based on trypanothione [N^1,N^8-bis(glutathionyl)spermidine; $T(SH)_2$] (72) and trypanothione reductase (TR), which keeps the dithiol in the reduced form (for reviews see refs. 73, 126). $T(SH)_2$ is a much more efficient reducing agent than GSH although the redox potentials of these thiols are very similar (73) (Table 2). Since $T(SH)_2$ is a dithiol, formation of an intramolecular

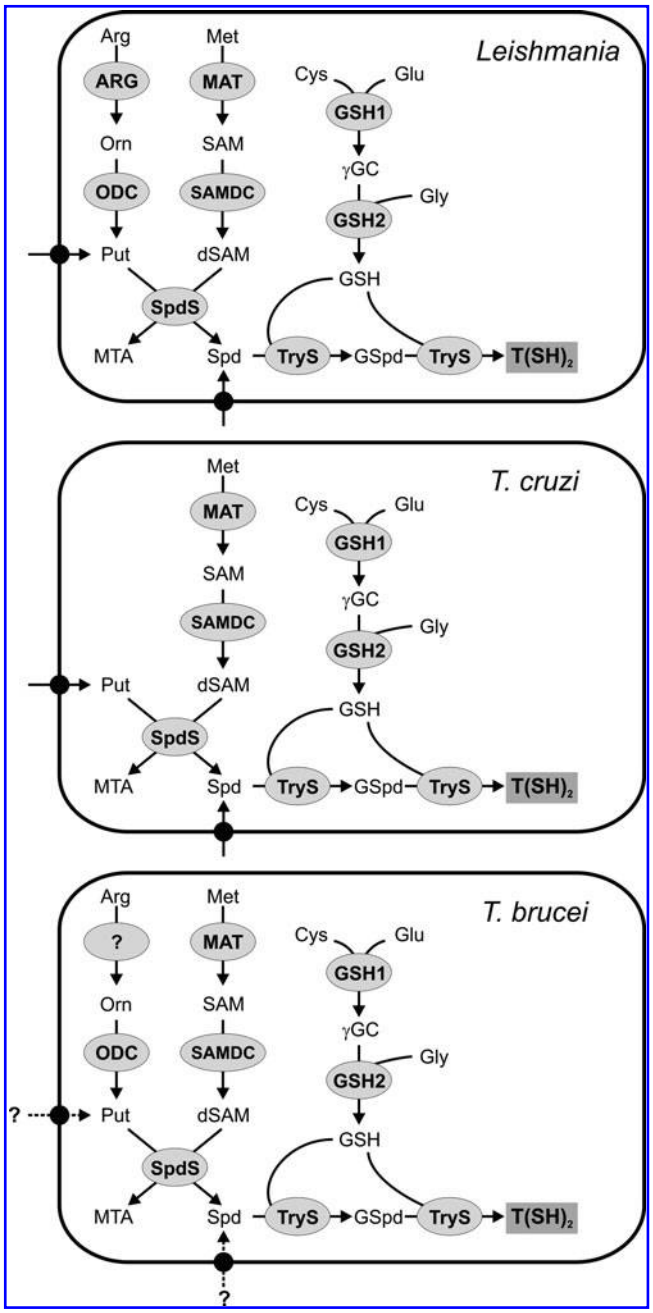
Table 2. Physicochemical Properties of Cellular Low-Molecular-Mass Thiols

	E_0'	*Thiol pK*
Cysteine/cystine (114, 145)	−220	8.1–8.9
GSH/GSSG	−230–250	8.1–8.9
$T(SH)_2/TS_2$ (73, 166)	−242	7.4
OvoSH/Ovo disulfide (264)	−92	1.42

$T(SH)_2$, trypanothione; TS_2, trypanothione disulfide.

disulfide is kinetically favored when compared with the intermolecular oxidation of two GSH molecules (85, 166). Moreover, the positive charge in the spermidine (Spd) bridge shifts the thiol pK-value to the physiological pH range (166) (Table 2), which renders $T(SH)_2$ highly reactive in thiol-disulfide exchange reactions (85).

TR is the only enzyme that connects the NADPH- and the thiol-based redox systems in these parasites. The enzyme has been biochemically characterized in the insect parasite *Crithidia fasciculata* (224), as well as the pathogenic *T. cruzi* (127), *Leishmania* (56), and *T. brucei* (115, 236). TR is a representative of the flavin adenine dinucleotide (FAD)-cystine-oxidoreductases and shares many physical and chemical properties with GR. However, both enzymes have a mutually exclusive specificity toward their disulfide substrate (127, 224). Although *Leishmania donovani* promastigotes expressing 15% of wild-type TR activity grow normally in axenic culture, they are highly sensitive toward oxidative stress and exhibit a significantly impaired ability to survive inside activated macrophages (66, 248). In conditional knockout cell lines of bloodstream *T. brucei*, the TR activity could be lowered to less than 10% of wild-type cells (131). This caused a remarkable growth arrest and later the appearance of revertants. In addition, the TR-deficient parasites became highly sensitive toward exogenous hydrogen peroxide and were not able to infect mice (131). Therefore, TR plays an essential role in trypanosomatids and is considered to be an interesting drug target (130).



Trypanothione biosynthesis in different trypanosomatid organisms

The biosynthesis of trypanothione can be dissected into three parts: Spd, GSH, and trypanothione syntheses (Fig. 5). The pathways for GSH and Spd biosynthesis correspond to those in mammals. The last step is parasite specific and comprises the amide bond formation between the glycine residues of two GSH molecules and the primary amines of Spd.

The polyamine pathway begins with the transformation of L-arginine into ornithine catalyzed by arginase (ARG, Fig. 5). The enzyme is present in *Leishmania amazonensis* (57), *Leishmania mexicana* (213), and *L. major* (209). ARG null mutants of promastigote *L. mexicana* and *L. major* fail to grow in semi-defined media (209, 213), but the proliferation defect can be rescued by supplementation with putrescine, Spd, or ornithine. The ARG null mutants retain the ability to infect Balb/c mice, although the animals show a slower progression of the lesions (82, 209). This suggests that the ornithine and/or polyamine levels inside the parasitophorous vacuole of the host cell are sufficient to sustain parasite growth. In trypanosomes, the origin of ornithine is still a matter of debate. Early studies were not able to show ARG activity in *T. cruzi*, *T. conorhini*, and *T. mega* cell extracts (35). A homolog of the

FIG. 5. Trypanothione biosynthesis in different trypanosomatids. The biosynthesis of trypanothione links the GSH and the polyamine pathways. Although all three pathogenic trypanosomatids, *Leishmania*, *T. brucei*, and *T. cruzi*, require Spd for $T(SH)_2$ production, the supply pathways present significant differences. *Leishmania* guarantee their Spd requirements by *de novo* synthesis and by salvage of putrescine or Spd from the environment. In contrast, *T. cruzi* is auxotrophic for putrescine and relies solely on the acquisition of the polyamines from the medium. *T. brucei* performs *de novo* synthesis of Spd; however, there is no experimental evidence for the existence of ARG and polyamine transporters (depicted by question marks). The enzymes involved are depicted with grey ovals and the polyamine transporters with black circles. dSAM, decarboxylated S-adenosylmethionine; MTA, methylthioadenosine; Orn, ornithine; Put, putrescine; Spd, spermidine; γ-GC, γ-glutamylcysteine; GSpd, glutathionylspermidine; $T(SH)_2$, trypanothione; MAT, methionine adenosyltransferase; SAMDC, S-adenosylmethionine decarboxylase; ARG, arginase; ODC, ornithine decarboxylase; SpdS, spermidine synthase; GSH1, γ-glutamylcysteine synthetase; GSH2, glutathione synthetase; TryS, trypanothione synthetase.

functionally characterized leishmanial ARG is absent from the trypanosome genomes. However, a gene encoding a putative agmatinase/ARG-like protein is present in all trypanosomes and *Leishmania* (19, 70, 110). The functionality of the latter protein needs to be assessed before concluding that *T. brucei* is auxotrophic for ornithine.

L-Ornithine is converted into putrescine by ornithine decarboxylase (ODC, Fig. 5). This enzyme occurs in *Leishmania* (94) and *T. brucei* (197) but not in the American trypanosome (39, 103), which renders *T. cruzi* auxotrophic for putrescine. In human cells ODC undergoes a rapid turnover with half-lives of less than 1 h (84). In contrast, in *T. brucei* (83, 196) and *Leishmania* (94) the turnover of ODC is much slower (for a review see ref. 195). The different half-lives of the mammalian and *T. brucei gambiense* ODC form probably the basis for the specificity of the drug eflornitine (difluoromethylornithine [DFMO]), which acts as an irreversible inhibitor of ODC. The genetic disruption of the *ODC* gene is lethal for promastigote *L. donovani* (113) or procyclic *T. brucei* (140), but the parasites grow normally in putrescine-supplemented media. More recently, RNA interference in bloodstream *T. brucei* bloodstream showed that ODC is also essential in the infectious mammalian stage. Interestingly, wild-type proliferation is rescued by putrescine but not Spd supplementation (282). This suggests that putrescine is not only required for Spd production, but may play a so far unknown role for parasite survival. *L. donovani* ODC null mutants are severely compromised in their ability to establish an infection in Balb/c mice. The average parasite load in the liver and spleen of the animals is 3–6 orders of magnitude lower after infection with ODC knockout compared to wild-type parasites (26). This shows that *L. donovani* amastigotes are not able to scavenge enough putrescine or Spd from the host to properly sustain proliferation.

SAM is provided by methionine adenosyltransferase (MAT, Fig. 5) (for a review see ref. 210). The partially purified enzyme from *T. brucei* presented biphasic kinetics for ATP and methionine and, unlike the mammalian counterpart, was only poorly inhibited by SAM (284). Recombinant MATs from *Leishmania infantum* (208) and *L. donovani* (193) have been cloned and functionally expressed. It has been proposed that MAT may be implicated in the resistance of *Leishmania* against methotrexate (65, 93).

In the next step, SAM is decarboxylated by S-adenosylmethionine decarboxylase (SAMDC, Fig. 5), a self-processing enzyme with an active site pyruvoyl group. SAMDC catalyses the production of decarboxylated SAM (dSAM), which provides the aminopropyl group in the synthesis of Spd (69). SAMDC activity has been studied in *T. brucei* extracts (23, 243) and the *L. donovani* (212, 240) and *T. cruzi* (121, 194) enzymes have been cloned and characterized. In contrast to the short half-life of the mammalian isoform, the SAMDCs from *Leishmania* and *T. brucei* seem to turn over much more slowly (212, 222). Although trypanosomal SAMDCs are partially activated by putrescine (20, 194, 243), the enzyme requires the formation of a heterodimer with a catalytically inactive homolog to become fully active (275). Pharmacological inhibition of SAMDC by irreversible inhibitors can cure *T. b. brucei* and *T. b. rhodesiense* infections in mice (15, 22). Moreover, SAMDC null mutants of *L. donovani* revealed that the enzyme is an absolute prerequisite for cell growth in the absence of Spd (212).

The final step of Spd biosynthesis involves the transfer of an aminopropyl group from dSAM to putrescine catalyzed by spermidine synthase (SpdS, Fig. 5). The enzyme has only been biochemically characterized in *T. brucei* (24, 242). RNA interference-mediated silencing showed that SpdS is essential and that the proliferation of the parasites could not be recovered by Spd supplementation (242). More recently, using a different medium composition, another group demonstrated that the lethal phenotype caused by SpdS knockdown in bloodstream *T. brucei* can be overcome by supplementation with 100 μM Spd (282). Taking into account that the plasma Spd concentration is 300 n*M* (254), it is, however, likely that *in vivo* SpdS is essential. *L. donovani* SpdS null mutants are auxotrophic for Spd (87, 211). Moreover, the ability of *L. donovani* SpdS null mutants to infect mice is severely compromised. The average parasite load in the liver and spleen of infected animals is 2–3 orders of magnitude lower when compared to an infection with wild-type parasites (87), which makes SpdS an interesting drug target. Little is known about *T. cruzi* SpdS. The conversion of [^{3}H]putrescine into Spd and spermine as well as of [^{3}H]cadaverine into aminopropylcadaverine has been established in the American trypanosome (103). A 2.5Å X-ray structure of *T. cruzi* SpdS has been deposited at the protein data bank (3BWB).

The polyamine requirements of the parasites could also be satisfied by acquisition from the environment. *T. cruzi* is auxotrophic for polyamines. The epimastigote insect form possesses a specific putrescine transporter whose protein levels are regulated by the extracellular putrescine concentration (89, 138). A *T. cruzi* Spd–specific transporter, TcPAT12, was cloned and biochemically characterized (38). The presence of two different transport systems for Spd and putrescine was also observed in *L. donovani* and *L. mexicana* (16, 116). It was proposed that *L. mexicana* promastigotes and amastigotes possess different polyamine transporters that are adapted to work at different pH optima (16). A polyamine permease, LmPOT1, was cloned from *L. major*. The protein is present exclusively in promastigotes and can efficiently transport both putrescine and Spd (95). Finally, in *T. brucei* at least a low efficient transport system for Spd and/or putrescine should exist to explain the growth recovery of the ODC and SpdS knockdown mutants when cultured in polyamine-supplemented media (282). However, due to the low polyamine levels in the plasma it is very likely that under *in vivo* conditions bloodstream *T. brucei* rely on the biosynthesis of polyamines.

GSH in *Trypanosoma* and *Leishmania* is synthesized by two enzymes that are common to mammalian cells. The first step catalyzed by GSH1 (Fig. 5) ligates Cys and Glu to produce γ-glutamylcysteine. The reaction is considered to be the rate limiting step of GSH biosynthesis because overexpression of GSH1 in bloodstream *T. brucei* or *Leishmania tarentolae* is associated with a significant increase in the GSH and $T(SH)_2$ levels (91, 223). The single-copy gene of *T. brucei* GSH1 has been cloned and the recombinant enzyme has been functionally characterized. As the mammalian enzyme, *T. brucei* GSH1 is inhibited by GSH (150). Different genetic strategies revealed that GSH1 is essential for *T. brucei* and *L. infantum* (107, 168). This is in agreement with the fact that BSO, a potent inhibitor of GSH1, can cure or prolong survival of mice infected with *T. brucei* (12).

The second step in the biosynthesis of GSH, namely, the linkage of γ-GC and glycine, is catalyzed by GSH2 (Fig. 5). In antimonite-resistant *L. tarentolae*, GSH2 expression is

upregulated when compared to a sensitive strain (93). The crystal structure of *T. brucei* GSH2, solved at 3.15Å resolution, showed that the active sites of the trypanosomal and mammalian enzymes are almost identical (80). More recently, the biochemical characterization of recombinant *T. cruzi* GSH2 revealed a normal hyperbolic kinetic (178) in contrast to the cooperative binding of γ-GC, typical for the mammalian enzymes (155).

The last step in the biosynthesis of $T(SH)_2$ is the conjugation of two GSH molecules with the N^1- and N^8-terminal amino groups of Spd (Fig. 5). Originally, the mechanism was studied in *C. fasciculata* which was shown to possess a glutathionylspermidine synthetase (GspdS) and a trypanothione synthetase (TryS) to catalyze the two consecutive steps (49, 246). However, since the TrySs of *T. cruzi*, *T. brucei* and *L. major* catalyze the conversion of GSH and Spd into $T(SH)_2$ (181, 184, 185), it is likely that in the pathogenic trypanosomatids $T(SH)_2$ biosynthesis is accomplished by a single enzyme. No GSpdS homolog has been found in the *T. brucei* genome and the one present in *L. major* is a pseudogene (184). Nevertheless, putative genes for GSpdS were described for *T. cruzi* and *L. infantum* though the functionality of the proteins remains to be proved (183). *T. cruzi* TryS displays a rather broad substrate specificity. It also accepts aminopropylcadaverine and spermine, thus producing homotrypanothione [N^1,N^9-bis(glutathionyl)aminopropylcadaverine] and N^1,N^{12}-bis(glutathionyl)spermine, respectively (10, 103, 185). Nonetheless, the preferred physiological product is $T(SH)_2$. GSpdS and TryS are composed of a C-terminal synthetase domain and an N-terminal amidase-domain that can hydrolyze the conjugates to restore GSH and Spd (81, 181, 182). The paradox activity, rather than being a futile cycle, has been suggested to regulate the intracellular polyamine and/or GSH levels under specific conditions (27, 227). Despite some attempts to verify this hypothesis, the fact that the amidase activity is not required for the viability or infectivity of bloodstream *T. brucei* (280) leaves its physiological role an open question. *T. brucei* TryS is essential for parasite survival and has been validated as a drug target by both inverse genetic (9, 50) and chemical approaches (247).

Despite a lot of work, it is still not clear whether the initial conjugation of GSH occurs at the N^1- or N^8-position of Spd. N^8-GSpd is almost undetectable in crithidial extracts but *in vitro* studies showed that the k_{cat}/K_m ratio of *C. fasciculata* TryS for N^8-GSpd is an order of magnitude higher than that for the N^1-isomer. This has led to the suggestion that both isomers are generated during the normal catalytic cycle of the enzyme (97). However, when taking into account that *Escherichia coli* GSpdS exclusively produces N^1-GSpd (239) and that modifications of the 3-aminopropyl but not of the 4-aminobutyl substituent abrogate the activity (27), it is likely that the parasite TrySs also mainly generate N^1-GSpd. This assumption is strengthened by the fact that N^8-acetylspermidine is almost as efficiently used by *T. cruzi* TryS as Spd while the N^1-acetyl derivative is only a poor substrate (10).

Trypanothione-dependent pathways in trypanosomatids

$T(SH)_2$ spontaneously reduces dehydroascorbate (DHA) at least two orders of magnitude faster than GSH (128) (Fig. 6). $T(SH)_2$ can also directly reduce GSSG and ovothiol disulfide (8, 165, 228). Furthermore, this unique dithiol displays much higher propensity than GSH to intercept NO in a dinitrosyl-trypanothionyl-iron complex that does not need to be sequestered by proteins, because it is harmless to the parasite even in its free form (25). $T(SH)_2$ may be involved in the defense against toxic xenobiotics. In fact, the eukaryotic elongation factor 1B complex from *L. major* (259), localized to the endoplasmic reticulum (ER) surface, displays trypanothione-S-transferase activity as well as low peroxidase activity toward hydrophobic peroxides (259). Finally, $T(SH)_2$ participates as a cofactor in the detoxification of reactive ketoaldehydes such as methylglyoxal. In contrast to the GSH-dependent mammalian GLXs, the trypanosomatid GLX I and GLX II enzymes are trypanothione-dependent (109, 258). *T. cruzi* (90), *L. major* (11, 258), and *L. donovani* (186, 187) possess a complete GLX system. In contrast, a GLX I gene is absent from the genome of African trypanosome (19) and GLX II does not seem to be essential for the proliferation of bloodstream and procyclic *T. brucei* (265). As recombinant *T. brucei* GLX II hydrolyzes thioesters that can be derived by GLX I-independent reactions, a function not related to methylglyoxal detoxification has been proposed (265).

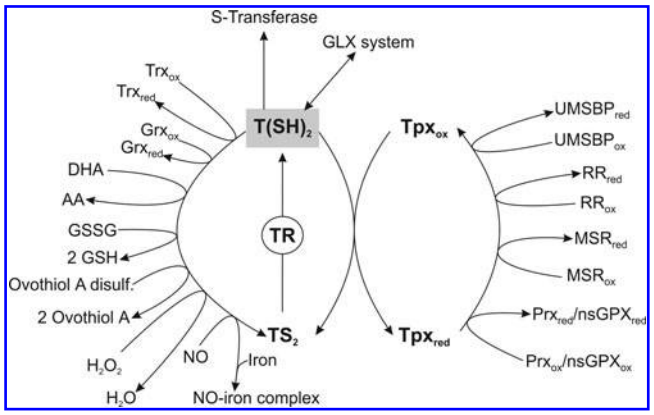


FIG. 6. Trypanothione-dependent pathways in trypanosomatids. $T(SH)_2$ directly reduces a broad range of low molecular compounds such as DHA, GSSG, ovothiol A disulfide, and H_2O_2. It intercepts NO in a trypanothionyl–iron complex and reduces redox proteins such as Trx, Grx, and Tpx. It can participate in the detoxification of ketoaldehyde by the GLX system and xenobiotics by trypanothione-S-transferases. Tpx is a multipurpose oxidoreductase that transfers reducing equivalents from $T(SH)_2$ to a variety of protein targets such as Prx, nsGPX, RR, UMSBP, and MSR. The TS_2 levels are kept low by the action of TR, which obtains its reducing equivalents from NADPH. AA, ascorbic acid; DHA, dehydroascorbate; MSR, methionine sulfoxide reductase; nsGPX, nonselenium glutathione peroxidase-type enzyme; Prx, peroxiredoxin; Tpx, tryparedoxin; TR, trypanothione reductase; TS_2, trypanothione disulfide; UMSBP, universal minicircle sequence-binding protein.

Many reactions of trypanothione are mediated by Tpx

Although at high concentrations $T(SH)_2$ directly undergoes in a wide variety of chemical reactions, many of the reactions are accelerated by the presence of Tpx. The protein is a distant relative of the superfamily of Trx-type thiol disulfide oxidoreductases (42, 88, 148, 149). Tpxs and Trxs share the same

core structure (5, 102) but show only 13% overall sequence identity. Moreover, the trypanosomatid-specific Tpxs have a WCPPCR active site motif [instead of the typical WCG(A)PC motif of Trxs] and with 16 kDa are substantially larger (162). Functionally, Tpxs share many properties with Trxs and Grxs (88, 149, 174, 231). *In vitro* Tpx can be reduced by mammalian TrxR. However, the enzyme is absent and $T(SH)_2$ is the *in vivo* reducing agent. Both the redox potential and the thiol pK-value of Tpx are every similar to the respective values of $T(SH)_2$ (Table 2) and the Tpx/$T(SH)_2$ couple is the determining factor for the intracellular redox milieu of the parasites (206). Two types of Tpx occur in trypanosomatids. The first class includes the functionally characterized mitochondrial and cytosolic isoforms in *L. infantum* (mTpx and cTpx) (42) and *T. brucei* cTpx (149,206,245). The class II Tpxs are unlikely to function as normal Tpxs owing to a number of amino acid substitutions (41).

In *T. brucei*, downregulation of the cTpx expression affects cell growth (51, 268) and is accompanied by transiently elevated levels of low-molecular-mass thiols (51). The cTpx is also essential for *L. infantum* (214), but the mTpx homozygous knockout promastigotes do not show any growth retardation or morphological alterations when compared to wild-type parasites (41). Functionally, Tpx acts as a multipurpose oxidoreductase that transfers reducing equivalents from $T(SH)_2$ to a variety of protein targets such as (i) RR, (ii) the universal minicircle sequence-binding protein (UMSBP), (iii) methionine sulfoxide reductase (MSR), and (iv) 2-Cys-Prxs and nonselenium glutathione peroxidase-type enzyme (nsGPX) (Fig. 6). $T(SH)_2$, but not the monothiols GSH or GSpd, can serve as direct donor of reducing equivalents for *T. brucei* RR. Nevertheless, the presence of Tpx increases the RR activity almost 10 times, which suggests that *in vivo* the $T(SH)_2$/Tpx couple acts as electron donor in the synthesis of DNA precursors (64). UMSBP is a zinc finger protein that plays a key role in the replication of mitochondrial DNA in trypanosomatids. The binding of UMSBP to the kinetoplast DNA is significantly affected by the redox state and Tpx has been shown to act as the reducing agent (161, 164, 179, 220). MSRs are involved in protein repair by catalyzing the reduction of methionine sulfoxide residues to methionine. Recently, *T. brucei* and *T. cruzi* have been shown to possess Tpx-dependent MSRs (7).

Trypanosomatids lack catalase and classical GPXs (reviewed in ref. 77). The parasite hydroperoxide metabolism is linked to $T(SH)_2$, but trypanothione-dependent peroxidases were not detected (37, 96, 191, 192). Later it was shown that hydroperoxide detoxification occurs by a sophisticated cascade of reactions in which $T(SH)_2$, TR, and Tpx play central roles as the carriers of reducing equivalents from NADPH onto two types of peroxidases, namely, 2-Cys-Prxs and nsGPXs (100, 174; for reviews see refs. 45, 124) (Fig. 6).

The genomes of *T. brucei*, *T. cruzi*, and *L. major* (19, 70, 110) encode multiple, almost identical copies for cytosolic 2-Cys-Prxs at one locus and on another chromosome, a single copy gene for the mitochondrial analog. The cytosolic and mitochondrial localization of the proteins has been experimentally verified in *T. cruzi* (274), *T. brucei* (245), *L. amazonensis* (144), and *L. infantum* (43). In *Leishmania* spp., one of the genes carries a glycosomal targeting signal, suggesting that the enzyme may also localize to this peroxisome-like organelle (45). Trypanosomatid 2-Cys-Prxs preferably reduce H_2O_2 and some of the enzymes are even inactivated by lipid-derived hydroperoxides (34, 40, 76). The 2-Cys-Prxs take also part in the decomposition of peroxynitrite ($ONOO^-$) (6, 198, 249). In comparison to Tpx and $T(SH)_2$, which react with $ONOO^-$ at rates that are within the range of typical thiols, the *T. brucei* and *T. cruzi* 2-Cys-Prxs reduce peroxynitrite 100 times faster (249; for a review see ref. 250).

In the infective form of *T. brucei*, the cytosolic, but not the mitochondrial, 2-Cys-Prx is crucial for parasite survival and protection against H_2O_2-mediated oxidative stress (268). However, *T. cruzi* epimastigotes overexpressing either the cytosolic or the mitochondrial isozyme are more resistant toward hydrogen peroxide, *t*-butyl hydroperoxide, and peroxynitrite (6, 198, 274). Due to the correlation between the 2-Cys-Prx protein levels in different *T. cruzi* strains and the parasitemia of infected mice, the enzymes are thought to be virulence factors (199). This hypothesis is strengthened by the fact that *T. cruzi* overexpressing the cytosolic 2-Cys-Prx can overcome the macrophage-derived cytotoxicity and display higher parasitemia and more prominent inflammatory tissue infiltrates in a mice model (6). In *L. infantum*, overexpression of the cytosolic and mitochondrial 2-Cys-Prx increases the resistance of promastigotes toward H_2O_2 and *t*-butyl hydroperoxide, respectively (43). Very recently, the mitochondrial 2-Cys-Prx has been shown to play an essential role for the infection of the mammalian host by *Leishmania*. Interestingly, the protein functions as a chaperon in a reaction independent of peroxidase activity (44). Moreover, the leishmanial Prxs seem to be implicated in drug resistance. Overexpression of the cytosolic enzyme in *L. tarentolae* increases resistance toward antimonials (281) and increased Prxs levels have been found in antimony unresponsive *L. donovani* field isolates (279).

The second class of peroxidases in trypanosomatids that obtain their reducing equivalents from the $T(SH)_2$/Tpx couple are nsGPXs. A genomic locus encodes a cluster of two or three nearly identical nsGPXs and at a separate locus an additional open reading frame for a distantly related nsGPX. As proposed by Castro and Tomas (45), the two types of enzymes will be called nsGPXs-A and nsGPXs-B, respectively. The only partially characterized parasite nsGPX-B is the *T. cruzi* protein. It is localized in the ER. The recombinant protein has been reported to have low GSH-dependent peroxidase activity with linoleic acid and phosphatidylcholine hydroperoxides and not to accept Tpx as reducing agent (273). It has been hypothesized that the protein plays a role in preventing or minimizing lipid peroxidation within the ER.

In trypanosomes and *Leishmania*, the nsGPXs-A are almost identical proteins, the only differences are the absence/presence of mitochondrial and glycosomal targeting signals. In *T. cruzi*, the nsGPX-As are found in the cytosol and glycosomes (270). In *T. brucei*, a cell fractionation revealed nsGPX-As clearly in the mitochondrial and cytosolic fractions (216).

Like mammalian GPX4, the trypanosomal and leishmanial nsGPX-A proteins are monomers (123, 216) and are probably mainly responsible for lipid hydroperoxide detoxification (61, 269). RNA interference studies proved that the enzymes are essential in both bloodstream and procyclic *T. brucei* (216, 268). The lethal phenotype of bloodstream cells in which the genes for the cytosolic nsGPX-As have been deleted is abolished by supplementing the culture medium with α-tocopherol or Trolox (61). The main physiological role of the

trypanosomatid nsGPX-As appears to be the prevention of lipid peroxidation as it is the case for mammalian GPX4 (219, 285).

$T(SH)_2$ is also the physiological reducing agent for the parasite Trx and Grxs

Despite the lack of GRs and TrxRs, *T. brucei*, *T. cruzi*, and *L. major* encode genes for Trxs as well as Grxs (19, 70, 110). The *Trx* gene is transcribed in both bloodstream and procyclic *T. brucei* (207), but the protein was not detectable by Western blot analysis even not in cells that expressed an ectopic copy of the gene at a level 30-fold higher than the wild-type transcript (217). In addition, deletion of both trx alleles in bloodstream *T. brucei* did not result in a growth phenotype at least under culture conditions (217). In *T. cruzi* epimastigotes, Trx was also only detectable after enrichment by immunoprecipitation. Immunohistochemistry showed a diffuse cytoplasmic staining with extension to the flagellum (200). *In vitro*, *T. brucei* Trx is an electron donor for RR (207, 218), 2-Cys-Prxs (207), and nsGPXs (100) as well as for 1-C-Grx1 (75). $T(SH)_2$ reduces the disulfide form of Trx, but the rate is at least 100 times slower than that of the reaction with Tpx (88, 218). Taking into account that all reactions observed for Trx are also accomplished by Tpx, which is present at a much higher intracellular concentration, the role of Trx in trypanosomatids remains to be elucidated.

Structurally, Grxs have been classified into three categories: (i) classical Grxs with the CPYC active site motif and Trx/Grx fold; (ii) GST-related proteins that possess Grx activities but have a structural organization similar to GSTs; (iii) 1-C-Grxs that contain a single cysteine in their active site (usually CGFS). Two dithiol Grxs have been characterized in *T. brucei*. Grx1 is a cytosolic protein, while Grx2 is probably located in the intermembrane space of the mitochondrion. Both proteins are reduced by $T(SH)_2$ and strongly catalyze the dithiol/disulfide exchange between $T(SH)_2$ and GSSG. While Grx2 is more active as protein disulfide reductase, Grx1 can form an iron–sulfur complex and prefers protein-GSH mixed disulfides as substrates (46). A dithiol Grx has also been characterized from *T. cruzi*. The protein seems to be involved in the dithiol/disulfide exchange between $T(SH)_2$ and GSSG and is likely to take part in the reduction of protein-GSH mixed disulfides (157). Three 1-C-Grxs are encoded in the *T. brucei*, *T. cruzi*, and *L. major* genomes (19, 70, 110). The trypanosomatid 1-C-Grx1 and 1-C-Grx2 are single domain proteins with a predicted mitochondrial presequence, whereas 1-C-Grx3 is a Trx/1-C-Grx fusion protein with no evident targeting signal. In bloodstream and procyclic *T. brucei*, 1-C-Grx1 and 1-C-Grx3 are abundant proteins that present highest intracellular concentration when the parasites are grown to the stationary/starvation phase (52). Complementation studies in Grx5-deficient *Saccharomyces cerevisiae* targeting the *T. brucei* proteins into the mitochondria revealed that only 1-C-Grx1 modestly rescues the mutant phenotype (75). Moreover, 1-C-Grx1 can coordinate an iron–sulfur cluster using GSH as ligand and plays a crucial role in the iron and redox homeostasis of the parasites (52). The Tritryp genomes (19, 70, 110) also encode genes for putative GST-related Grxs [*T. brucei* (Tb927.7.3500), *L. major* (LmjF.14.1480), and *T. cruzi* (Tc00.1047053508265.10)], but none of the proteins has been characterized so far.

Some Trypanosomatid Parasites Contain Ovothiol A

Ovothiols are a group of 4-mercaptohistidines that were first discovered in marine invertebrate eggs giving them their trivial name (252). Ovothiol A stands for 1-methyl-4-mercaptohistidine (Fig. 7), and ovothiol B and C are the respective N^{α}-methyl and N^{α},N^{α}-dimethyl derivatives. Upon fertilization, sea urchin eggs form a protective envelop that is cross-linked by dityrosyl residues, a reaction requiring H_2O_2 as extracellular oxidant. The eggs contain 5 m*M* ovothiol C, which reacts with H_2O_2, and the resulting disulphide is then reduced by GSH. Thus, ovothiol can act as a nonenzymatic GSH peroxidase system protecting the early embryo from oxidative stress (251).

In 1994, Steenkamp and Spiess reported for the first time on the occurrence of ovothiol A in a trypanosomatid organism (229). Shortly after its detection in the insect parasite *C. fasciculata*, it became obvious that the aromatic thiol is also present in *Leishmania* (229) and other pathogenic trypanosomatids (8). The thorough analysis of its prevalence in different *Leishmania* species, *T. cruzi* and *T. brucei*, revealed ovothiol A in all insect stages with cellular concentrations ranging from >1 m*M* in *Leishmania* to at least 10-fold lower levels in trypanosomes (8). Interestingly, the mammalian stages of the parasites contain significantly less ovothiol A and bloodstream *T. brucei* as well as amastigote *L. major* lack detectable amounts.

The biosynthesis of ovothiol A has partially been unraveled (Fig. 7). In the presence of His, Cys, Fe^{2+}, and pyridoxal phosphate, cell free extracts of *C. fasciculata* catalyze the formation of 4-mercaptohistidine (260). The methyl group of

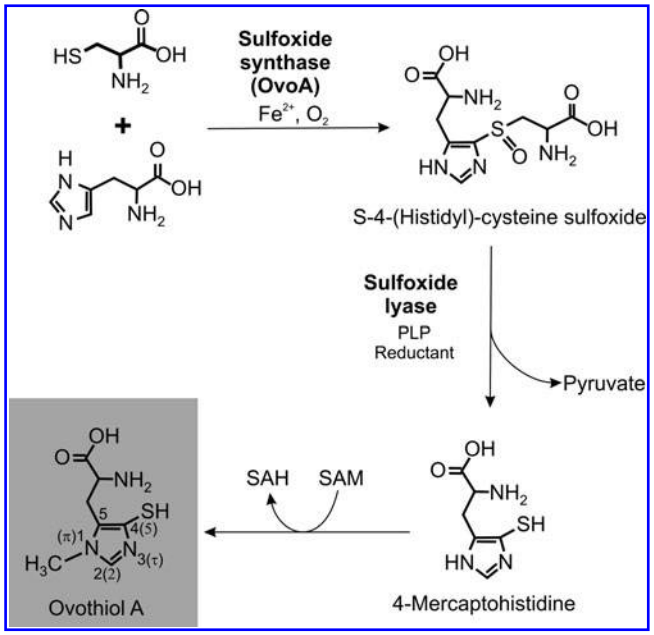


FIG. 7. Biosynthesis of ovothiol A. The biosynthesis of ovothiol A starts from cysteine and histidine. In the first step catalyzed by OvoA, S-4-(histidyl)-cysteine sulfoxide is formed. Cleavage by a so far uncharacterized lyase generates 4-mercaptohistidine. The final methylation by SAM results in the formation of ovothiol A (1-methyl-4-mercaptohistidine or, according to IUPAC nomenclature, N-methyl-5-thiohistidine) and SAH. SAH, S-adenosylhomocysteine.

ovothiol A is most likely derived from SAM (228, 230). Very recently, putative genes for S-4-(histidyl)-cysteine sulfoxide synthase (OvoAs), the first enzyme of the biosynthetic pathway, have been identified in different eukaryotic kingdoms and bacterial phyla. The recombinant enzymes from the γ-proteobacterium *Erwinia tasmaniensis* as well as from *T. cruzi* catalyze—although with modest efficiency—the iron-dependent formation of OvoA from His and Cys (29). The enzymes catalyzing the following steps, namely, the cleavage into 4-mercaptohistidine and the final methylation still await their characterization.

The physiological role of ovothiol A in trypanosomatids is not known. With a thiol pK-value of 1.42 (264), ovothiol is in the thiolate state throughout the physiological pH range. Although ovothiols can act as nonenzymatic scavengers of hydrogen peroxide, the reaction is less efficient than that by $T(SH)_2$ (8). In addition, the parasites possess efficient trypanothione-dependent peroxidases (see above). These findings render ovothiol A unlikely to play a major role in the cellular H_2O_2 metabolism of trypanosomatids. The disulphide form of ovothiol is not a substrate of TR but is readily reduced in the presence of trace amounts of $T(SH)_2$, suggesting that ovothiol A is kept reduced by the spontaneous reaction with the dithiol (8). This is corroborated by the fact that the redox potential of ovothiol is −92 mV (264) and thus 150 mV more positive than that of trypanothione (−242 mV) (73). *Leishmania* as well as *T. cruzi* proliferate within macrophages and have to deal with the innate immune system of their mammalian host. In mice, expression of inducible nitric oxide synthase is essential for the elimination of *L. donovani* (172), suggesting an important role for NO in the elimination of intracellular trypanosomatids. Ovothiols are efficient radical scavengers. Ovothiol A decomposes S-nitrosoglutathione with a second-order rate constant three orders of magnitude higher compared with GSH. This has led to the suggestion that ovothiol A in synergy with $T(SH)_2$ provides the parasites with an efficient system for the nonenzymatic decomposition of nitrosothiols (261). Since the mammalian stages of the parasites contain only very low levels of ovothiol (8), the physiological significance of this reaction remains to be elucidated. In addition, as described above, $T(SH)_2$ itself can sequester NO in form of a harmless and stable iron complex (25).

Metazoan Parasites Have GSH-Based Redox Systems

Filarial worms secrete several GSH-dependent enzymes

The antioxidant defense in filarial nematodes such as *Wucheria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus* largely corresponds to that in higher organisms (221). The main low-molecular-mass thiol is GSH (Table 1). The GSH1 and GR from *O. volvulus* exert structural and kinetic properties comparable to those of the enzymes from other origins (151, 170).

Nematodes possess superoxide dismutases, nsGPXs, GSTs, and Trx. As a peculiarity, these redox proteins, together with other proteins, are partially secreted as a potential immune evasion strategy of these parasites (18, 99, 136, 221). The nsGPX of *Brugia pahangi* is glycosylated. It is specifically expressed after infection of the mammalian host and represents the major surface protein of adult lymphatic filarial parasites (53, 241). The recombinant enzyme reduces lipid-derived hydroperoxides with GSH as electron donor and is inactive with a bacterial Trx system as reducing system (241). The GSTs of nematodes are a focus of recent antifilarial drug development approaches (14).

The GSH and Trx systems of flatworms are linked by a single reductase

In platyhelminths (flatworms), to which belong *Schistosoma*, *Echinococci*, and *Taenia*, the causative agents of schistosomiasis, hydatic disease, and cysticercosis, respectively, cellular redox homeostasis and antioxidant defense are based on both GSH and Trx systems. However, parasitic platyhelminths—but not free living flatworms (180)—lack conventional GRs and TrxRs. Instead, the parasites rely exclusively on a single enzyme that provides reducing equivalents for both pathways (4). This thioredoxin glutathione reductase (TGR) is a fusion protein composed of an N-terminal Grx domain that is linked with a TrxR/GR-type NADPH-oxidoreductase. TGR has GR, TrxR, and Grx activities (Fig. 8) (4). In mammals this type of oxidoreductase is specifically found in testes (237). As mammalian TrxRs, TGRs are selenoproteins with a C-terminal Gly-Cys-SeCys-Gly motif. The parasite TGR occurs in the cytosol and mitochondria of the worms (2, 28, 92). It is essential for *Schistosoma* survival and is currently investigated as a target for specific antischistosomal chemotherapy (137).

In parasitic helminths, GSH probably plays an important role in the removal of heavy metals. Recently, a *Schistosoma mansoni* phytochelatin synthase (PCS) has been characterized (205). The enzyme catalyzes the conversion of GSH into phytochelatines. These oligopeptides with the general formula $(\gamma\text{-GC})_n\text{-Gly}$ ($n = 2$–11) efficiently sequester heavy metals. PCS occurs in all mammalian stages of *S. mansoni* and its expression increases in response to the presence of heavy metals. It has been hypothesized that the enzyme plays a role in the detoxification of iron generated by the breakdown of host hemoglobin. Three PCS transcripts are produced in *S. mansoni* by alternative splicing, probably generating two cytosolic and a mitochondrial version of the enzyme (205). PCS genes exist also in nematodes such as *B. malayi* as well as many other organisms but not in mammals.

S. mansoni possesses a selenocysteine-containing GPX that catalyzes the GSH-dependent reduction of hydrogen peroxide (159). Adult worms express the highest activity, which renders them more resistant to oxidative stress compared

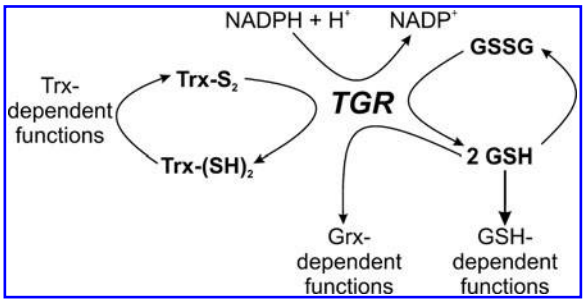


FIG. 8. Reactions catalyzed by TGR. The unique TGR of helminth parasites catalyzes the NADPH-dependent disulfide reduction of Trx as well as glutathione (GSSG) and has Grx activities. TGR, thioredoxin glutathione reductase.

with other developmental stages. The physiological function of the parasite enzyme is probably that of a phospholipid hydroperoxide GPX (156).

Ascorbate as an Antioxidant in Parasitic Organisms

Ascorbate can directly metabolize ROS, maintain α-tocopherol (vitamin E) in its reduced state, and mediate the electron transfer to ascorbate-dependent peroxidases (272). Most eukaryotes can synthesize ascorbate. Among mammals, only humans, some other primates, and guinea pigs have lost this ability because they lack an active gulonolactone oxidase that catalyzes the final step of mammalian ascorbate biosynthesis. Few studies have been published on the role of ascorbate in parasitic organisms. Mouse erythrocytes infected with the rodent malarial parasite *P. vinckei* show an increased uptake of ascorbate compared with uninfected cells (234), whereas both normal and infected cells rapidly take up DHA and reduce it to ascorbate. Parasitized but not control cells release most of the ascorbate formed that could in part explain the higher plasma levels of ascorbate found in infected animals (108). Mice erythrocytes infected with *P. vinckei* contain higher levels of ascorbate when compared with uninfected cells, and it has been suggested that in late stage infections the ascorbate content and redox state is altered to keep vitamin E reduced and thus to protect the cell from hemolysis (233). Nevertheless, the physiological significance of ascorbate in malarial infection is not clear. Knockout mice that are unable to synthesize ascorbate develop the same parasitemia upon infection with the rodent parasite *P. berghei* as wild-type mice (98). In the case of the human malarial parasite *P. falciparum*, ascorbate has been even reported to be destructive to different intraerythrocytic stages (158).

In extracts of *T. vaginalis* ascorbate peroxidase activity has been measured, but the protein has not been characterized (188). Trophozoites of *E. histolytica* require cysteine and ascorbate in the medium for optimal attachment and growth (86), but the underlying molecular mechanism has not been revealed. *B. malayi* depends on exogenous vitamin C for larval molting and it has been suggested that the filarial parasite does not synthesize ascorbate. During larval development, ascorbic acid does not seem to act as a general antioxidant but to play a specific role (204). A distinct function of ascorbate or the (in)ability for biosynthesis of ascorbate in these parasites has not yet been reported.

Ascorbate supply and metabolism in trypanosomatids

Trypanosomatids have the capacity to synthesize vitamin C (Fig. 9). *T. brucei* arabinonolactone oxidase (ALO) and *T. cruzi* galactonolactone oxidase (GAL) share 60% of all residues (147, 272). The enzymes are localized in the glycosomes, unique peroxisome-like organelles that harbor the first seven enzymes of glycolysis as well as ether lipid biosynthesis and some other pathways in trypanosomatids (272). The so far uncharacterized *Leishmania* ALO homolog lacks an obvious glycosomal targeting motif. The recombinant *T. brucei* and *T. cruzi* enzymes accept both D-arabino-1,4-lactone and L-galactono-1,4-lactone as substrates (133, 147, 272). Recently, the cofactor of *T. cruzi* GAL has been identified as FAD (133) and not flavin mononucleotide as suggested previously (147). Despite the double-substrate specificity of the parasite ALO/GAL, a genomic analysis suggests that ascorbate is synthesized *via* L-galactose and L-galactono-1,4-lactone as it is the case in plants (Fig. 9) (272). In bloodstream *T. brucei*, deletion of both alleles of ALO results in cells that, after a transient growth defect, show wild-type *in vitro* proliferation and can produce a lethal *in vivo* mice infection. Depletion of ascorbate from the medium causes a transient growth defect in both mutant and wild-type *T. brucei*. Taken together, the data suggest that ALO is not essential for African trypanosomes but that the parasites can take up ascorbate from its environment (272). This seems not to be the case for *T. cruzi*, which has been reported to be incapable of scavenging exogenous ascorbate (147). Both epimastigote and trypomastigote *T. cruzi* contain ascorbate, the concentration in the latter mammalian infective form being two- to four-fold higher than in the insect form (47). In trypanosomatids, ascorbate is most probably kept in its reduced state spontaneously by $T(SH)_2$, (128).

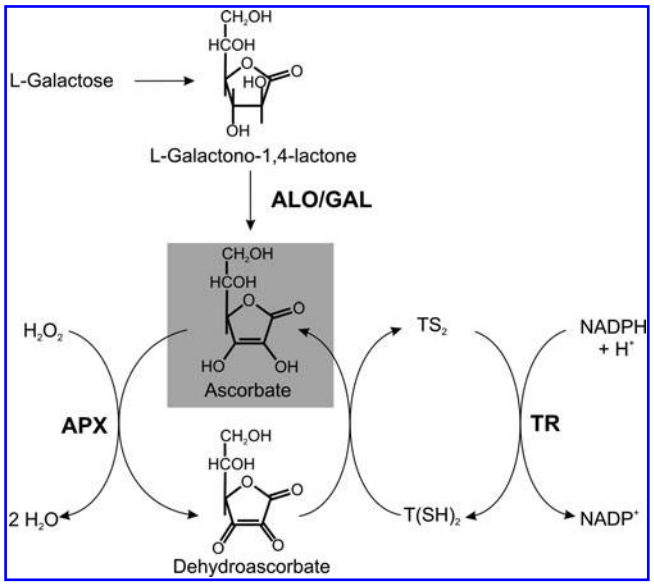


FIG. 9. Ascorbate biosynthesis and metabolism in trypanosomatids. *T. brucei* ALO and *T. cruzi* GAL catalyze oxidation of both galactono-1,4-lactone and arabinono-1,4-lactone. An APX occurs in *T. cruzi* and *Leishmania* species but not in *T. brucei*. APX, ascorbate-dependent heme peroxidase; ALO, arabinonolactone oxidase; GAL, galactonolactone oxidase.

T. cruzi and *L. major*, but not *T. brucei*, possess a plant-like ascorbate-dependent heme peroxidase (APX) (1, 271). In epimastigote *T. cruzi*, APX colocalizes with the chaperone protein BiP, suggesting a localization in the ER (271). *L. major* APX has an overall sequence identity of 63% with the *T. cruzi* peroxidase. It has been shown to be a mitochondrial protein, localized in the intermembrane space side of the inner membrane (62). *T. cruzi* APX catalyzes the ascorbate-dependent reduction of hydrogen peroxide but not of cumene or *t*-butyl hydroperoxides (271). Both *T. cruzi* and *Leishmania* APX undergo a time-dependent inactivation by hydrogen peroxide (1, 271).

Wild-type *L. major* promastigotes cultured in the presence of 250 μM H_2O_2 express APX at levels about sixfold higher

compared with unstressed cells (62). Overexpression of APX in *T. cruzi* or *L. major* results in an increased resistance toward exogenous H_2O_2 (63, 271). In addition, APX overexpression in *L. major* results in a significantly reduced intracellular H_2O_2 concentration and renders the cells resistant to cardiolipin oxidation induced by different mitochondrial ROS-generating drugs (62).

Recently, APX knockout mutants of *L. major* have been generated. The cells contain higher cellular concentrations of H_2O_2 and are more susceptible to exogenous H_2O_2 (189). Interestingly, the *Leishmania* knockout mutants exhibit a much higher internalization rate and improved survival in macrophages and cause a more severe mice infection compared with wild-type parasites. In contrast, APX-overexpressing parasites are practically avirulent. This may be due to the following mechanism. The virulent inoculum of *Leishmania* promastigotes contains a high ratio of annexin A5-binding apoptotic parasites. When these cells are depleted from the virulent population, the remaining parasites are no longer infectious (255, 262). This suggests that apoptotic promastigotes, in an altruistic way, enable the intracellular survival of the viable parasites. Indeed, cultures of APX-deficient *L. major* promastigotes contain a higher ratio of metacyclic and apoptotic cells compared to wild-type or APX-overexpressing cells. APX may thus play a major role in cellular differentiation of *L. major* and protect the parasite from apoptosis. Downregulation of APX in *Leishmania* seems to be crucial for disease development (189).

Summary and Outlook

Parasitic protozoa to which belong the causative agents of numerous, mainly neglected tropical diseases employ distinct small thiols as main cellular antioxidants. The redox potentials of these compounds, namely, cysteine, GSH, and trypanothione, are very similar and thus cannot account for this diversity (Table 2). In the case of *Entamoeba*, *Trichomonas*, and *Giardia*, life under microaerophilic conditions probably does not require a specific low-molecular-mass thiol, and Cys can act as both direct antioxidant and regulator of antioxidant enzymes. Metazoan parasites as well as *Plasmodia*, the causative agents of malaria, possess a GSH metabolism as it is the case in the mammalian host. In contrast, trypanosomatids developed a unique trypanothione-based system. $T(SH)_2$ with a pK-value close to the physiological pH is a highly reactive natural dithiol that undergoes a variety of spontaneous reactions that are not fulfilled by GSH or occur at rates that are by orders of magnitude lower.

It is tempting to speculate if or which of the parasite (specific) pathways might be exploited for an antiparasitic drug development. As outlined in this review, several parasites are capable of synthesizing cysteine, but these pathways do not appear to be first choice targets. Either, as shown for *Leishmania* and *T. cruzi*, two biosynthetic pathways exist or, in the case of *E. histolytica* and *T. vaginalis*, cysteine can alternatively be taken up from the environment.

In the case of malaria, lowering the GSH levels in infected erythrocytes is supposed to mimic the protecting effect observed in natural glucose-6-phosphate dehydrogenase deficiency. In the rodent malarial parasite *P. berghei*, gene disruption approaches revealed that the intraerythrocytic forms require either GSH biosynthesis or GSSG reduction. This would suggest that interference with one of these pathways may not be sufficient to impair parasite proliferation. Yet, it is not clear if this is also true for human malarial parasites. The development of *P. falciparum* is severely affected in erythrocytes depleted of GR activity (286, 287) or GSH *de novo* synthesis (153). On the other hand, the fact that the activity has to be lowered by >90% renders GR a difficult target molecule (117, 286, 287). Nevertheless, a very recent study shows that GR plays a crucial role in the bioactivation of antimalarial naphthoquinones (171).

In trypanosomatids, nearly all enzymes involved in the synthesis and metabolism of $T(SH)_2$ are essential and thus fulfill a prerequisite for a drug target molecule. With more than 60 publications, TR is so far the most intensively studied putative target protein. A drawback is that, as with GR and malaria, the activity of TR has to be lowered by >85% to impair parasite proliferation, which requires the development of highly potent inhibitors (131). Other enzymes of the trypanothione metabolism such as those involved in the synthesis of the dithiol may be even more suitable targets (178). Indeed, BSO, a specific inhibitor of GSH1, cures rodents from infection with *T. brucei* (12). Importantly, DFMO, a drug in clinical use for the treatment of late-stage sleeping sickness caused by *T. b. gambiense* is a specific inhibitor of ODC. Impairment of $T(SH)_2$ biosynthesis is at least one crucial consequence of ODC inactivation (see Fig. 5). An attractive approach may be the development of compounds that simultaneously interfere with two steps of the pathway, for instance with TryS and TR.

A promising current drug development approach against *Schistosoma* and related platyhelminths focuses on TGR (137), an enzyme essential for parasite survival. Since inhibitors of the parasite TGR such as several gold complexes also interfere with human TrxR and mammalian testes express a TGR-type enzyme as well (137), it remains to be shown if parasite-specific compounds can be developed.

In conclusion, essentiality is a prerequisite but by no means sufficient for a protein to be a drug target. Other aspects such as the degree of inhibition required, the availability of a suitable *in vitro* test system, oral availability of the drug, and, in some cases, the ability of the compound to cross the blood brain barrier are crucial points that must be taken into account when aiming at a clinically successful drug (178, 278). Future work will show which of the parasite redox systems indeed fulfils these demands.

References

1. Adak S and Datta AK. *Leishmania major* encodes an unusual peroxidase that is a close homologue of plant ascorbate peroxidase: a novel role of the transmembrane domain. *Biochem J* 390: 465–474, 2005.
2. Agorio A, Chalar C, Cardozo S, and Salinas G. Alternative mRNAs arising from trans-splicing code for mitochondrial and cytosolic variants of *Echinococcus granulosus* thioredoxin Glutathione reductase. *J Biol Chem* 278: 12920–12928, 2003.
3. Akerman SE and Müller S. 2-Cys peroxiredoxin PfTrx-Px1 is involved in the antioxidant defence of *Plasmodium falciparum*. *Mol Biochem Parasitol* 130: 75–81, 2003.
4. Alger HM and Williams DL. The disulfide redox system of *Schistosoma mansoni* and the importance of a multifunctional

enzyme, thioredoxin glutathione reductase. *Mol Biochem Parasitol* 121: 129–139, 2002.
5. Alphey MS, Leonard GA, Gourley DG, Tetaud E, Fairlamb AH, and Hunter WN. The high resolution crystal structure of recombinant *Crithidia fasciculata* tryparedoxin-I. *J Biol Chem* 274: 25613–25622, 1999.
6. Alvarez MN, Peluffo G, Piacenza L, and Radi R. Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized *Trypanosoma cruzi*: consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. *J Biol Chem* 286: 6627–6640, 2011.
7. Arias DG, Cabeza MS, Erben ED, Carranza PG, Lujan HD, Tellez Inon MT, Iglesias AA, and Guerrero SA. Functional characterization of methionine sulfoxide reductase A from *Trypanosoma* spp. *Free Radic Biol Med* 50: 37–46, 2011.
8. Ariyanayagam MR and Fairlamb AH. Ovothiol and trypanothione as antioxidants in trypanosomatids. *Mol Biochem Parasitol* 115: 189–198, 2001.
9. Ariyanayagam MR, Oza SL, Guther ML, and Fairlamb AH. Phenotypic analysis of trypanothione synthetase knockdown in the African trypanosome. *Biochem J* 391: 425–432, 2005.
10. Ariyanayagam MR, Oza SL, Mehlert A, and Fairlamb AH. Bis(glutathionyl)spermine and other novel trypanothione analogues in *Trypanosoma cruzi*. *J Biol Chem* 278: 27612–27619, 2003.
11. Ariza A, Vickers TJ, Greig N, Armour KA, Dixon MJ, Eggleston IM, Fairlamb AH, and Bond CS. Specificity of the trypanothione-dependent *Leishmania major* glyoxalase I: structure and biochemical comparison with the human enzyme. *Mol Microbiol* 59: 1239–1248, 2006.
12. Arrick BA, Griffith OW, and Cerami A. Inhibition of glutathione synthesis as a chemotherapeutic strategy for trypanosomiasis. *J Exp Med* 153: 720–725, 1981.
13. Atamna H and Ginsburg H. The malaria parasite supplies glutathione to its host cell—investigation of glutathione transport and metabolism in human erythrocytes infected with *Plasmodium falciparum*. *Eur J Biochem* 250: 670–679, 1997.
14. Azeez S, Babu RO, Aykkal R, and Narayanan R. Virtual screening and *in vitro* assay of potential drug like inhibitors from spices against glutathione-S-transferase of filarial nematodes. *J Mol Model* 2011 [Epub ahead of print]. DOI: 10.1007/s00894-011-1035-2.
15. Barker RH Jr., Liu H, Hirth B, Celatka CA, Fitzpatrick R, Xiang Y, Willert EK, Phillips MA, Kaiser M, Bacchi CJ, Rodriguez A, Yarlett N, Klinger JD, and Sybertz E. Novel S-adenosylmethionine decarboxylase inhibitors for the treatment of human African trypanosomiasis. *Antimicrob Agents Chemother* 53: 2052–2058, 2009.
16. Basselin M, Coombs GH, and Barrett MP. Putrescine and spermidine transport in *Leishmania*. *Mol Biochem Parasitol* 109: 37–46, 2000.
17. Becker K, Rahlfs S, Nickel C, and Schirmer RH. Glutathione—functions and metabolism in the malarial parasite *Plasmodium falciparum*. *Biol Chem* 384: 551–566, 2003.
18. Bennuru S, Semnani R, Meng Z, Ribeiro JM, Veenstra TD, and Nutman TB. *Brugia malayi* excreted/secreted proteins at the host/parasite interface: stage- and gender-specific proteomic profiling. *PLoS Negl Trop Dis* 3: e410, 2009.
19. Berriman M, Ghedin E, Hertz-Fowler C, Blandin G, Renauld H, Bartholomeu DC, Lennard NJ, Caler E, Hamlin NE, Haas B, Bohme U, Hannick L, Aslett MA, Shallom J, Marcello L, Hou L, Wickstead B, Alsmark UC, Arrowsmith C, Atkin RJ, Barron AJ, Bringaud F, Brooks K, Carrington M, Cherevach I, Chillingworth TJ, Churcher C, Clark LN, Corton CH, Cronin A, Davies RM, Doggett J, Djikeng A, Feldblyum T, Field MC, Fraser A, Goodhead I, Hance Z, Harper D, Harris BR, Hauser H, Hostetler J, Ivens A, Jagels K, Johnson D, Johnson J, Jones K, Kerhornou AX, Koo H, Larke N, Landfear S, Larkin C, Leech V, Line A, Lord A, Macleod A, Mooney PJ, Moule S, Martin DM, Morgan GW, Mungall K, Norbertczak H, Ormond D, Pai G, Peacock CS, Peterson J, Quail MA, Rabbinowitsch E, Rajandream MA, Reitter C, Salzberg SL, Sanders M, Schobel S, Sharp S, Simmonds M, Simpson AJ, Tallon L, Turner CM, Tait A, Tivey AR, Van Aken S, Walker D, Wanless D, Wang S, White B, White O, Whitehead S, Woodward J, Wortman J, Adams MD, Embley TM, Gull K, Ullu E, Barry JD, Fairlamb AH, Opperdoes F, Barrell BG, Donelson JE, Hall N, Fraser CM, Melville SE, and El-Sayed NM. The genome of the African trypanosome *Trypanosoma brucei*. *Science* 309: 416–422, 2005.
20. Beswick TC, Willert EK, and Phillips MA. Mechanisms of allosteric regulation of *Trypanosoma cruzi* S-adenosylmethionine decarboxylase. *Biochemistry* 45: 7797–7807, 2006.
21. Bhattacharya J and Swarup-Mitra S. Reduction in erythrocytic GSH level and stability in *Plasmodium vivax* malaria. *Trans R Soc Trop Med Hyg* 81: 64–66, 1987.
22. Bitonti AJ, Byers TL, Bush TL, Casara PJ, Bacchi CJ, Clarkson AB Jr., McCann PP, and Sjoerdsma A. Cure of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense* infections in mice with an irreversible inhibitor of S-adenosylmethionine decarboxylase. *Antimicrob Agents Chemother* 34: 1485–1490, 1990.
23. Bitonti AJ, Dumont JA, and McCann PP. Characterization of *Trypanosoma brucei brucei* S-adenosyl-L-methionine decarboxylase and its inhibition by Berenil, pentamidine and methylglyoxal bis(guanylhydrazone). *Biochem J* 237: 685–689, 1986.
24. Bitonti AJ, Kelly SE, and McCann PP. Characterization of spermidine synthase from *Trypanosoma brucei brucei*. *Mol Biochem Parasitol* 13: 21–28, 1984.
25. Bocedi A, Dawood KF, Fabrini R, Federici G, Gradoni L, Pedersen JZ, and Ricci G. Trypanothione efficiently intercepts nitric oxide as a harmless iron complex in trypanosomatid parasites. *FASEB J* 24: 1035–1042, 2010.
26. Boitz JM, Yates PA, Kline C, Gaur U, Wilson ME, Ullman B, and Roberts SC. *Leishmania donovani* ornithine decarboxylase is indispensable for parasite survival in the mammalian host. *Infect Immun* 77: 756–763, 2009.
27. Bollinger JM Jr., Kwon DS, Huisman GW, Kolter R, and Walsh CT. Glutathionylspermidine metabolism in *Escherichia coli*. Purification, cloning, overproduction, and characterization of a bifunctional glutathionylspermidine synthetase/amidase. *J Biol Chem* 270: 14031–14041, 1995.
28. Bonilla M, Denicola A, Novoselov SV, Turanov AA, Protasio A, Izmendi D, Gladyshev VN, and Salinas G. Platyhelminth mitochondrial and cytosolic redox homeostasis is controlled by a single thioredoxin glutathione reductase and dependent on selenium and glutathione. *J Biol Chem* 283: 17898–17907, 2008.
29. Braunshausen A and Seebeck FP. Identification and characterization of the first ovothiol biosynthetic enzyme. *J Am Chem Soc* 133: 1757–1759, 2011.
30. Brown DM, Upcroft JA, and Upcroft P. Cysteine is the major low-molecular weight thiol in *Giardia duodenalis*. *Mol Biochem Parasitol* 61: 155–158, 1993.

31. Brown DM, Upcroft JA, and Upcroft P. A thioredoxin reductase-class of disulphide reductase in the protozoan parasite *Giardia duodenalis*. *Mol Biochem Parasitol* 83: 211–220, 1996.
32. Bruchhaus I, Richter S, and Tannich E. Recombinant expression and biochemical characterization of an NADPH:flavin oxidoreductase from *Entamoeba histolytica*. *Biochem J* 330 (Pt 3): 1217–1221, 1998.
33. Buchholz K, Putrianti ED, Rahlfs S, Schirmer RH, Becker K, and Matuschewski K. Molecular genetics evidence for the *in vivo* roles of the two major NADPH-dependent disulfide reductases in the malaria parasite. *J Biol Chem* 285: 37388–37395, 2010.
34. Budde H, Flohé L, Hecht HJ, Hofmann B, Stehr M, Wissing J, and Lunsdorf H. Kinetics and redox-sensitive oligomerisation reveal negative subunit cooperativity in tryparedoxin peroxidase of *Trypanosoma brucei brucei*. *Biol Chem* 384: 619–633, 2003.
35. Camargo EP, Coelho JA, Moraes G, and Figueiredo EN. *Trypanosoma spp.*, *Leishmania spp.* and *Leptomonas spp.*: enzymes of ornithine-arginine metabolism. *Exp Parasitol* 46: 141–144, 1978.
36. Canepa GE, Bouvier LA, Miranda MR, Uttaro AD, and Pereira CA. Characterization of *Trypanosoma cruzi* L-cysteine transport mechanisms and their adaptive regulation. *FEMS Microbiol Lett* 292: 27–32, 2009.
37. Carnieri EG, Moreno SN, and Docampo R. Trypanothione-dependent peroxide metabolism in *Trypanosoma cruzi* different stages. *Mol Biochem Parasitol* 61: 79–86, 1993.
38. Carrillo C, Canepa GE, Algranati ID, and Pereira CA. Molecular and functional characterization of a spermidine transporter (TcPAT12) from *Trypanosoma cruzi*. *Biochem Biophys Res Commun* 344: 936–940, 2006.
39. Carrillo C, Cejas S, Gonzalez NS, and Algranati ID. *Trypanosoma cruzi* epimastigotes lack ornithine decarboxylase but can express a foreign gene encoding this enzyme. *FEBS Lett* 454: 192–196, 1999.
40. Castro H, Budde H, Flohé L, Hofmann B, Lunsdorf H, Wissing J, and Tomás AM. Specificity and kinetics of a mitochondrial peroxiredoxin of *Leishmania infantum*. *Free Radic Biol Med* 33: 1563–1574, 2002.
41. Castro H, Romao S, Carvalho S, Teixeira F, Sousa C, and Tomás AM. Mitochondrial redox metabolism in trypanosomatids is independent of tryparedoxin activity. *PLoS One* 5: e12607, 2010.
42. Castro H, Sousa C, Novais M, Santos M, Budde H, Cordeiro-da-Silva A, Flohé L, and Tomás AM. Two linked genes of *Leishmania infantum* encode tryparedoxins localised to cytosol and mitochondrion. *Mol Biochem Parasitol* 136: 137–147, 2004.
43. Castro H, Sousa C, Santos M, Cordeiro-da-Silva A, Flohé L, and Tomás AM. Complementary antioxidant defense by cytoplasmic and mitochondrial peroxiredoxins in *Leishmania infantum*. *Free Radic Biol Med* 33: 1552–1562, 2002.
44. Castro H, Teixeira F, Romao S, Santos M, Cruz T, Flórido M, Appelberg R, Oliveira P, Ferreira-da-Silva F, and Tomás AM. *Leishmania* mitochondrial peroxiredoxin plays a crucial peroxidase-unrelated role during infection: insight into its novel chaperone activity. *PLoS Pathog* 7: e1002325, 2011.
45. Castro H and Tomás AM. Peroxidases of trypanosomatids. *Antioxid Redox Signal* 10: 1593–1606, 2008.
46. Ceylan S, Seidel V, Ziebart N, Berndt C, Dirdjaja N, and Krauth-Siegel RL. The dithiol glutaredoxins of African trypanosomes have distinct roles and are closely linked to the unique trypanothione metabolism. *J Biol Chem* 285: 35224–35237, 2010.
47. Clark D, Albrecht M, and Arevalo J. Ascorbate variations and dehydroascorbate reductase activity in *Trypanosoma cruzi* epimastigotes and trypomastigotes. *Mol Biochem Parasitol* 66: 143–145, 1994.
48. Cobbold SA, Martin RE, and Kirk K. Methionine transport in the malaria parasite *Plasmodium falciparum*. *Int J Parasitol* 41: 125–135, 2011.
49. Comini M, Menge U, Wissing J, and Flohé L. Trypanothione synthesis in crithidia revisited. *J Biol Chem* 280: 6850–6860, 2005.
50. Comini MA, Guerrero SA, Haile S, Menge U, Lunsdorf H, and Flohé L. Validation of *Trypanosoma brucei* trypanothione synthetase as drug target. *Free Radic Biol Med* 36: 1289–1302, 2004.
51. Comini MA, Krauth-Siegel RL, and Flohé L. Depletion of the thioredoxin homologue tryparedoxin impairs antioxidative defence in African trypanosomes. *Biochem J* 402: 43–49, 2007.
52. Comini MA, Rettig J, Dirdjaja N, Hanschmann EM, Berndt C, and Krauth-Siegel RL. Monothiol glutaredoxin-1 is an essential iron-sulfur protein in the mitochondrion of African trypanosomes. *J Biol Chem* 283: 27785–27798, 2008.
53. Cookson E, Blaxter ML, and Selkirk ME. Identification of the major soluble cuticular glycoprotein of lymphatic filarial nematode parasites (gp29) as a secretory homolog of glutathione peroxidase. *Proc Natl Acad Sci U S A* 89: 5837–5841, 1992.
54. Coombs GH, Westrop GD, Suchan P, Puzova G, Hirt RP, Embley TM, Mottram JC, and Müller S. The amitochondriate eukaryote *Trichomonas vaginalis* contains a divergent thioredoxin-linked peroxiredoxin antioxidant system. *J Biol Chem* 279: 5249–5256, 2004.
55. Cruz F and Ferry JG. Interaction of iron-sulfur flavoprotein with oxygen and hydrogen peroxide. *Biochim Biophys Acta* 1760: 858–864, 2006.
56. Cunningham ML and Fairlamb AH. Trypanothione reductase from *Leishmania donovani*. Purification, characterisation and inhibition by trivalent antimonials. *Eur J Biochem* 230: 460–468, 1995.
57. da Silva ER, da Silva MF, Fischer H, Mortara RA, Mayer MG, Framesqui K, Silber AM, and Floeter-Winter LM. Biochemical and biophysical properties of a highly active recombinant arginase from *Leishmania (Leishmania) amazonensis* and subcellular localization of native enzyme. *Mol Biochem Parasitol* 159: 104–111, 2008.
58. Dalle-Donne I, Milzani A, Gagliano N, Colombo R, Giustarini D, and Rossi R. Molecular mechanisms and potential clinical significance of S-glutathionylation. *Antioxid Redox Signal* 10: 445–473, 2008.
59. Deponte M, Rahlfs S, and Becker K. Peroxiredoxin systems of protozoal parasites. *Subcell Biochem* 44: 219–229, 2007.
60. Di Matteo A, Scandurra FM, Testa F, Forte E, Sarti P, Brunori M, and Giuffre A. The O_2-scavenging flavodiiron protein in the human parasite *Giardia intestinalis*. *J Biol Chem* 283: 4061–4068, 2008.
61. Diechtierow M and Krauth-Siegel RL. A tryparedoxin-dependent peroxidase protects African trypanosomes from membrane damage. *Free Radic Biol Med* 51: 856–868, 2011.
62. Dolai S, Yadav RK, Pal S, and Adak S. *Leishmania major* ascorbate peroxidase overexpression protects cells against reactive oxygen species-mediated cardiolipin oxidation. *Free Radic Biol Med* 45: 1520–1529, 2008.

63. Dolai S, Yadav RK, Pal S, and Adak S. Overexpression of mitochondrial *Leishmania major* ascorbate peroxidase enhances tolerance to oxidative stress-induced programmed cell death and protein damage. *Eukaryot Cell* 8: 1721–1731, 2009.
64. Dormeyer M, Reckenfelderbaumer N, Lüdemann H, and Krauth-Siegel RL. Trypanothione-dependent synthesis of deoxyribonucleotides by *Trypanosoma brucei* ribonucleotide reductase. *J Biol Chem* 276: 10602–10606, 2001.
65. Drummelsmith J, Girard I, Trudel N, and Ouellette M. Differential protein expression analysis of *Leishmania major* reveals novel roles for methionine adenosyltransferase and S-adenosylmethionine in methotrexate resistance. *J Biol Chem* 279: 33273–33280, 2004.
66. Dumas C, Ouellette M, Tovar J, Cunningham ML, Fairlamb AH, Tamar S, Olivier M, and Papadopoulou B. Disruption of the trypanothione reductase gene of *Leishmania* decreases its ability to survive oxidative stress in macrophages. *EMBO J* 16: 2590–2598, 1997.
67. Duszenko M, Muhlstadt K, and Broder A. Cysteine is an essential growth factor for *Trypanosoma brucei* bloodstream forms. *Mol Biochem Parasitol* 50: 269–273, 1992.
68. Eckman JR and Eaton JW. Dependence of plasmodial glutathione metabolism on the host cell. *Nature* 278: 754–756, 1979.
69. Ekstrom JL, Tolbert WD, Xiong H, Pegg AE, and Ealick SE. Structure of a human S-adenosylmethionine decarboxylase self-processing ester intermediate and mechanism of putrescine stimulation of processing as revealed by the H243A mutant. *Biochemistry* 40: 9495–9504, 2001.
70. El-Sayed NM, Myler PJ, Bartholomeu DC, Nilsson D, Aggarwal G, Tran AN, Ghedin E, Worthey EA, Delcher AL, Blandin G, Westenberger SJ, Caler E, Cerqueira GC, Branche C, Haas B, Anupama A, Arner E, Aslund L, Attipoe P, Bontempi E, Bringaud F, Burton P, Cadag E, Campbell DA, Carrington M, Crabtree J, Darban H, da Silveira JF, de Jong P, Edwards K, Englund PT, Fazelina G, Feldblyum T, Ferella M, Frasch AC, Gull K, Horn D, Hou L, Huang Y, Kindlund E, Klingbeil M, Kluge S, Koo H, Lacerda D, Levin MJ, Lorenzi H, Louie T, Machado CR, McCulloch R, McKenna A, Mizuno Y, Mottram JC, Nelson S, Ochaya S, Osoegawa K, Pai G, Parsons M, Pentony M, Pettersson U, Pop M, Ramirez JL, Rinta J, Robertson L, Salzberg SL, Sanchez DO, Seyler A, Sharma R, Shetty J, Simpson AJ, Sisk E, Tammi MT, Tarleton R, Teixeira S, Van Aken S, Vogt C, Ward PN, Wickstead B, Wortman J, White O, Fraser CM, Stuart KD, and Andersson B. The genome sequence of *Trypanosoma cruzi*, etiologic agent of Chagas disease. *Science* 309: 409–415, 2005.
71. Fahey RC, Newton GL, Arrick B, Overdank-Bogart T, and Aley SB. *Entamoeba histolytica*: a eukaryote without glutathione metabolism. *Science* 224: 70–72, 1984.
72. Fairlamb AH, Blackburn P, Ulrich P, Chait BT, and Cerami A. Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. *Science* 227: 1485–1487, 1985.
73. Fairlamb AH and Cerami A. Metabolism and functions of trypanothione in the Kinetoplastida. *Annu Rev Microbiol* 46: 695–729, 1992.
74. Färber PM, Becker K, Müller S, Schirmer RH, and Franklin RM. Molecular cloning and characterization of a putative glutathione reductase gene, the PfGR2 gene, from *Plasmodium falciparum*. *Eur J Biochem* 239: 655–661, 1996.
75. Filser M, Comini MA, Molina-Navarro MM, Dirdjaja N, Herrero E, and Krauth-Siegel RL. Cloning, functional analysis, and mitochondrial localization of *Trypanosoma brucei* monothiol glutaredoxin-1. *Biol Chem* 389: 21–32, 2008.
76. Flohé L, Budde H, Bruns K, Castro H, Clos J, Hofmann B, Kansal-Kalavar S, Krumme D, Menge U, Plank-Schumacher K, Sztajer H, Wissing J, Wylegalla C, and Hecht HJ. Tryparedoxin peroxidase of *Leishmania donovani*: molecular cloning, heterologous expression, specificity, and catalytic mechanism. *Arch Biochem Biophys* 397: 324–335, 2002.
77. Flohé L, Hecht HJ, and Steinert P. Glutathione and trypanothione in parasitic hydroperoxide metabolism. *Free Radic Biol Med* 27: 966–984, 1999.
78. Francis SE, Sullivan DJ Jr., and Goldberg DE. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annu Rev Microbiol* 51: 97–123, 1997.
79. Fritz-Wolf K, Becker A, Rahlfs S, Harwaldt P, Schirmer RH, Kabsch W, and Becker K. X-ray structure of glutathione S-transferase from the malarial parasite *Plasmodium falciparum*. *Proc Natl Acad Sci U S A* 100: 13821–13826, 2003.
80. Fyfe PK, Alphey MS, and Hunter WN. Structure of *Trypanosoma brucei* glutathione synthetase: domain and loop alterations in the catalytic cycle of a highly conserved enzyme. *Mol Biochem Parasitol* 170: 93–99, 2010.
81. Fyfe PK, Oza SL, Fairlamb AH, and Hunter WN. Leishmania trypanothione synthetase-amidase structure reveals a basis for regulation of conflicting synthetic and hydrolytic activities. *J Biol Chem* 283: 17672–17680, 2008.
82. Gaur U, Roberts SC, Dalvi RP, Corraliza I, Ullman B, and Wilson ME. An effect of parasite-encoded arginase on the outcome of murine cutaneous leishmaniasis. *J Immunol* 179: 8446–8453, 2007.
83. Ghoda L, Phillips MA, Bass KE, Wang CC, and Coffino P. Trypanosome ornithine decarboxylase is stable because it lacks sequences found in the carboxyl terminus of the mouse enzyme which target the latter for intracellular degradation. *J Biol Chem* 265: 11823–11826, 1990.
84. Ghoda L, van Daalen Wetters T, Macrae M, Ascherman D, and Coffino P. Prevention of rapid intracellular degradation of ODC by a carboxyl-terminal truncation. *Science* 243: 1493–1495, 1989.
85. Gilbert HF. Molecular and cellular aspects of thiol-disulfide exchange. *Adv Enzymol Relat Areas Mol Biol* 63: 69–172, 1990.
86. Gillin FD and Diamond LS. Attachment of *Entamoeba histolytica* to glass in a defined maintenance medium: specific requirement for cysteine and ascorbic acid. *J Protozool* 27: 474–478, 1980.
87. Gilroy C, Olenyik T, Roberts SC, and Ullman B. Spermidine synthase is required for virulence of *Leishmania donovani*. *Infect Immun* 79: 2764–2769, 2011.
88. Gommel DU, Nogoceke E, Morr M, Kiess M, Kalisz HM, and Flohé L. Catalytic characteristics of tryparedoxin. *Eur J Biochem* 248: 913–918, 1997.
89. Gonzalez NS, Ceriani C, and Algranati ID. Differential regulation of putrescine uptake in *Trypanosoma cruzi* and other trypanosomatids. *Biochem Biophys Res Commun* 188: 120–128, 1992.
90. Greig N, Wyllie S, Vickers TJ, and Fairlamb AH. Trypanothione-dependent glyoxalase I in *Trypanosoma cruzi*. *Biochem J* 400: 217–223, 2006.
91. Grondin K, Haimeur A, Mukhopadhyay R, Rosen BP, and Ouellette M. Co-amplification of the gamma-glutamylcysteine synthetase gene gsh1 and of the ABC transporter gene

pgpA in arsenite-resistant *Leishmania tarentolae*. *EMBO J* 16: 3057–3065, 1997.
92. Guevara-Flores A, Del Arenal IP, Mendoza-Hernandez G, Pardo JP, Flores-Herrera O, and Rendon JL. Mitochondrial thioredoxin-glutathione reductase from larval *Taenia crassiceps* (Cysticerci). *J Parasitol Res* 2010: pii 719856, 2010.
93. Guimond C, Trudel N, Brochu C, Marquis N, El Fadili A, Peytavi R, Briand G, Richard D, Messier N, Papadopoulou B, Corbeil J, Bergeron MG, Legare D, and Ouellette M. Modulation of gene expression in *Leishmania* drug resistant mutants as determined by targeted DNA microarrays. *Nucleic Acids Res* 31: 5886–5896, 2003.
94. Hanson S, Adelman J, and Ullman B. Amplification and molecular cloning of the ornithine decarboxylase gene of *Leishmania donovani*. *J Biol Chem* 267: 2350–2559, 1992.
95. Hasne MP and Ullman B. Identification and characterization of a polyamine permease from the protozoan parasite *Leishmania major*. *J Biol Chem* 280: 15188–15194, 2005.
96. Henderson GB, Fairlamb AH, and Cerami A. Trypanothione dependent peroxide metabolism in *Crithidia fasciculata* and *Trypanosoma brucei*. *Mol Biochem Parasitol* 24: 39–45, 1987.
97. Henderson GB, Yamaguchi M, Novoa L, Fairlamb AH, and Cerami A. Biosynthesis of the trypanosomatid metabolite trypanothione: purification and characterization of trypanothione synthetase from *Crithidia fasciculata*. *Biochemistry* 29: 3924–3929, 1990.
98. Herbas MS and Suzuki H. Vitamin C deficiency fails to protect mice from malaria. *Exp Anim* 59: 239–243, 2010.
99. Hewitson JP, Harcus YM, Curwen RS, Dowle AA, Atmadja AK, Ashton PD, Wilson A, and Maizels RM. The secretome of the filarial parasite, *Brugia malayi*: proteomic profile of adult excretory-secretory products. *Mol Biochem Parasitol* 160: 8–21, 2008.
100. Hillebrand H, Schmidt A, and Krauth-Siegel RL. A second class of peroxidases linked to the trypanothione metabolism. *J Biol Chem* 278: 6809–6815, 2003.
101. Hiller N, Fritz-Wolf K, Deponte M, Wende W, Zimmermann H, and Becker K. *Plasmodium falciparum* glutathione S-transferase—structural and mechanistic studies on ligand binding and enzyme inhibition. *Protein Sci* 15: 281–289, 2006.
102. Hofmann B, Budde H, Bruns K, Guerrero SA, Kalisz HM, Menge U, Montemartini M, Nogoceke E, Steinert P, Wissing JB, Flohé L, and Hecht HJ. Structures of tryparedoxins revealing interaction with trypanothione. *Biol Chem* 382: 459–471, 2001.
103. Hunter KJ, Le Quesne SA, and Fairlamb AH. Identification and biosynthesis of N^1,N^9-bis(glutathionyl)aminopropylcadaverine (homotrypanothione) in *Trypanosoma cruzi*. *Eur J Biochem* 226: 1019–1027, 1994.
104. Husain A, Jeelani G, Sato D, and Nozaki T. Global analysis of gene expression in response to L-Cysteine deprivation in the anaerobic protozoan parasite *Entamoeba histolytica*. *BMC Genomics* 12: 275, 2011.
105. Husain A, Sato D, Jeelani G, Mi-ichi F, Ali V, Suematsu M, Soga T, and Nozaki T. Metabolome analysis revealed increase in S-methylcysteine and phosphatidylisopropanolamine synthesis upon L-cysteine deprivation in the anaerobic protozoan parasite *Entamoeba histolytica*. *J Biol Chem* 285: 39160–39170, 2010.
106. Hussain S, Ali V, Jeelani G, and Nozaki T. Isoform-dependent feedback regulation of serine O-acetyltransferase isoenzymes involved in L-cysteine biosynthesis of *Entamoeba histolytica*. *Mol Biochem Parasitol* 163: 39–47, 2009.
107. Huynh TT, Huynh VT, Harmon MA, and Phillips MA. Gene knockdown of gamma-glutamylcysteine synthetase by RNAi in the parasitic protozoa *Trypanosoma brucei* demonstrates that it is an essential enzyme. *J Biol Chem* 278: 39794–39800, 2003.
108. Iheanacho EN, Stocker R, and Hunt NH. Redox metabolism of vitamin C in blood of normal and malaria-infected mice. *Biochim Biophys Acta* 1182: 15–21, 1993.
109. Irsch T and Krauth-Siegel RL. Glyoxalase II of African trypanosomes is trypanothione-dependent. *J Biol Chem* 279: 22209–22217, 2004.
110. Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, Sisk E, Rajandream MA, Adlem E, Aert R, Anupama A, Apostolou Z, Attipoe P, Bason N, Bauser C, Beck A, Beverley SM, Bianchettin G, Borzym K, Bothe G, Bruschi CV, Collins M, Cadag E, Ciarloni L, Clayton C, Coulson RM, Cronin A, Cruz AK, Davies RM, De Gaudenzi J, Dobson DE, Duesterhoeft A, Fazelina G, Fosker N, Frasch AC, Fraser A, Fuchs M, Gabel C, Goble A, Goffeau A, Harris D, Hertz-Fowler C, Hilbert H, Horn D, Huang Y, Klages S, Knights A, Kube M, Larke N, Litvin L, Lord A, Louie T, Marra M, Masuy D, Matthews K, Michaeli S, Mottram JC, Muller-Auer S, Munden H, Nelson S, Norbertczak H, Oliver K, O'Neil S, Pentony M, Pohl TM, Price C, Purnelle B, Quail MA, Rabbinowitsch E, Reinhardt R, Rieger M, Rinta J, Robben J, Robertson L, Ruiz JC, Rutter S, Saunders D, Schafer M, Schein J, Schwartz DC, Seeger K, Seyler A, Sharp S, Shin H, Sivam D, Squares R, Squares S, Tosato V, Vogt C, Volckaert G, Wambutt R, Warren T, Wedler H, Woodward J, Zhou S, Zimmermann W, Smith DF, Blackwell JM, Stuart KD, Barrell B, and Myler PJ. The genome of the kinetoplastid parasite, *Leishmania major*. *Science* 309: 436–442, 2005.
111. Jaeger T and Flohé L. The thiol-based redox networks of pathogens: unexploited targets in the search for new drugs. *Biofactors* 27: 109–120, 2006.
112. Jeelani G, Husain A, Sato D, Ali V, Suematsu M, Soga T, and Nozaki T. Two atypical L-cysteine-regulated NADPH-dependent oxidoreductases involved in redox maintenance, L-cystine and iron reduction, and metronidazole activation in the enteric protozoan *Entamoeba histolytica*. *J Biol Chem* 285: 26889–26899, 2010.
113. Jiang Y, Roberts SC, Jardim A, Carter NS, Shih S, Ariyanayagam M, Fairlamb AH, and Ullman B. Ornithine decarboxylase gene deletion mutants of *Leishmania donovani*. *J Biol Chem* 274: 3781–3788, 1999.
114. Jocelyn PC. The standard redox potential of cysteine-cystine from the thiol-disulphide exchange reaction with glutathione and lipoic acid. *Eur J Biochem* 2: 327–331, 1967.
115. Jones DC, Ariza A, Chow WH, Oza SL, and Fairlamb AH. Comparative structural, kinetic and inhibitor studies of *Trypanosoma brucei* trypanothione reductase with *T. cruzi*. *Mol Biochem Parasitol* 169: 12–19, 2010.
116. Kandpal M and Tekwani BL. Polyamine transport systems of *Leishmania donovani* promastigotes. *Life Sci* 60: 1793–1801, 1997.
117. Kanzok SM, Schirmer RH, Turbachova I, Iozef R, and Becker K. The thioredoxin system of the malaria parasite *Plasmodium falciparum*. Glutathione reduction revisited. *J Biol Chem* 275: 40180–40186, 2000.
118. Kawazu S, Komaki K, Tsuji N, Kawai S, Ikenoue N, Hatabu T, Ishikawa H, Matsumoto Y, Himeno K, and Kano S.

Molecular characterization of a 2-Cys peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. *Mol Biochem Parasitol* 116: 73–79, 2001.
119. Kehr S, Jortzik E, Delahunty C, Yates JR, Rahlfs S, and Becker K. Protein S-glutathionylation in malaria parasites. *Antioxid Redox Signal* 15: 2855–2865, 2011.
120. Kehr S, Sturm N, Rahlfs S, Przyborski JM, and Becker K. Compartmentation of redox metabolism in malaria parasites. *PLoS Pathog* 6: e1001242, 2010.
121. Kinch LN, Scott JR, Ullman B, and Phillips MA. Cloning and kinetic characterization of the *Trypanosoma cruzi* S-adenosylmethionine decarboxylase. *Mol Biochem Parasitol* 101: 1–11, 1999.
122. Komaki-Yasuda K, Kawazu S, and Kano S. Disruption of the *Plasmodium falciparum* 2-Cys peroxiredoxin gene renders parasites hypersensitive to reactive oxygen and nitrogen species. *FEBS Lett* 547: 140–144, 2003.
123. König J and Fairlamb AH. A comparative study of type I and type II tryparedoxin peroxidases in *Leishmania major*. *FEBS J* 274: 5643–5658, 2007.
124. Krauth-Siegel LR, Comini MA, and Schlecker T. The trypanothione system. *Subcell Biochem* 44: 231–251, 2007.
125. Krauth-Siegel RL, Bauer H, and Schirmer RH. Dithiol proteins as guardians of the intracellular redox milieu in parasites: old and new drug targets in trypanosomes and malaria-causing plasmodia. *Angew Chem Int Ed Engl* 44: 690–715, 2005.
126. Krauth-Siegel RL and Comini MA. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim Biophys Acta* 1780: 1236–1248, 2008.
127. Krauth-Siegel RL, Enders B, Henderson GB, Fairlamb AH, and Schirmer RH. Trypanothione reductase from *Trypanosoma cruzi*. Purification and characterization of the crystalline enzyme. *Eur J Biochem* 164: 123–128, 1987.
128. Krauth-Siegel RL and Lüdemann H. Reduction of dehydroascorbate by trypanothione. *Mol Biochem Parasitol* 80: 203–208, 1996.
129. Krauth-Siegel RL, Müller JG, Lottspeich F, and Schirmer RH. Glutathione reductase and glutamate dehydrogenase of *Plasmodium falciparum*, the causative agent of tropical malaria. *Eur J Biochem* 235: 345–350, 1996.
130. Krauth-Siegel RL and Schöneck R. Flavoprotein structure and mechanism. 5. Trypanothione reductase and lipoamide dehydrogenase as targets for a structure-based drug design. *FASEB J* 9: 1138–1146, 1995.
131. Krieger S, Schwarz W, Ariyanayagam MR, Fairlamb AH, Krauth-Siegel RL, and Clayton C. Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. *Mol Microbiol* 35: 542–552, 2000.
132. Krnajski Z, Walter RD, and Müller S. Isolation and functional analysis of two thioredoxin peroxidases (peroxiredoxins) from *Plasmodium falciparum*. *Mol Biochem Parasitol* 113: 303–308, 2001.
133. Kudryashova EV, Leferink NG, Slot IG, and van Berkel WJ. Galactonolactone oxidoreductase from *Trypanosoma cruzi* employs a FAD cofactor for the synthesis of vitamin C. *Biochim Biophys Acta* 1814: 545–552, 2011.
134. Kumar B, Chaubey S, Shah P, Tanveer A, Charan M, Siddiqi MI, and Habib S. Interaction between sulphur mobilisation proteins SufB and SufC: evidence for an iron-sulphur cluster biogenesis pathway in the apicoplast of *Plasmodium falciparum*. *Int J Parasitol* 41: 991–999, 2011.
135. Kumar C, Igbaria A, D'Autreaux B, Planson AG, Junot C, Godat E, Bachhawat AK, Delaunay-Moisan A, and Toledano MB. Glutathione revisited: a vital function in iron metabolism and ancillary role in thiol-redox control. *EMBO J* 30: 2044–2056, 2011.
136. Kunchithapautham K, Padmavathi B, Narayanan RB, Kaliraj P, and Scott AL. Thioredoxin from *Brugia malayi*: defining a 16-kilodalton class of thioredoxins from nematodes. *Infect Immun* 71: 4119–4126, 2003.
137. Kuntz AN, Davioud-Charvet E, Sayed AA, Califf LL, Dessolin J, Arner ES, and Williams DL. Thioredoxin glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *PLoS Med* 4: e206, 2007.
138. Le Quesne SA and Fairlamb AH. Regulation of a high-affinity diamine transport system in *Trypanosoma cruzi* epimastigotes. *Biochem J* 316 (Pt 2): 481–486, 1996.
139. Leitsch D, Kolarich D, Binder M, Stadlmann J, Altmann F, and Duchene M. *Trichomonas vaginalis*: metronidazole and other nitroimidazole drugs are reduced by the flavin enzyme thioredoxin reductase and disrupt the cellular redox system. Implications for nitroimidazole toxicity and resistance. *Mol Microbiol* 72: 518–536, 2009.
140. Li F, Hua SB, Wang CC, and Gottesdiener KM. Procyclic *Trypanosoma brucei* cell lines deficient in ornithine decarboxylase activity. *Mol Biochem Parasitol* 78: 227–236, 1996.
141. Liebau E, Dawood KF, Fabrini R, Fischer-Riepe L, Perbandt M, Stella L, Pedersen JZ, Bocedi A, Petrarca P, Federici G, and Ricci G. Tetramerization and cooperativity in *Plasmodium falciparum* glutathione S-transferase are mediated by atypic loop 113–119. *J Biol Chem* 284: 22133–22139, 2009.
142. Liebau E, De Maria F, Burmeister C, Perbandt M, Turella P, Antonini G, Federici G, Giansanti F, Stella L, Lo Bello M, Caccuri AM, and Ricci G. Cooperativity and pseudo-cooperativity in the glutathione S-transferase from *Plasmodium falciparum*. *J Biol Chem* 280: 26121–26128, 2005.
143. Lill R and Muhlenhoff U. Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. *Annu Rev Biochem* 77: 669–700, 2008.
144. Lin YC, Hsu JY, Chiang SC, and Lee ST. Distinct overexpression of cytosolic and mitochondrial tryparedoxin peroxidases results in preferential detoxification of different oxidants in arsenite-resistant *Leishmania amazonensis* with and without DNA amplification. *Mol Biochem Parasitol* 142: 66–75, 2005.
145. Lindley H. A study of the kinetics of the reaction between thiol compounds and choloracetamide. *Biochem J* 74: 577–584, 1960.
146. Loftus B, Anderson I, Davies R, Alsmark UC, Samuelson J, Amedeo P, Roncaglia P, Berriman M, Hirt RP, Mann BJ, Nozaki T, Suh B, Pop M, Duchene M, Ackers J, Tannich E, Leippe M, Hofer M, Bruchhaus I, Willhoeft U, Bhattacharya A, Chillingworth T, Churcher C, Hance Z, Harris B, Harris D, Jagels K, Moule S, Mungall K, Ormond D, Squares R, Whitehead S, Quail MA, Rabbinowitsch E, Norbertczak H, Price C, Wang Z, Guillen N, Gilchrist C, Stroup SE, Bhattacharya S, Lohia A, Foster PG, Sicheritz-Ponten T, Weber C, Singh U, Mukherjee C, El-Sayed NM, Petri WA, Jr., Clark CG, Embley TM, Barrell B, Fraser CM, and Hall N. The genome of the protist parasite *Entamoeba histolytica*. *Nature* 433: 865–868, 2005.

147. Logan FJ, Taylor MC, Wilkinson SR, Kaur H, and Kelly JM. The terminal step in vitamin C biosynthesis in *Trypanosoma cruzi* is mediated by a FMN-dependent galactonolactone oxidase. *Biochem J* 407: 419–426, 2007.
148. Lopez JA, Carvalho TU, de Souza W, Flohé L, Guerrero SA, Montemartini M, Kalisz HM, Nogoceke E, Singh M, Alves MJ, and Colli W. Evidence for a trypanothione-dependent peroxidase system in *Trypanosoma cruzi*. *Free Radic Biol Med* 28: 767–772, 2000.
149. Lüdemann H, Dormeyer M, Sticherling C, Stallmann D, Follmann H, and Krauth-Siegel RL. *Trypanosoma brucei* tryparedoxin, a thioredoxin-like protein in African trypanosomes. *FEBS Lett* 431: 381–385, 1998.
150. Lueder DV and Phillips MA. Characterization of *Trypanosoma brucei* gamma-glutamylcysteine synthetase, an essential enzyme in the biosynthesis of trypanothione (diglutathionylspermidine). *J Biol Chem* 271: 17485–17490, 1996.
151. Lüersen K, Müller S, Hussein A, Liebau E, and Walter RD. The gamma-glutamylcysteine synthetase of *Onchocerca volvulus*. *Mol Biochem Parasitol* 111: 243–251, 2000.
152. Lüersen K, Walter RD, and Müller S. The putative gamma-glutamylcysteine synthetase from *Plasmodium falciparum* contains large insertions and a variable tandem repeat. *Mol Biochem Parasitol* 98: 131–142, 1999.
153. Lüersen K, Walter RD, and Müller S. *Plasmodium falciparum*-infected red blood cells depend on a functional glutathione *de novo* synthesis attributable to an enhanced loss of glutathione. *Biochem J* 346 Pt 2: 545–552, 2000.
154. Lujan HD and Nash TE. The uptake and metabolism of cysteine by *Giardia lamblia* trophozoites. *J Eukaryot Microbiol* 41: 169–175, 1994.
155. Luo JL, Huang CS, Babaoglu K, and Anderson ME. Novel kinetics of mammalian glutathione synthetase: characterization of gamma-glutamyl substrate cooperative binding. *Biochem Biophys Res Commun* 275: 577–581, 2000.
156. Maiorino M, Pierce R, and Flohé L. Product of the *Schistosoma mansoni* glutathione peroxidase gene is a selenium containing phospholipid hydroperoxide glutathione peroxidase (PHGPx) sharing molecular weight and substrate specificity with its mammalian counterpart. *Biomed Environ Sci* 10: 209–213, 1997.
157. Marquez VE, Arias DG, Piattoni CV, Robello C, Iglesias AA, and Guerrero SA. Cloning, expression, and characterization of a dithiol glutaredoxin from *Trypanosoma cruzi*. *Antioxid Redox Signal* 12: 787–792, 2010.
158. Marva E, Golenser J, Cohen A, Kitrossky N, Har-el R, and Chevion M. The effects of ascorbate-induced free radicals on *Plasmodium falciparum*. *Trop Med Parasitol* 43: 17–23, 1992.
159. Mei H, Thakur A, Schwartz J, and Lo Verde PT. Expression and characterization of glutathione peroxidase activity in the human blood fluke *Schistosoma mansoni*. *Infect Immun* 64: 4299–4306, 1996.
160. Meierjohann S, Walter RD, and Müller S. Glutathione synthetase from *Plasmodium falciparum*. *Biochem J* 363: 833–838, 2002.
161. Milman N, Motyka SA, Englund PT, Robinson D, and Shlomai J. Mitochondrial origin-binding protein UMSBP mediates DNA replication and segregation in trypanosomes. *Proc Natl Acad Sci U S A* 104: 19250–19255, 2007.
162. Montemartini M, Nogoceke E, Singh M, Steinert P, Flohé L, and Kalisz HM. Sequence analysis of the tryparedoxin peroxidase gene from *Crithidia fasciculata* and its functional expression in *Escherichia coli*. *J Biol Chem* 273: 4864–4871, 1998.
163. Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, Best AA, Cande WZ, Chen F, Cipriano MJ, Davids BJ, Dawson SC, Elmendorf HG, Hehl AB, Holder ME, Huse SM, Kim UU, Lasek-Nesselquist E, Manning G, Nigam A, Nixon JE, Palm D, Passamaneck NE, Prabhu A, Reich CI, Reiner DS, Samuelson J, Svard SG, and Sogin ML. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. *Science* 317: 1921–1926, 2007.
164. Motyka SA, Drew ME, Yildirir G, and Englund PT. Overexpression of a cytochrome b5 reductase-like protein causes kinetoplast DNA loss in *Trypanosoma brucei*. *J Biol Chem* 281: 18499–18506, 2006.
165. Moutiez M, Aumercier M, Teissier E, Parmentier B, Tartar A, and Sergheraert C. Reduction of a trisulfide derivative of glutathione by glutathione reductase. *Biochem Biophys Res Commun* 202: 1380–1386, 1994.
166. Moutiez M, Meziene-Cherif D, Aumercier M, Sergheraert C, and Tartar A. Compared reactivities of trypanothione and glutathione in conjugation reactions. *Chem Pharm Bull* 42: 2641–2644, 1994.
167. Mueller EG. Trafficking in persulfides: delivering sulfur in biosynthetic pathways. *Nat Chem Biol* 2: 185–194, 2006.
168. Mukherjee A, Roy G, Guimond C, and Ouellette M. The gamma-glutamylcysteine synthetase gene of *Leishmania* is essential and involved in response to oxidants. *Mol Microbiol* 74: 914–927, 2009.
169. Müller S. Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. *Mol Microbiol* 53: 1291–1305, 2004.
170. Müller S, Gilberger TW, Fairlamb AH, and Walter RD. Molecular characterization and expression of *Onchocerca volvulus* glutathione reductase. *Biochem J* 325 (Pt 3): 645–651, 1997.
171. Müller T, Johann L, Jannack B, Bruckner M, Lanfranchi DA, Bauer H, Sanchez C, Yardley V, Deregnaucourt C, Schrevel J, Lanzer M, Schirmer RH, and Davioud-Charvet E. Glutathione reductase-catalyzed cascade of redox reactions to bioactivate potent antimalarial 1,4-naphthoquinones—a new strategy to combat malarial parasites. *J Am Chem Soc* 133: 11557–11571, 2011.
172. Murray HW and Nathan CF. Macrophage microbicidal mechanisms *in vivo*: reactive nitrogen versus oxygen intermediates in the killing of intracellular visceral *Leishmania donovani*. *J Exp Med* 189: 741–746, 1999.
173. Nickel C, Rahlfs S, Deponte M, Koncarevic S, and Becker K. Thioredoxin networks in the malarial parasite *Plasmodium falciparum*. *Antioxid Redox Signal* 8: 1227–1239, 2006.
174. Nogoceke E, Gommel DU, Kiess M, Kalisz HM, and Flohé L. A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. *Biol Chem* 378: 827–836, 1997.
175. Nozaki T, Ali V, and Tokoro M. Sulfur-containing amino acid metabolism in parasitic protozoa. *Adv Parasitol* 60: 1–99, 2005.
176. Nozaki T, Asai T, Sanchez LB, Kobayashi S, Nakazawa M, and Takeuchi T. Characterization of the gene encoding serine acetyltransferase, a regulated enzyme of cysteine biosynthesis from the protist parasites *Entamoeba histolytica* and *Entamoeba dispar*. Regulation and possible function of the cysteine biosynthetic pathway in *Entamoeba*. *J Biol Chem* 274: 32445–32452, 1999.

177. Nozaki T, Shigeta Y, Saito-Nakano Y, Imada M, and Kruger WD. Characterization of transsulfuration and cysteine biosynthetic pathways in the protozoan hemoflagellate, *Trypanosoma cruzi*. Isolation and molecular characterization of cystathionine beta-synthase and serine acetyltransferase from *Trypanosoma*. *J Biol Chem* 276: 6516–6523, 2001.
178. Olin-Sandoval V, Moreno-Sanchez R, and Saavedra E. Targeting trypanothione metabolism in trypanosomatid human parasites. *Curr Drug Targets* 11: 1614–1630, 2010.
179. Onn I, Milman-Shtepel N, and Shlomai J. Redox potential regulates binding of universal minicircle sequence binding protein at the kinetoplast DNA replication origin. *Eukaryot Cell* 3: 277–287, 2004.
180. Otero L, Bonilla M, Protasio AV, Fernandez C, Gladyshev VN, and Salinas G. Thioredoxin and glutathione systems differ in parasitic and free-living platyhelminths. *BMC Genomics* 11: 237, 2010.
181. Oza SL, Ariyanayagam MR, Aitcheson N, and Fairlamb AH. Properties of trypanothione synthetase from *Trypanosoma brucei*. *Mol Biochem Parasitol* 131: 25–33, 2003.
182. Oza SL, Ariyanayagam MR, and Fairlamb AH. Characterization of recombinant glutathionylspermidine synthetase/amidase from *Crithidia fasciculata*. *Biochem J* 364: 679–686, 2002.
183. Oza SL, Chen S, Wyllie S, Coward JK, and Fairlamb AH. ATP-dependent ligases in trypanothione biosynthesis—kinetics of catalysis and inhibition by phosphinic acid pseudopeptides. *FEBS J* 275: 5408–5421, 2008.
184. Oza SL, Shaw MP, Wyllie S, and Fairlamb AH. Trypanothione biosynthesis in *Leishmania major*. *Mol Biochem Parasitol* 139: 107–116, 2005.
185. Oza SL, Tetaud E, Ariyanayagam MR, Warnon SS, and Fairlamb AH. A single enzyme catalyses formation of trypanothione from glutathione and spermidine in *Trypanosoma cruzi*. *J Biol Chem* 277: 35853–35861, 2002.
186. Padmanabhan PK, Mukherjee A, and Madhubala R. Characterization of the gene encoding glyoxalase II from *Leishmania donovani*: a potential target for anti-parasite drugs. *Biochem J* 393: 227–234, 2006.
187. Padmanabhan PK, Mukherjee A, Singh S, Chattopadhyaya S, Gowri VS, Myler PJ, Srinivasan N, and Madhubala R. Glyoxalase I from *Leishmania donovani*: a potential target for anti-parasite drug. *Biochem Biophys Res Commun* 337: 1237–1248, 2005.
188. Page-Sharp M, Behm CA, and Smith GD. *Tritrichomonas foetus* and *Trichomonas vaginalis*: the pattern of inactivation of hydrogenase activity by oxygen and activities of catalase and ascorbate peroxidase. *Microbiology* 142 (Pt 1): 207–211, 1996.
189. Pal S, Dolai S, Yadav RK, and Adak S. Ascorbate peroxidase from *Leishmania major* controls the virulence of infective stage of promastigotes by regulating oxidative stress. *PLoS One* 5: e11271, 2010.
190. Pastrana-Mena R, Dinglasan RR, Franke-Fayard B, Vega-Rodriguez J, Fuentes-Caraballo M, Baerga-Ortiz A, Coppens I, Jacobs-Lorena M, Janse CJ, and Serrano AE. Glutathione reductase-null malaria parasites have normal blood stage growth but arrest during development in the mosquito. *J Biol Chem* 285: 27045–27056, 2010.
191. Penketh PG, Kennedy WP, Patton CL, and Sartorelli AC. Trypanosomatid hydrogen peroxide metabolism. *FEBS Lett* 221: 427–431, 1987.
192. Penketh PG and Klein RA. Hydrogen peroxide metabolism in *Trypanosoma brucei*. *Mol Biochem Parasitol* 20: 111–121, 1986.
193. Perez-Pertejo Y, Reguera RM, Villa H, Garcia-Estrada C, Balana-Fouce R, Pajares MA, and Ordonez D. *Leishmania donovani* methionine adenosyltransferase. Role of cysteine residues in the recombinant enzyme. *Eur J Biochem* 270: 28–35, 2003.
194. Persson K, Aslund L, Grahn B, Hanke J, and Heby O. *Trypanosoma cruzi* has not lost its S-adenosylmethionine decarboxylase: characterization of the gene and the encoded enzyme. *Biochem J* 333 (Pt 3): 527–537, 1998.
195. Persson L. Ornithine decarboxylase and S-adenosylmethionine decarboxylase in trypanosomatids. *Biochem Soc Trans* 35: 314–317, 2007.
196. Phillips MA, Coffino P, and Wang CC. Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei*. Implications for enzyme turnover and selective difluoromethylornithine inhibition. *J Biol Chem* 262: 8721–8727, 1987.
197. Phillips MA, Coffino P, and Wang CC. *Trypanosoma brucei* ornithine decarboxylase: enzyme purification, characterization, and expression in *Escherichia coli*. *J Biol Chem* 263: 17933–17941, 1988.
198. Piacenza L, Peluffo G, Alvarez MN, Kelly JM, Wilkinson SR, and Radi R. Peroxiredoxins play a major role in protecting *Trypanosoma cruzi* against macrophage- and endogenously-derived peroxynitrite. *Biochem J* 410: 359–368, 2008.
199. Piacenza L, Zago MP, Peluffo G, Alvarez MN, Basombrio MA, and Radi R. Enzymes of the antioxidant network as novel determiners of *Trypanosoma cruzi* virulence. *Int J Parasitol* 39: 1455–1464, 2009.
200. Piattoni CV, Blancato VS, Miglietta H, Iglesias AA, and Guerrero SA. On the occurrence of thioredoxin in *Trypanosoma cruzi*. *Acta Trop* 97: 151–160, 2006.
201. Piñeyro MD, Parodi-Talice A, Portela M, Arias DG, Guerrero SA, and Robello C. Molecular characterization and interactome analysis of *Trypanosoma cruzi* Tryparedoxin 1. *J Proteomics* 74: 1683–1692, 2011.
202. Rada P, Smid O, Sutak R, Dolezal P, Pyrih J, Zarsky V, Montagne JJ, Hrdy I, Camadro JM, and Tachezy J. The monothiol single-domain glutaredoxin is conserved in the highly reduced mitochondria of *Giardia intestinalis*. *Eukaryot Cell* 8: 1584–1591, 2009.
203. Rahlfs S, Fischer M, and Becker K. *Plasmodium falciparum* possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. *J Biol Chem* 276: 37133–37140, 2001.
204. Rajan TV, Paciorkowski N, Kalajzic I, and McGuiness C. Ascorbic acid is a requirement for the morphogenesis of the human filarial parasite *Brugia malayi*. *J Parasitol* 89: 868–870, 2003.
205. Ray D and Williams DL. Characterization of the phytochelatin synthase of *Schistosoma mansoni*. *PLoS Negl Trop Dis* 5: e1168, 2011.
206. Reckenfelderbäumer N and Krauth-Siegel RL. Catalytic properties, thiol pK value, and redox potential of *Trypanosoma brucei* tryparedoxin. *J Biol Chem* 277: 17548–17555, 2002.
207. Reckenfelderbäumer N, Lüdemann H, Schmidt H, Steverding D, and Krauth-Siegel RL. Identification and functional characterization of thioredoxin from *Trypanosoma brucei brucei*. *J Biol Chem* 275: 7547–7552, 2000.
208. Reguera RM, Balana-Fouce R, Perez-Pertejo Y, Fernandez FJ, Garcia-Estrada C, Cubria JC, Ordonez C, and Ordonez D. Cloning expression and characterization of methionine

adenosyltransferase in *Leishmania infantum* promastigotes. *J Biol Chem* 277: 3158–3167, 2002.
209. Reguera RM, Balana-Fouce R, Showalter M, Hickerson S, and Beverley SM. *Leishmania major* lacking arginase (ARG) are auxotrophic for polyamines but retain infectivity to susceptible BALB/c mice. *Mol Biochem Parasitol* 165: 48–56, 2009.
210. Reguera RM, Redondo CM, Perez-Pertejo Y, and Balana-Fouce R. S-Adenosylmethionine in protozoan parasites: functions, synthesis and regulation. *Mol Biochem Parasitol* 152: 1–10, 2007.
211. Roberts SC, Jiang Y, Jardim A, Carter NS, Heby O, and Ullman B. Genetic analysis of spermidine synthase from *Leishmania donovani*. *Mol Biochem Parasitol* 115: 217–226, 2001.
212. Roberts SC, Scott J, Gasteier JE, Jiang Y, Brooks B, Jardim A, Carter NS, Heby O, and Ullman B. S-adenosylmethionine decarboxylase from *Leishmania donovani*. Molecular, genetic, and biochemical characterization of null mutants and overproducers. *J Biol Chem* 277: 5902–5909, 2002.
213. Roberts SC, Tancer MJ, Polinsky MR, Gibson KM, Heby O, and Ullman B. Arginase plays a pivotal role in polyamine precursor metabolism in *Leishmania*. Characterization of gene deletion mutants. *J Biol Chem* 279: 23668–23678, 2004.
214. Romao S, Castro H, Sousa C, Carvalho S, and Tomas AM. The cytosolic tryparedoxin of *Leishmania infantum* is essential for parasite survival. *Int J Parasitol* 39: 703–711, 2009.
215. Sarma GN, Savvides SN, Becker K, Schirmer M, Schirmer RH, and Karplus PA. Glutathione reductase of the malarial parasite *Plasmodium falciparum*: crystal structure and inhibitor development. *J Mol Biol* 328: 893–907, 2003.
216. Schlecker T, Schmidt A, Dirdjaja N, Voncken F, Clayton C, and Krauth-Siegel RL. Substrate specificity, localization, and essential role of the glutathione peroxidase-type tryparedoxin peroxidases in *Trypanosoma brucei*. *J Biol Chem* 280: 14385–14394, 2005.
217. Schmidt A, Clayton CE, and Krauth-Siegel RL. Silencing of the thioredoxin gene in *Trypanosoma brucei brucei*. *Mol Biochem Parasitol* 125: 207–210, 2002.
218. Schmidt H and Krauth-Siegel RL. Functional and physicochemical characterization of the thioredoxin system in *Trypanosoma brucei*. *J Biol Chem* 278: 46329–46336, 2003.
219. Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culmsee C, Plesnila N, Kremmer E, Radmark O, Wurst W, Bornkamm GW, Schweizer U, and Conrad M. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab* 8: 237–248, 2008.
220. Sela D, Yaffe N, and Shlomai J. Enzymatic mechanism controls redox-mediated protein-DNA interactions at the replication origin of kinetoplast DNA minicircles. *J Biol Chem* 283: 32034–32044, 2008.
221. Selkirk ME, Smith VP, Thomas GR, and Gounaris K. Resistance of filarial nematode parasites to oxidative stress. *Int J Parasitol* 28: 1315–1332, 1998.
222. Selzer PM, Hesse F, Hamm-Kunzelmann B, Muhlstadt K, Echner H, and Duszenko M. Down regulation of S-adenosyl-L-methionine decarboxylase activity of *Trypanosoma brucei* during transition from long slender to short stumpy-like forms in axenic culture. *Eur J Cell Biol* 69: 173–179, 1996.
223. Shahi SK, Krauth-Siegel RL, and Clayton CE. Overexpression of the putative thiol conjugate transporter TbMRPA causes melarsoprol resistance in *Trypanosoma brucei*. *Mol Microbiol* 43: 1129–1138, 2002.
224. Shames SL, Fairlamb AH, Cerami A, and Walsh CT. Purification and characterization of trypanothione reductase from *Crithidia fasciculata*, a newly discovered member of the family of disulfide-containing flavoprotein reductases. *Biochemistry* 25: 3519–3526, 1986.
225. Sherman IW. Transport of amino acids and nucleic acid precursors in malarial parasites. *Bull World Health Organ* 55: 211–225, 1977.
226. Sherman IW. Biochemistry of *Plasmodium* (malarial parasites). *Microbiol Rev* 43: 453–495, 1979.
227. Shim H and Fairlamb AH. Levels of polyamines, glutathione and glutathione-spermidine conjugates during growth of the insect trypanosomatid *Crithidia fasciculata*. *J Gen Microbiol* 134: 807–817, 1988.
228. Steenkamp DJ. Trypanosomal antioxidants and emerging aspects of redox regulation in the trypanosomatids. *Antioxid Redox Signal* 4: 105–121, 2002.
229. Steenkamp DJ and Spies HS. Identification of a major low-molecular-mass thiol of the trypanosomatid *Crithidia fasciculata* as ovothiol A. Facile isolation and structural analysis of the bimane derivative. *Eur J Biochem* 223: 43–50, 1994.
230. Steenkamp DJ, Weldrick D, and Spies HS. Studies on the biosynthesis of ovothiol A. Identification of 4-mercaptohistidine as an intermediate. *Eur J Biochem* 242: 557–566, 1996.
231. Steinert P, Plank-Schumacher K, Montemartini M, Hecht HJ, and Flohé L. Permutation of the active site motif of tryparedoxin 2. *Biol Chem* 381: 211–219, 2000.
232. Stocker R, Hunt NH, Buffinton GD, Weidemann MJ, Lewis-Hughes PH, and Clark IA. Oxidative stress and protective mechanisms in erythrocytes in relation to *Plasmodium vinckei* load. *Proc Natl Acad Sci U S A* 82: 548–551, 1985.
233. Stocker R, Hunt NH, Weidemann MJ, and Clark IA. Protection of vitamin E from oxidation by increased ascorbic acid content within *Plasmodium vinckei*-infected erythrocytes. *Biochim Biophys Acta* 876: 294–299, 1986.
234. Stocker R, Weidemann MJ, and Hunt NH. Possible mechanisms responsible for the increased ascorbic acid content of Plasmodium vinckei-infected mouse erythrocytes. *Biochim Biophys Acta* 881: 391–397, 1986.
235. Sturm N, Jortzik E, Mailu BM, Koncarevic S, Deponte M, Forchhammer K, Rahlfs S, and Becker K. Identification of proteins targeted by the thioredoxin superfamily in *Plasmodium falciparum*. *PLoS Pathog* 5: e1000383, 2009.
236. Sullivan FX, Shames SL, and Walsh CT. Expression of *Trypanosoma congolense* trypanothione reductase in *Escherichia coli*: overproduction, purification, and characterization. *Biochemistry* 28: 4986–4992, 1989.
237. Sun QA, Kirnarsky L, Sherman S, and Gladyshev VN. Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. *Proc Natl Acad Sci U S A* 98: 3673–3678, 2001.
238. Sztajer H, Gamain B, Aumann KD, Slomianny C, Becker K, Brigelius-Flohe R, and Flohé L. The putative glutathione peroxidase gene of *Plasmodium falciparum* codes for a thioredoxin peroxidase. *J Biol Chem* 276: 7397–7403, 2001.
239. Tabor H and Tabor CW. Isolation, characterization, and turnover of glutathionylspermidine from *Escherichia coli*. *J Biol Chem* 250: 2648–2654, 1975.
240. Taladriz S, Ramiro MJ, Hanke T, and Larraga V. S-adenosylmethionine decarboxylase from *Leishmania infantum* promastigotes: molecular cloning and differential expression. *Parasitol Res* 88: 421–426, 2002.

241. Tang L, Gounaris K, Griffiths C, and Selkirk ME. Heterologous expression and enzymatic properties of a selenium-independent glutathione peroxidase from the parasitic nematode *Brugia pahangi*. *J Biol Chem* 270: 18313–18318, 1995.
242. Taylor MC, Kaur H, Blessington B, Kelly JM, and Wilkinson SR. Validation of spermidine synthase as a drug target in African trypanosomes. *Biochem J* 409: 563–569, 2008.
243. Tekwani BL, Bacchi CJ, and Pegg AE. Putrescine activated S-adenosylmethionine decarboxylase from *Trypanosoma brucei brucei*. *Mol Cell Biochem* 117: 53–61, 1992.
244. Tekwani BL and Mehlotra RK. Molecular basis of defence against oxidative stress in *Entamoeba histolytica* and *Giardia lamblia*. *Microbes Infect* 1: 385–394, 1999.
245. Tetaud E, Giroud C, Prescott AR, Parkin DW, Baltz D, Biteau N, Baltz T, and Fairlamb AH. Molecular characterisation of mitochondrial and cytosolic trypanothione-dependent tryparedoxin peroxidases in *Trypanosoma brucei*. *Mol Biochem Parasitol* 116: 171–183, 2001.
246. Tetaud E, Manai F, Barrett MP, Nadeau K, Walsh CT, and Fairlamb AH. Cloning and characterization of the two enzymes responsible for trypanothione biosynthesis in *Crithidia fasciculata*. *J Biol Chem* 273: 19383–19390, 1998.
247. Torrie LS, Wyllie S, Spinks D, Oza SL, Thompson S, Harrison JR, Gilbert IH, Wyatt PG, Fairlamb AH, and Frearson JA. Chemical validation of trypanothione synthetase: a potential drug target for human trypanosomiasis. *J Biol Chem* 284: 36137–36145, 2009.
248. Tovar J, Cunningham ML, Smith AC, Croft SL, and Fairlamb AH. Down-regulation of *Leishmania donovani* trypanothione reductase by heterologous expression of a trans-dominant mutant homologue: effect on parasite intracellular survival. *Proc Natl Acad Sci U S A* 95: 5311–5316, 1998.
249. Trujillo M, Budde H, Piñeyro MD, Stehr M, Robello C, Flohé L, and Radi R. *Trypanosoma brucei* and *Trypanosoma cruzi* tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. *J Biol Chem* 279: 34175–34182, 2004.
250. Trujillo M, Ferrer-Sueta G, Thomson L, Flohé L, and Radi R. Kinetics of peroxiredoxins and their role in the decomposition of peroxynitrite. *Subcell Biochem* 44: 83–113, 2007.
251. Turner E, Hager LJ, and Shapiro BM. Ovothiol replaces glutathione peroxidase as a hydrogen peroxide scavenger in sea urchin eggs. *Science* 242: 939–941, 1988.
252. Turner E, Klevit R, Hager LJ, and Shapiro BM. Ovothiols, a family of redox-active mercaptohistidine compounds from marine invertebrate eggs. *Biochemistry* 26: 4028–4036, 1987.
253. Urscher M, Alisch R, and Deponte M. The glyoxalase system of malaria parasites—implications for cell biology and general glyoxalase research. *Semin Cell Dev Biol* 22: 262–270, 2011.
254. Van Dobbenburgh OA, Houwen B, Jurjens H, Marrink J, Halie MR, and Nieweg HO. Plasma spermidine concentrations as early indication of response to therapy in human myeloma. *J Clin Pathol* 36: 804–807, 1983.
255. van Zandbergen G, Bollinger A, Wenzel A, Kamhawi S, Voll R, Klinger M, Müller A, Hölscher C, Herrmann M, Sacks D, Solbach W, and Laskay T. Leishmania disease development depends on the presence of apoptotic promastigotes in the virulent inoculum. *Proc Natl Acad Sci U S A* 103: 13837–13842, 2006.
256. Vega-Rodriguez J, Franke-Fayard B, Dinglasan RR, Janse CJ, Pastrana-Mena R, Waters AP, Coppens I, Rodriguez-Orengo JF, Srinivasan P, Jacobs-Lorena M, and Serrano AE. The glutathione biosynthetic pathway of *Plasmodium* is essential for mosquito transmission. *PLoS Pathog* 5: e1000302, 2009.
257. Vicente JB, Ehrenkaufer GM, Saraiva LM, Teixeira M, and Singh U. *Entamoeba histolytica* modulates a complex repertoire of novel genes in response to oxidative and nitrosative stresses: implications for amebic pathogenesis. *Cell Microbiol* 11: 51–69, 2009.
258. Vickers TJ, Greig N, and Fairlamb AH. A trypanothione-dependent glyoxalase I with a prokaryotic ancestry in *Leishmania major*. *Proc Natl Acad Sci U S A* 101: 13186–13191, 2004.
259. Vickers TJ, Wyllie S, and Fairlamb AH. *Leishmania major* elongation factor 1B complex has trypanothione S-transferase and peroxidase activity. *J Biol Chem* 279: 49003–49009, 2004.
260. Vogt RN, Spies HS, and Steenkamp DJ. The biosynthesis of ovothiol A (N-methyl-4-mercaptohistidine). Identification of S-(4′-L-histidyl)-L-cysteine sulfoxide as an intermediate and the products of the sulfoxide lyase reaction. *Eur J Biochem* 268: 5229–5241, 2001.
261. Vogt RN and Steenkamp DJ. The metabolism of S-nitrosothiols in the trypanosomatids: the role of ovothiol A and trypanothione. *Biochem J* 371: 49–59, 2003.
262. Wanderley JL, Pinto da Silva LH, Deolindo P, Soong L, Borges VM, Prates DB, de Souza AP, Barral A, Balanco JM, do Nascimento MT, Saraiva EM, and Barcinski MA. Cooperation between apoptotic and viable metacyclics enhances the pathogenesis of Leishmaniasis. *PLoS One* 4: e5733, 2009.
263. Wang XF and Cynader MS. Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. *J Neurosci* 21: 3322–3331, 2001.
264. Weaver KH and Rabenstein DL. Thiol/disulfide exchange reactions of ovothiol A with glutathione. *J Org Chem* 60: 1904–1907, 1995.
265. Wendler A, Irsch T, Rabbani N, Thornalley PJ, and Krauth-Siegel RL. Glyoxalase II does not support methylglyoxal detoxification but serves as a general trypanothione thioesterase in African trypanosomes. *Mol Biochem Parasitol* 163: 19–27, 2009.
266. Westrop GD, Georg I, and Coombs GH. The mercaptopyruvate sulfurtransferase of *Trichomonas vaginalis* links cysteine catabolism to the production of thioredoxin persulfide. *J Biol Chem* 284: 33485–33494, 2009.
267. Westrop GD, Goodall G, Mottram JC, and Coombs GH. Cysteine biosynthesis in *Trichomonas vaginalis* involves cysteine synthase utilizing O-phosphoserine. *J Biol Chem* 281: 25062–25075, 2006.
268. Wilkinson SR, Horn D, Prathalingam SR, and Kelly JM. RNA interference identifies two hydroperoxide metabolizing enzymes that are essential to the bloodstream form of the African trypanosome. *J Biol Chem* 278: 31640–31646, 2003.
269. Wilkinson SR, Meyer DJ, and Kelly JM. Biochemical characterization of a trypanosome enzyme with glutathione-dependent peroxidase activity. *Biochem J* 352: 755–761, 2000.
270. Wilkinson SR, Meyer DJ, Taylor MC, Bromley EV, Miles MA, and Kelly JM. The *Trypanosoma cruzi* enzyme TcGPXI is a glycosomal peroxidase and can be linked to trypanothione reduction by glutathione or tryparedoxin. *J Biol Chem* 277: 17062–17071, 2002.

271. Wilkinson SR, Obado SO, Mauricio IL, and Kelly JM. *Trypanosoma cruzi* expresses a plant-like ascorbate-dependent hemoperoxidase localized to the endoplasmic reticulum. *Proc Natl Acad Sci U S A* 99: 13453–13458, 2002.
272. Wilkinson SR, Prathalingam SR, Taylor MC, Horn D, and Kelly JM. Vitamin C biosynthesis in trypanosomes: a role for the glycosome. *Proc Natl Acad Sci U S A* 102: 11645–11650, 2005.
273. Wilkinson SR, Taylor MC, Touitha S, Mauricio IL, Meyer DJ, and Kelly JM. TcGPXII, a glutathione-dependent *Trypanosoma cruzi* peroxidase with substrate specificity restricted to fatty acid and phospholipid hydroperoxides, is localized to the endoplasmic reticulum. *Biochem J* 364: 787–794, 2002.
274. Wilkinson SR, Temperton NJ, Mondragon A, and Kelly JM. Distinct mitochondrial and cytosolic enzymes mediate trypanothione-dependent peroxide metabolism in *Trypanosoma cruzi*. *J Biol Chem* 275: 8220–8225, 2000.
275. Willert EK, Fitzpatrick R, and Phillips MA. Allosteric regulation of an essential trypanosome polyamine biosynthetic enzyme by a catalytically dead homolog. *Proc Natl Acad Sci U S A* 104: 8275–8280, 2007.
276. Williams RA, Kelly SM, Mottram JC, and Coombs GH. 3-Mercaptopyruvate sulfurtransferase of *Leishmania* contains an unusual C-terminal extension and is involved in thioredoxin and antioxidant metabolism. *J Biol Chem* 278: 1480–1486, 2003.
277. Williams RA, Westrop GD, and Coombs GH. Two pathways for cysteine biosynthesis in *Leishmania major*. *Biochem J* 420: 451–462, 2009.
278. Wyatt PG, Gilbert IH, Read KD, and Fairlamb AH. Target validation: linking target and chemical properties to desired product profile. *Curr Top Med Chem* 11: 1275–1283, 2011.
279. Wyllie S, Mandal G, Singh N, Sundar S, Fairlamb AH, and Chatterjee M. Elevated levels of tryparedoxin peroxidase in antimony unresponsive *Leishmania donovani* field isolates. *Mol Biochem Parasitol* 173: 162–164, 2010.
280. Wyllie S, Oza SL, Patterson S, Spinks D, Thompson S, and Fairlamb AH. Dissecting the essentiality of the bifunctional trypanothione synthetase-amidase in *Trypanosoma brucei* using chemical and genetic methods. *Mol Microbiol* 74: 529–540, 2009.
281. Wyllie S, Vickers TJ, and Fairlamb AH. Roles of trypanothione S-transferase and tryparedoxin peroxidase in resistance to antimonials. *Antimicrob Agents Chemother* 52: 1359–1365, 2008.
282. Xiao Y, McCloskey DE, and Phillips MA. RNA interference-mediated silencing of ornithine decarboxylase and spermidine synthase genes in *Trypanosoma brucei* provides insight into regulation of polyamine biosynthesis. *Eukaryot Cell* 8: 747–755, 2009.
283. Xue M, Rabbani N, and Thornalley PJ. Glyoxalase in ageing. *Semin Cell Dev Biol* 22: 293–301, 2011.
284. Yarlett N, Garofalo J, Goldberg B, Ciminelli MA, Ruggiero V, Sufrin JR, and Bacchi CJ. S-adenosylmethionine synthetase in bloodstream *Trypanosoma brucei*. *Biochim Biophys Acta* 1181: 68–76, 1993.
285. Yoo MH, Gu X, Xu XM, Kim JY, Carlson BA, Patterson AD, Cai H, Gladyshev VN, and Hatfield DL. Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage and in Alzheimer's disease. *Antioxid Redox Signal* 12: 819–827, 2010.
286. Zhang Y, Konig I, and Schirmer RH. Glutathione reductase-deficient erythrocytes as host cells of malarial parasites. *Biochem Pharmacol* 37: 861–865, 1988.
287. Zhang YA, Hempelmann E, and Schirmer RH. Glutathione reductase inhibitors as potential antimalarial drugs. Effects of nitrosoureas on *Plasmodium falciparum in vitro*. *Biochem Pharmacol* 37: 855–860, 1988.

Address correspondence to:
Dr. R. Luise Krauth-Siegel
Biochemie-Zentrum der Universität Heidelberg (BZH)
Im Neuenheimer Feld 328
D-69120 Heidelberg
Germany

E-mail: luise.krauth-siegel@bzh.uni-heidelberg.de

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Abbreviations Used

1-C-Grx = monothiol glutaredoxin
2-Cys-Prx = 2-Cys-peroxiredoxin
AA = ascorbic acid
ALO = arabinolactone oxidase
AOP = antioxidant protein
APX = ascorbate-dependent heme peroxidase
ARG = arginase
Asp-AT = aspartate aminotransferase
BSO = buthionine sulfoximine
CBS = cystathionine-β-synthase
CD = cysteine desulfurase
CGL = cystathionine-γ-lyase
CS = cysteine synthase
DFMO = difluoromethylornithine
DHA = dehydroascorbate
dNDPs = deoxyribonucleotides
dSAM = decarboxylated S-adenosylmethionine
ER = endoplasmic reticulum
FAD = flavin adenine dinucleotide
FDP = flavin diiron protein
GAL = galactonolactone oxidase
GLX = glyoxalase
GR = glutathione reductase
Grx = glutaredoxin
GSH = glutathione
GSH1 = γ-glutamylcysteine synthetase
GSH2 = glutathione synthetase
GSpd = glutathionylspermidine
GSpdS = glutathionylspermidine synthetase
GSSG = glutathione disulfide
GST = glutathione-S-transferase
ISF = iron-sulphur flavoprotein
MAT = methionine adenosyltransferase
MFS = major facilitator superfamily
MGL = methionine-γ-lyase
MSR = methionine sulfoxide reductase
MST = mercaptopyruvate sulfurtransferase
nsGPX = nonselenium glutathione peroxidase-type enzyme

Abbreviations Used (Cont.)

OAS = O-acetylserine
ODC = ornithine decarboxylase
OvoA = S-4-(histidyl)-cysteine sulfoxide synthase
PCS = phytochelatin synthase
Prx = peroxiredoxin
ROS = reactive oxygen species
RR = ribonucleotide reductase
RTS = reverse *trans*-sulfuration
SAH = S-adenosylhomocysteine
SAM = S-adenosylmethionine
SAMDC = S-adenosylmethionine decarboxylase
SAT = serine acetyl-CoA transferase
SeCys = selenocysteine
SMC = S-methylcysteine
Spd = spermidine
SpdS = spermidine synthase
$T(SH)_2$ = trypanothione
TGR = thioredoxin glutathione reductase
Tpx = tryparedoxin
TR = trypanothione reductase
Trx = thioredoxin
TrxR = thioredoxin reductase
TryS = trypanothione synthetase
TS_2 = trypanothione disulfide
UMSBP = universal minicircle sequence-binding protein
γ-GC = γ-glutamylcysteine

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1. Leopold Flohé. 2012. The trypanothione system and its implications in the therapy of trypanosomatid diseases. *International Journal of Medical Microbiology* **302**:4-5, 216-220. [CrossRef]
2. Marta Sousa Silva, António E.N. Ferreira, Ricardo Gomes, Ana M. Tomás, Ana Ponces Freire, Carlos Cordeiro. 2012. The glyoxalase pathway in protozoan parasites. *International Journal of Medical Microbiology* **302**:4-5, 225-229. [CrossRef]
3. Viridiana Olin-Sandoval, Zabdi González-Chávez, Miriam Berzunza-Cruz, Ignacio Martínez, Ricardo Jasso-Chávez, Ingeborg Becker, Bertha Espinoza, Rafael Moreno-Sánchez, Emma Saavedra. 2012. Drug target validation of the trypanothione pathway enzymes through metabolic modelling. *FEBS Journal* **279**:10, 1811-1833. [CrossRef]