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Review

The thioredoxin system of *Plasmodium falciparum* and other parasites

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Abstract. Antioxidant defence plays a crucial role in rapidly growing and multiplying organisms, including parasites and tumor cells. Apart from reactive oxygen species (ROS) produced in endogenous reactions, parasites are usually exposed to high ROS concentrations imposed by the host immune system. The glutathione and thioredoxin systems represent the two major antioxidant defence lines in most eukaryotes and prokaryotes. Trypanosomatids, however, are characterized by their unique trypanothione system. These systems are NADPH-dependent and based on the catalytic activity of the flavoenzymes glutathione reductase, trypanothione reductase and thioredoxin reductase (TrxR), respectively. TrxR reduces the 12-kDa protein thioredoxin (Trx), which in turn provides electrons to ribonucleotide reductase, thioredoxin peroxidases (TPxs), certain transcription factors and other target molecules. Comparing the thioredoxin systems of different parasites and their respective host cells enhances our understanding of parasite biology and evolution, of parasite-host interactions and mechanisms of drug resistance. It furthermore opens avenues for the development of novel antiparasitic compounds. Here we review the current knowledge on the Trx systems of eukaryotic parasites, finally focusing on the malarial parasite *Plasmodium falciparum*.

Key words. Thioredoxin; thioredoxin reductase; malaria; peroxiredoxin; *Plasmodium falciparum.*

Introduction

Oxidative stress

Toxic derivatives of oxygen and nitrogen, like hydrogen peroxide, nitric oxide, superoxide radicals, peroxynitrite and hydroxyl radicals, are continuously generated in living organisms. These so called reactive oxygen species (ROS) or reactive nitrogen species (RNS) are produced as a by-product in cell respiration, as defence agents against infections, during detoxification of xenobiotics and by ultraviolet (UV)-radiation. ROS and NOS directly or indirectly damage biological macromolecules. Oxidation of proteins can impair enzyme function and induce false cross-linking, lipid peroxidation disturbs membrane integrity, and oxidative DNA damage can impair protein synthesis and cell division. Against this oxidative stress as a whole but also against individual ROS, a number of antioxidant defence systems have evolved which include antioxidant enzymes and low molecular weight antioxidants [1].

Many parasites multiply rapidly in an environment of high oxygen tension. The blood stages of the malarial parasite *Plasmodium falciparum*, e.g. produce up to 16 merozoites within 48 h [2]. This is impressively reflected by the fact that glucose uptake and utilization of a parasitized erythrocyte is increased by a factor of 100 when compared with noninfected cells. Furthermore, malarial parasites degrade and metabolize 60–80% of their host

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cells' hemoglobin [3], a process that takes place in an acidic digestive vacuole. During proteolysis superoxide radicals and heme (as hemin) are released and must be detoxified – O_2^- by dismutation and heme by polymerization and by reaction with reduced glutathione, respectively [4].

The glutathione system

The glutathione and thioredoxin (Trx) systems represent the two cornerstones of cellular antioxidant defence of both parasite and host cell compartment [5]. The cysteine-containing tripeptide glutathione is kept in the reduced state by glutathione reductase (GR), a flavoenzyme which catalyses the reaction GSSG + NADPH + $H^+ \rightarrow$ 2 GSH + $NADP^+$. *P. falciparum* GR has been cloned [6], and the recombinant protein as well as the enzyme purified from malarial parasites have been characterized in detail and shown to be inhibited by the antimalarial agent methylene blue in therapeutic concentrations [6–8]. A number of other glutathione-dependent proteins contribute to essential cellular functions, including glyoxalases, glutathione peroxidases (GPxs), possibly an *S*-nitrosoglutathione reductase, glutaredoxin (Grx) and glutathione-*S*-transferases [1, 9–13]. Glucose-6-phosphate dehydrogenase [14] and glutamate dehydrogenase as major sources of NADPH also indirectly contribute to antioxidant defence [14].

The Trx system

In parallel to the glutathione system, the Trx system consists of NADPH, thioredoxin reductase (TrxR), and further proteins, enzymes and metabolites that depend on reduced Trx. Trxs are a group of small (~12 kDa) redox-active proteins belonging to the Trx superfamily, of which Grx, tryparedoxin and protein disulfide isomerase are further members [9]. All members of this family show a similar structure, the 'Trx or Grx fold', which consists of a central four-stranded β sheet surrounded by α helices. As shown for yeast, at least one out of four Trx and Grx genes has to be present for viability [15]. Classical Trxs have a typical active-site motif with two conserved cysteine residues (Cys-Gly-Pro-Cys) (fig. 1); typical Grxs are characterized by the sequence Cys-Pro-Tyr-Cys [9, 16]. Many organisms, including *Escherichia coli*, yeast and humans have more than one Trx; often a second mitochondrial Trx/TrxR system exists in parallel to the cytosolic system [17, 18].

Trx contributes to a range of essential cellular functions. It is an important redox active protein that protects against oxidative damage. It supplies reducing equivalents to enzymes such as ribonucleotide reductase and thioredoxin peroxidase (TPx), but it also reduces cysteine residues in other proteins, including certain transcription factors, which results in their increased binding to DNA [9, 16, 19]. Mammalian Trxs have also been shown to function as cellular growth factors, to inhibit apoptosis and to be highly expressed and secreted by certain tumor cells. Furthermore, extracellular Trx plays a role in inflammatory joint diseases [16, 20–24]. The first – of only a few – parasitic Trxs was discovered in *Echinococcus granulosus* [25].

TrxR

Trx is reduced by the NADPH-dependent flavoenzyme TrxR. TrxR belongs to a family of pyridine dinucleotide oxidoreductases that further includes GR, trypanothione reductase, mercuric reductase, NADH peroxidase, lipoamide dehydrogenase, rubredoxin reductase and adrenodoxin reductase [26, 27]. High molecular weight (55 kDa per subunit) TrxRs occur in mammalian cells, *Drosophila melanogaster*, *Caenorhabditis elegans* and in malarial parasites [28–32]. They are structurally and mechanistically closely related to GR but differ significantly from low molecular weight (35 kDa per subunit) bacterial TrxRs [27, 33]. A major feature distinguishing large TrxRs from GR is an additional C-terminal redox center (figs 2, 3) which is essentially involved in catalysis and consists of a cysteine-selenocysteine sequence in mammalian TrxRs, of a Cys-Cys sequence in *Drosophila* and of a Cys-Cys pair interrupted by four amino acids in *P. falciparum* [16, 27, 28, 31, 32, 34, 35]. In contrast to GR, which specifically reduces glutathione disulfide (GSSG), large TrxRs have a broad substrate spectrum, including low molecular weight compounds such as 5,5′-dithio-bis (2-nitrobenzoate) (DTNB) as well as proteins. GSSG, however, is not accepted as substrate by TrxRs. As recently demonstrated, *D. melanogaster*, the malaria vector *Anopheles gambiae*, and probably also other insects lack a genuine GR. In these organisms GSSG reduction is likely to be maintained by a chemical reaction between GSSG and reduced Trx, underlining the importance of the Trx system [28]. In certain metabolic situations this reaction can play a role also in GR-containing species, including *P. falciparum* [31].

TPxs

Peroxiredoxins (Prxs) form a recently discovered and ubiquitously distributed family of antioxidant enzymes that act as peroxidases by reducing hydrogen peroxide and organic hydroperoxides to water or the corresponding alcohol [36, 37]. In contrast to many other peroxidases, the function of Prxs does not depend on redox cofactors such as metals or prosthetic groups [38]. The Prx superfamily – which includes alkyl hydroperoxidases and tryparedoxin peroxidase [39] – was first discovered in yeast [19, 40]. Later, these antioxidant enzymes were

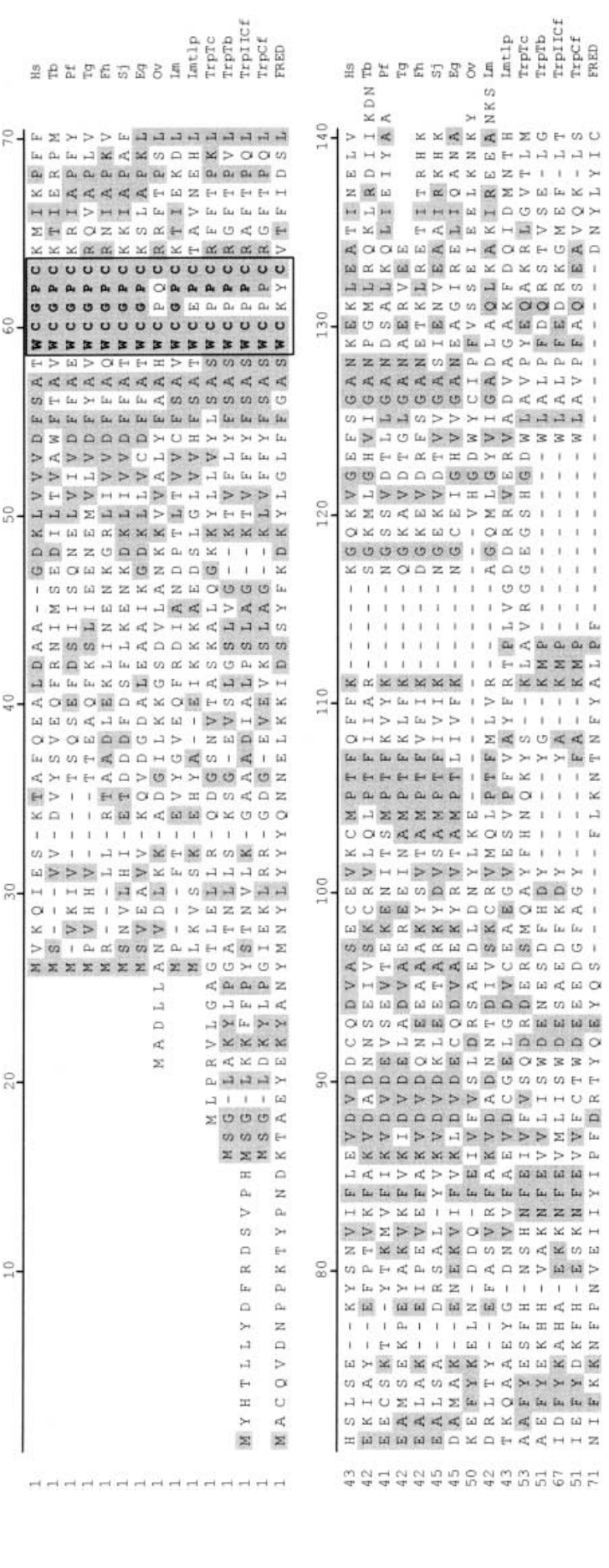

Figure 1. Parasitic Trxs and tryparedoxins. Human Trx-1 (as a host protein) is included. The sources of the Trxs are Hs, human (JH0568); Tb, *Trypansoma brucei brucei* (CAB81781); Pf, *Plasmodium falciparum* (AAF34541); Tg, *Toxoplasma gondii* (BG657266); Fh, *Fasciola hepatica* (AAF14217); Sj, *Schistosoma japonicum* (AAD52699); Eg, *Echinococcus granulosus* (AAC14584); Ov, *Onchocerca volvulus* (AI130160); Lm, *Leishmania major* (AAC24637); Lmtlp, Trx-like protein of *L. major* (AAG10802); TrpTc, tryparedoxin of *Trypanosoma cruzi* (AAF04973); TrpTb, tryparedoxin of *T. brucei* (CAA07003); TrpCf, tryparedoxin of *Crithidia fasciculata* (AAC72299); TrpIICf, tryparedoxinII of *C. fasciculata* (AAC61984); Fred, Trx-like redox-active protein of *P. falciparum* (AAF87222). In the NCBI database, a second Trx protein sequence of *F. hepatica* was found which, however, differs from the first Trx in only two amino acids (V3L and A5R). Incomplete or atypical Trx-like sequences have been detected in *L. major*, *Trichinella spiralis* and *Eimeria tenella*.

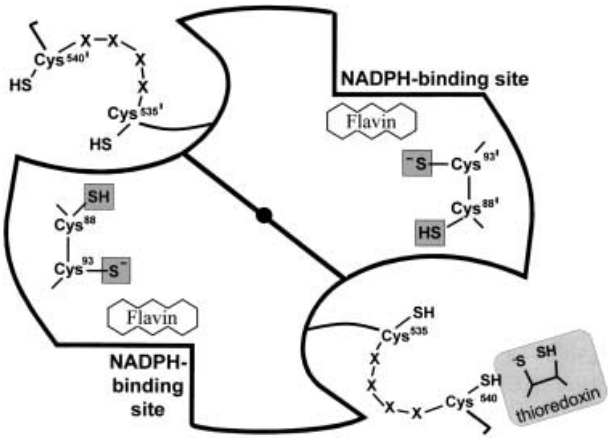

Figure 2. Sketch of homodimeric *P. falciparum* TrxR (after Holger Bauer, with kind permission). Electrons are transferred from NADPH to flavin adenine dinucleotide (FAD) and from there to the active-site disulfide, which is in redox communication with the C-terminal redox center of the other subunit. This second redox center is located on a flexible arm and donates reducing equivalents to the various substrates.

identified in a wide range of species, including helminths, protozoa, bacteria, fungi, vertebrates and plants [36, 41]. Prxs can be divided into two subgroups, the 1-Cys Prxs and the 2-Cys Prxs (figs 4a, b), depending on the presence of one or two conserved cysteine-containing motifs [19, 36, 37]. 2-Cys Prxs use electrons provided by the small protein Trx and were thus also named TPxs (formerly called thiol-specific antioxidants) [36, 42]. As the first exception to this rule, recently a 1-Cys Prx with TPx activity was reported in yeast [43]. Other 1-Cys Prxs characterized so far proved to accept glutathione as reducing substrate [44–46]. An adequate classification of the novel and heterogeneous class of Prxs remains to be established (for review see [47]).

In this review article we summarize the present knowledge of the Trx systems of different eukaryotic parasites. The article focuses on the major components of the system, namely Trx, TrxR and TPxs. We will furthermore concentrate on those genes and proteins which have been published or at least annotated, and are therefore accessible in the databases. However, we should like to emphasize that the genome projects presently carried out on parasites offer unique opportunities to discover further proteins involved in Trx metabolism.

Anaerobic Diplomonadea

Giardia

The anaerobic protozoan parasite *Giardia duodenalis* is reported to possess a soluble dimeric FAD containing NADPH-dependent disulfide reductase consisting of two 35-kDa subunits and a partially purified 12-kDa protein, a putative Trx, which enhances disulfide reductase activity sixfold [48]. The reductase was shown to cleave cystine, oxidized glutathione and DTNB. Primary structure and biochemical properties place the enzyme into the class of small TrxRs present in bacteria and some lower eukaryotes, such as *Streptomyces clavuligerus* [49] and *Penicillium chrysogenum* [50]. *G. duodenalis* lacks mitochondria, superoxide dismutase, catalase, reduced glutathione – cysteine is the major low-molecular mass thiol – and glutathione-dependent peroxidase (GPx), as well as GR activities. Therefore, the putative Trx-like system of *Giardia* is thought to constitute a major part of the antioxidant defence in this organism. Furthermore, within *Giardia* a sequence with homology to TPx has been detected (see fig. 4); however, no further literature on this protein is available. The identification of this putative TPx is in good agreement with the presence of a putative Trx and a TrxR in *Giardia*. Therefore, this species is likely to have a complete Trx system consisting of NADPH, Trx, a low molecular weight TrxR indicating a close relation to bacteria, and a TPx.

Several biochemical and molecular characteristics of *Giardia* led to the proposal that the Metamonada (including *Giardia*) should be placed into a new superkingdom called Archezoa [51, 52], which bridges the empires of the Bacteria and the Eukaryota. The Trx system of *Giardia* as known so far supports this proposal [53].

Amoebozoa

Entamoeba histolytica

In contrast to *Giardia* the anaerobic protozoan parasite *Entamoeba histolytica* contains an iron-dependent superoxide dismutase, but also lacks detectable activities of catalase, glutathione transferase, GPx and GR. In 1995, a protein homologous to prokaryotic disulfide oxidoreductases was described [54]. This 34-kDa protein shows 38% amino acid sequence identity to TrxRs of bacteria. This first report of a small, bacterial-type TrxR in a eukaryote was followed by the detection of the first Prx (previously named thiol-specific antioxidant protein) in a pathogenic eukaryote, namely in *E. histolytica* [55]. The Trx dependency of this peroxidase was biochemically verified. Furthermore, a second TPx that differed in its N-terminal sequence from the first enzyme was described in the parasite (P19476).

The growth of the two ancient anaerobic protozoan parasites *Giardia* and *Entamoeba* was shown to be completely inhibited by the use of 30 μg/ml of allicin, a compound contained in freshly crushed garlic [56, for review see 57]. This antiparasitic effect is likely to be based on an interaction of allicin with thiol-containing proteins such as TrxR [57]. Since there is only a limited number of antioxidant systems operating in these organisms (GSH and

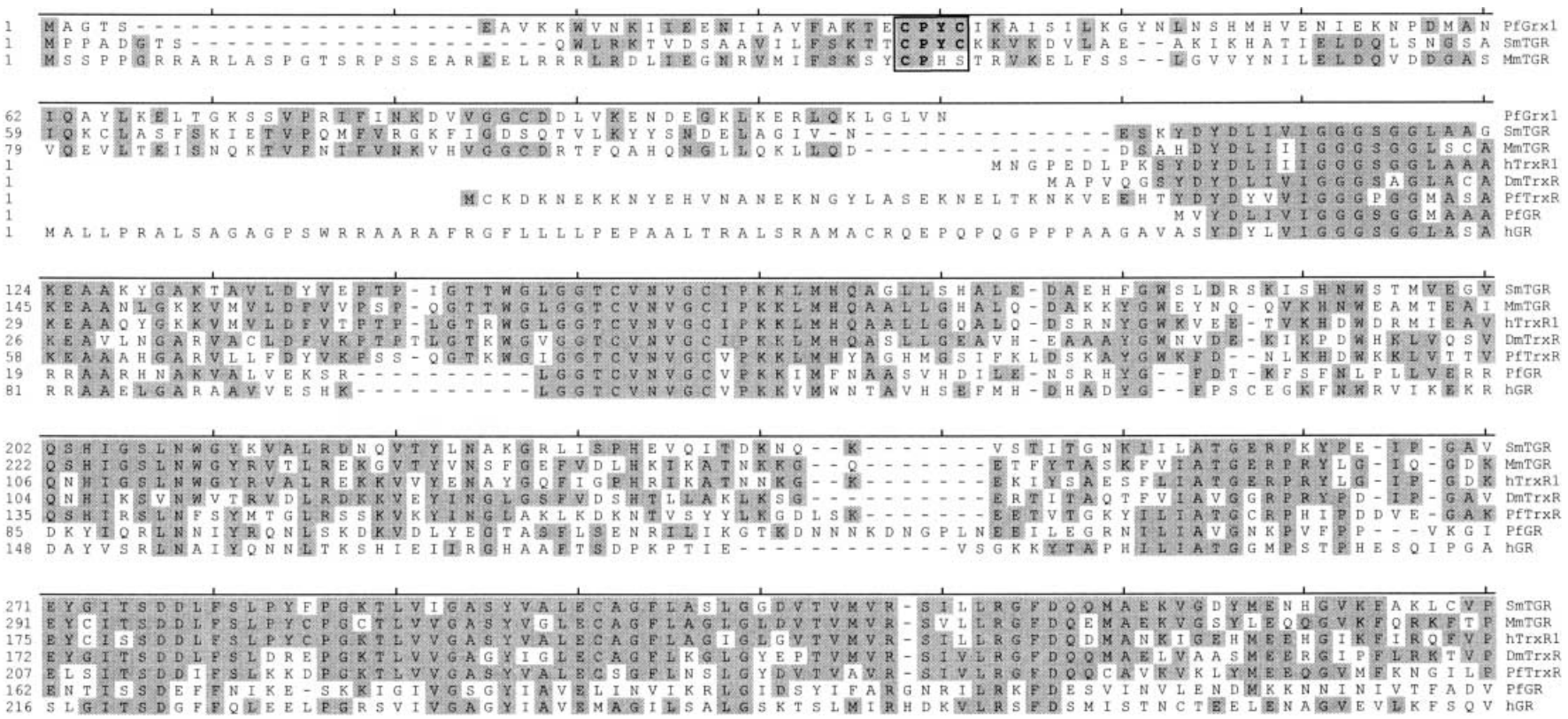

Figure 3. Alignment of different oxidoreductases (TrxRs and GRs). In addition to GRs and TrxRs, the two recently described TGRs, which reduce glutathione disulfide as well as Trx disulfide, are included in this alignment. Furthermore the *P. falciparum* Grx1 sequence is given to illustrate the similarity of the N-terminal region of TGRs with Grxs. PfGrx1, *P. falciparum* Grx-1 (AAK91589); MmTGR, mouse TGR (AAK31172); hTrxR1, human TrxR (S79851); SmTGR, *Schistosoma mansoni* TGR (AAK85233); DmTrxR1, *Drosophila melanogaster* TrxR (AF301144); PfTrxR, *P. falciparum* TrxR (CAA60574); PfGR, *P. falciparum* GR (CAA63747); hGR, mitochondrial human GR (XP_005119): the cytosolic enzyme starts at M44. *T. cruzi* trypanothione reductase (not shown) represents a further member of this enzyme family.

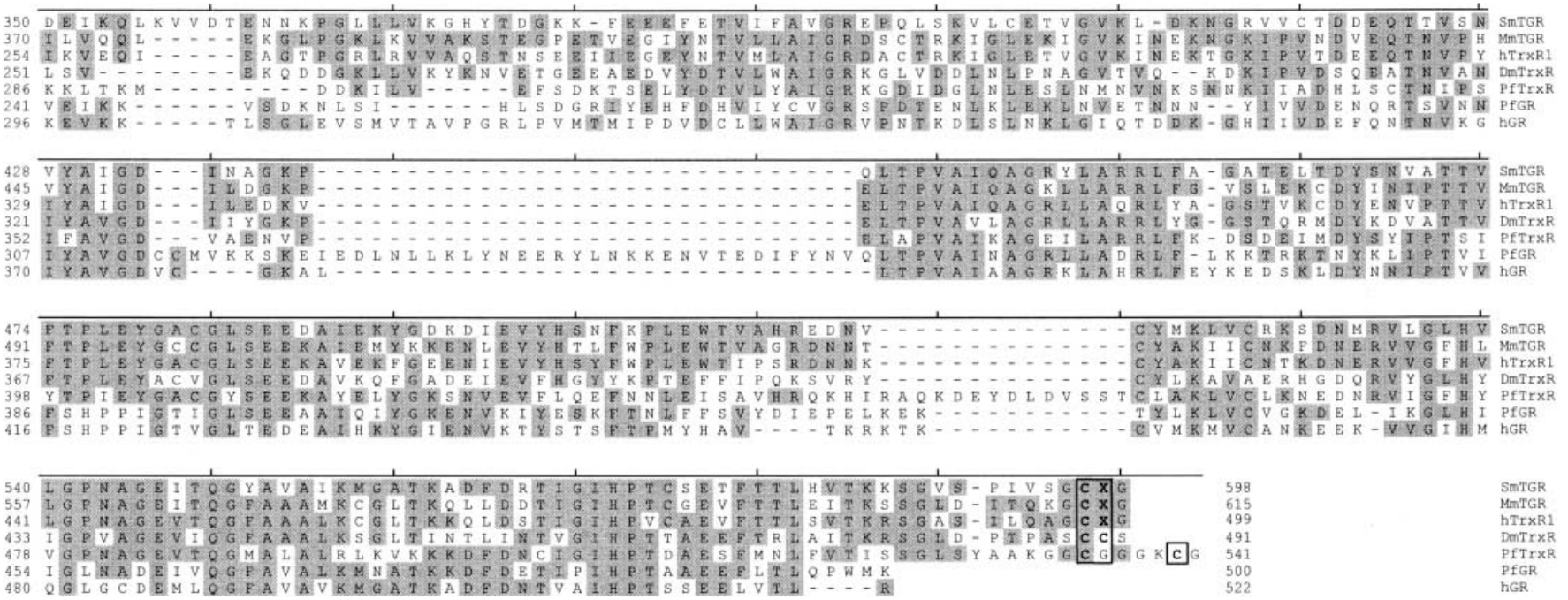

Figure 3 (continued)

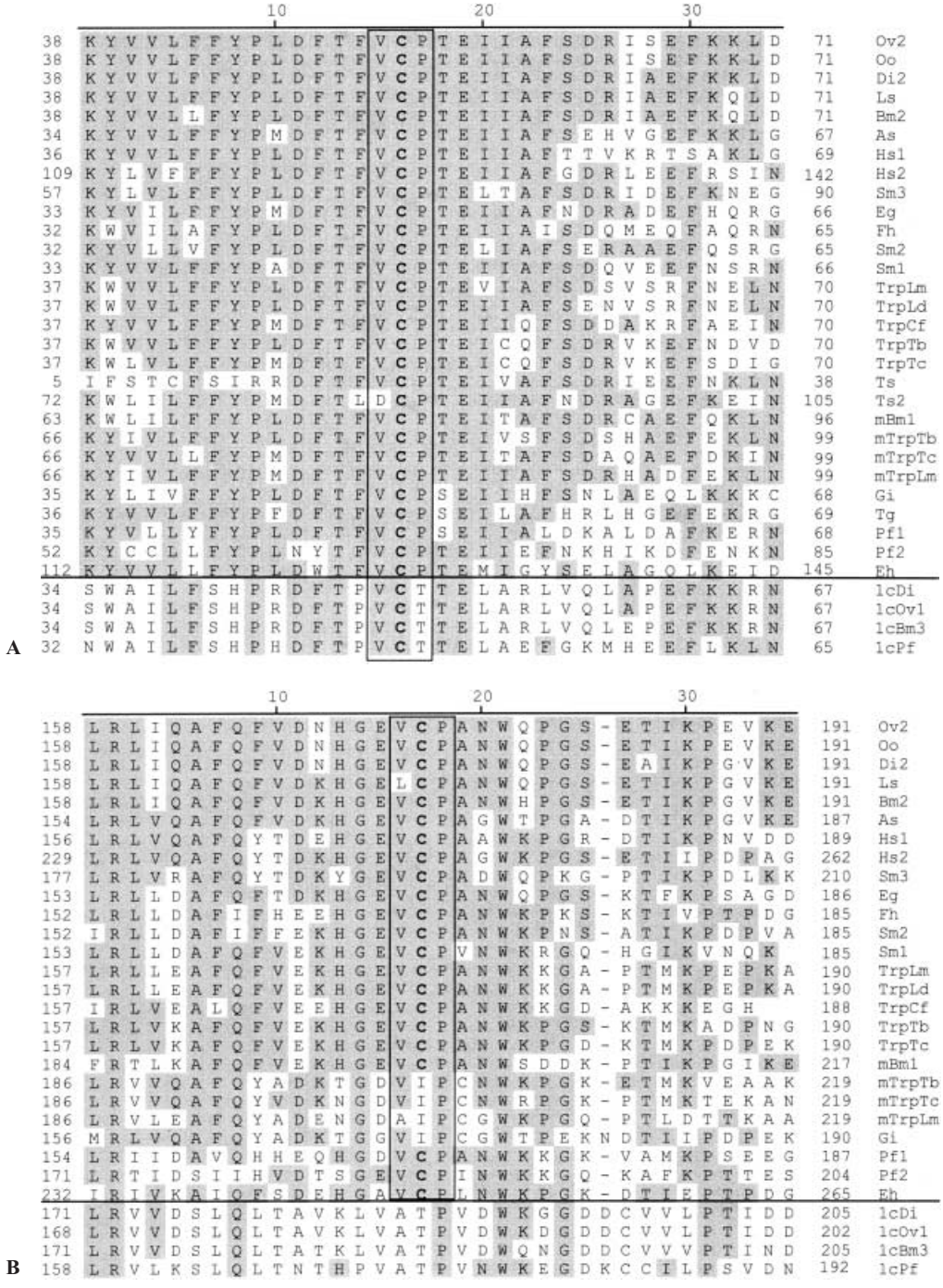

Figure 4. Alignment of parasitic Trx- and tryparedoxin peroxidases as Prxs. Panels *A* and *B* show the sequence area around the first (second) active-site cysteine. Two human TPxs are included. Hs1, TPx-1 of human (NM_005809); Hs2, TPx-2 of human (NM_006406); Pf1, TPx-1 of *P. falciparum* (AAF67110); Pf2, putative TPx-2 of *P. falciparum* (AAK20024); 1cPf, 1-Cys Prx of *P. falciparum* (BAA78369); Tg, TPx of *Toxoplasma gondii* (BG660585); As, TPx of *Ascaris suum* (BAA90476); Fh, TPx of *F. hepatica* (AAB71727); Gi, TPx of *Giardia intestinalis* (AAD51093); Sm2, TPx-2 of *S. mansoni* (AAD40685); Sm1, TPx-1 of *S. mansoni* (AAD17299); Sm3, TPx-3 of *S. mansoni* (AAG15506); Eg, TPx of *E. granulosus* (AAD02002 and BI244260; see below); Ts1, TPx-1 of *T. spiralis* (BG353012); Ts2, TPx-2 of *T. spiralis* (BG520721); Eh, thiol-specific antioxidant of *Entamoeba histolytica* (A43862); 1cDi, 1-Cys peroxiredoxin of *Dirofilaria immitis* (AAF21097); Di2, TPx-2 of *D. immitis* (AAC38831 and AF001007); 1cOv1TSA, 1-Cys peroxiredoxin-1 of *O. volvulus* (AAC27392); Ov2, TPx-2 of *O. volvulus* (AF029247 and AF043415); Oo, TPx of *Onchocerca ochengi* (AF068946); Ls, TPx of *Litomosoides sigmodontis* (AF105258); mBm1, mitochondrial TPx-1 of *Brugia malayi* (AAC23701); Bm2, TPx-2 of *B. malayi* (U47100); 1cBm3, 1-Cys Prx-3 of *B. malayi* (AAF21098); Tryparedoxin peroxidases: trpLm, *L. major* (AAC79432); mtrpLm, *L. major* (CAB58299); trpLd, *Leishmania donovani* (AAK00633); trpTc, *T. cruzi* (AAF04974); mtrpTc, *T. cruzi* (CAA06923); trpTb, *Trypanosoma brucei rhodesiense* (Q26695), sequence is identical to trp of *T. brucei brucei* (AAK69531); mtrpTb, *T. brucei* (AAG28496); trpCf, *C. fasciculata* (AAC15095). A second TPx nucleotide sequence of *A. suum* (BG733737) was found to lead to only one exchange (M5V); this second sequence arose from an EST clone and is likely to lack the 3′-terminal sequence. The *E. granulosus* TPx sequence is combined from two database entries. Two sequences of TPx of *T. spiralis* and one of *T. gondii* are likely to be incomplete.

related enzymes are missing), the loss of TrxR function has significant antiparasitic effects. The host organism as a higher eukaryote is, in contrast, less impaired.

Trematoda

Fasciola hepatica

F. hepatica is a hermaphroditic *Echinostomatia* that infects sheep, goats, cattle, and also humans. A clone encoding a putative Prx was isolated first from a complementary DNA (cDNA) library [58], heterologous expression in *E. coli* and functional analysis were performed later [59]. The 2-Cys Prx with a molecular mass of 21.7 kDa per subunit was expressed as glutathione S-transferase fusion protein and showed antioxidant properties in a metal-catalysed oxidation (MCO) assay [59]. Furthermore, immunological studies showed that the protein is able to form dimers and represents a component of the excretory-secretory extract. After the identification of a Trx in *F. hepatica* [60], the Trx dependency of this peroxidase was demonstrated.

Trx of *F. hepatica* has been identified as a tegument component in both the juvenile and the adult fluke [61]. Recombinant Trx was shown to stimulate bovine T cells specifically but weakly, and is therefore not a promising candidate for inducing resistance to *F. hepatica* [61]. FhTrx with a molecular mass of 11.4 kDa was recombinantly produced as a fusion protein in *E. coli* and proven to be biologically active [60]. Like the TPx of *F. hepatica*, FhTrx was shown to be part of the excretory-secretory extract. Until now, no catalase and only trace GPx activity have been described in the parasite [62, 63], pointing to an important role of the Trx system.

Schistosoma mansoni

Database information on this trematode indicates three different 2-Cys TPx genes; one of them (SmTPx1) – the first TPx reported in schistosomes – has been further characterized [64]. Recombinant SmTPx1 is enzymatically active and was found to have a Trx-dependent hydrogen peroxide activity of 4.5 U/mg of protein. TPx activity in adult worm homogenisates was 48 mU/mg of protein, compared with 47 mU/mg for GPx. Western blot analysis showed that TPx was expressed in both male and female worms. As shown by the same group, SmTPx1 is a T and B cell egg antigen in schistosome-infected mice [65]; both native and recombinant TPx1 stimulate a significant $CD4^+$ T cell proliferation in different mice strains. Western blot analysis showed that eggs have a higher level of TPx1 protein than both adult male and adult female worms, and that TPx1 is localized to the von Lichtenberg's envelope surrounding the miracidium. TPx1 is furthermore present in egg secretory products. Trx and TrxR messenger RNAs (mRNAs) were already detected in the parasite as well as TrxR protein in schistosome eggs (unpublished data, cited in [65]). These observations point to the presence of the complete Trx system in *Schistosoma mansoni*. The differential gene expression in different compartments and developmental stages of the parasite will be addressed in further studies.

Interestingly, for *S. mansoni* the gene of a chimeric protein consisting of a highly conserved TrxR sequence preceded by an (N-terminal) glutaredoxin domain has been described (see fig. 3). A protein of high similarity was recently reported in mouse and shown to reduce both Trx and glutathione at the expense of NADPH [66] (fig. 3). These chimeric enzymes, named TGRs, represent a new type of disulfide oxidoreductases, and bridge the Trx and the glutathione system. The TGR of both mouse and *S. mansoni* is likely to contain a catalytically active selenocysteine residue. Apart from TGR, a selenocysteine-containing GPx has been detected in *S. mansoni* [67–70]. To our knowledge, *Schistosoma* is the first parasite for which the existence of selenium-dependent enzymes has been demonstrated. For many other organisms, including mammals (e.g. man, rat, cattle and sheep) [71], prokaryotes (e.g. *E. coli, Clostridium barkeri*) [72], archaea (e.g. *Methanopyrus kandleri, Methanococcus jannaschii*) [73, 74], as well as the nematode *C. elegans* [29, 75], the importance of selenium in antioxidant defence is already established.

For *Schistosoma japonicum* a Trx sequence has been entered into the databases (see fig. 1) but no further data on the Trx system of this parasite are available.

Cestoda

Echinococcus granulosus

This helminthic parasite possesses a Cu/Zn-dependent superoxide dismutase, but no catalase or GPx activity has been detected so far [76, 77]. A Prx gene was, however, cloned from cDNA. The deduced amino acid sequence with a predicted molecular mass of 21.4 kDa has two conserved cysteine-containing motifs; therefore, the protein is likely to represent a member of the 2-Cys peroxidase family of Prxs [77]. Also a Trx gene was identified in a cDNA library of *Echinococcus granulosus* [25]. Recombinant protein was used to raise antibodies that clearly detected a 12-kDa band in protoscolex total protein extracts. The EgTrx was found to be active in the insulin assay. Southern and Northern blot analyses indicated the presence of two Trx genes or a splice variant.

Nematoda (for review see [78, 79])

Trichinella spiralis

On the basis of a nematode expressed sequence tag (EST) sequencing project, cDNAs coding for two proteins related to Trx were detected in muscle stage larvae (BG353677 and BG302300). The active-site motif typical for Trxs is, however, missing in both deduced proteins. In addition, two – probably incomplete – putative TPx sequences were detected (fig. 4). Whether they represent 1- or 2-Cys Prxs needs to be clarified. Since May 2001 there are three different partially overlapping cDNA sequences of a putative oxidoreductase in *Trichinella spiralis* available (BG520773, BG353264, BG438462). Although the highest homologies exist with TrxRs, it is at the moment not possible to further classify the respective enzyme.

Ascaris suum

Ascaris was shown to have a 22.6-kDa 2-Cys peroxidase member of the Prx family [80]. A general peroxidase activity of this protein was demonstrated by using the MCO system, but no further biochemical data, like dependency upon Trx, are presently available. The enzyme is expressed at all life stages of the parasite, and immunological analyses suggest the presence of two different proteins reacting with anti-AsPrx serum.

At this point it should be mentioned that in some reports 2-Cys Prxs are classified as TPx2 (although there might not exist a TPx1) because of their two conserved cysteine-containing sequence motifs. Other authors name the first Prx identified in an organism TPx1 and the second Prx (although it might be a 1-Cys Prx) TPx2. This heterogeneous use of nomenclature – as found in the filarial nematodes *Brugia malayi*, *D. immitis*, and *O. volvulus* (for review see [79]) – requires a standardization in analogy to Table 1.

Brugia malayi

Apart from a non-selenium-dependent GPx, in this human filarial nematode three different Prxs have been described: a 1-Cys peroxidase and two 2-Cys peroxidases, one of them carrying a putative mitochondrial target sequence of 30 N-terminal amino acids [81]. This represents the first putative mitochondrial Prx described in parasitic nematodes. The respective gene, *mtpx1*, was found to be constitutively transcribed in all stages of the parasite present in the mammalian host. The gene was recombinantly expressed in *E. coli*, and antibodies were raised against the protein obtained. A 25-kDa translation product was found in parasitic extracts of all developmental stages studied. Immunohistochemical, immunofluorescent and immunoprecipitation studies showed that the peroxidase is localized in the cells of the hypodermis/lateral chord in adult parasites but not at the surface or in excretory/secretory products [81]. Antioxidant activity of recombinant mTPx1 was demonstrated by an MCO assay. The other 2-Cys Prx, Bm-TPx2, was detected in larval stages of *B. malayi* [82]. This enzyme may represent – in analogy to *O. volvulus* and *D. immitis* TPx2 (see below) – an isoenzyme exported to the surface or the excretory-secretory compartment [81]. No biochemical data are available on the 1-Cys Prx mentioned above.

Dirofilaria immitis

Two Prxs are known for this filarial parasite, which infects dogs and cats: a 1-Cys Prx and two 2-Cys Prxs which differ in six amino acids only. Therefore, only one of these two sequences is shown in the alignment (fig. 4), but both accession numbers are given in the legend.

The nucleic acid sequence of the 2-Cys peroxidase was isolated from a fourth-stage larval cDNA library and recombinantly expressed in *E. coli* [83]. The protein shows peroxidase activity by its ability to protect DNA from oxidative nicking in a metal-catalysed oxidation system. Polyclonal antibodies directed against the protein reacted with a 22-kDa protein in *D. immitis* larval and adult parasite extracts, and also in adult but not in larval excretory-secretory products.

The 1-Cys Prx [84] was also expressed in *E. coli*. A kinetic characterization revealed a K_M of 16.3 mM for H_2O_2 and a V_{max} of 16 µmol min^{-1} mg^{-1}under the experimental conditions chosen. Antibodies identified a 27-kDa antigen in parasite extracts, and larval as well as adult excretory-secretory products. The Prx was localized to the lateral hypodermal chords of both male and female worms and, in addition, to afibrillar muscle cells in male worms and some areas of the uterine wall in female worms. This 1-Cys Prx is the first parasite Prx to be shown to exogenously detoxify added H_2O_2 in an in vitro system with dithiothreitol (DTT) [84]. The fact that both peroxidases of *D. immitis* were detected in excretory-secretory products is surprising because neither protein contains a signal leader sequence, possibly pointing to other transport mechanisms [85–87]. Like *B. malayi*, *D. immitis* possesses a putative non-selenium-containing GPx, but also a glutathione-*S*-transferase and a superoxide dismutase [88–90].

Onchocerca volvulus

Also in this nematode two Prxs have been described: a 1-Cys Prx and two 2-Cys peroxidases which differ in only six amino acids. Therefore, only one of these two sequences is shown in the alignment (fig. 4), but both accession numbers are given in the legend. The 2-Cys peroxidase was found in an *O. volvulus* L3 larval cDNA library [91] in a content of 2.5%, indicating an upregu-

lated expression and an important role in vivo. The gene of this 2-Cys-TPx was expressed in *E. coli*, and the protein obtained was shown to have antioxidant activity in an MCO assay. Respective antibodies recognized a protein from both larval and adult worm crude extracts with a molecular weight of 22 kDa. In larvae, this protein was predominantly localized in the hypodermis and the cuticle, and in adult worms in the uterine epithelium and the intestine, which may indicate a secretion of this protein.

The 1-Cys Prx gene was isolated from an *O. volvulus* adult worm cDNA library. It has antioxidant activity in the MCO assay, and its localization in the lateral hypodermal chords of adult *O. volvulus* also suggests its secretion in vivo [92]. The same enzyme has been characterized by another group as an immunomodulatory protein in the serum of an onchocerciasis patient [93].

A putative Trx has been detected in a cDNA library of *O. volvulus* larvae. The respective protein has not yet been biochemically characterized and contains the atypical active site motif WCPQC instead of WCGPC for Trxs, or WCPYC for Grxs. However, the existence of the gene points to a complete Trx system in *O. volvulus* – and probably other filarial nematodes. In both *Onchocerca ochengi* and *Litomosoides sigmodontis,* a putative 2-Cys Prx sequence has been found (fig. 4).

Trypanosomatidae

Trypanosomes and Leishmania are the causative agents of severe tropical diseases, examples being African sleeping sickness (*Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*), Nagana cattle disease (*Trypanosoma congolense* and *Trypanosoma brucei brucei*), Chagas disease (*Trypanosoma cruzi*) and the three manifestations of leishmaniasis (*Leishmania donovani*, *Leishmania major, Leishmania mexicana).* All these parasitic protozoa have a thiol metabolism that completely differs from that of other eukaryotes and prokaryotes. They lack the glutathione reductase system as well as GPx and catalase[1]. Trypanothione [N^1,N^8-bis(glutathionyl)spermidine] and monoglutathionylspermidine are the main low molecular mass thiols [96, 97]. These glutathionylspermidine conjugates are kept in the reduced state by trypanothione reductase and NADPH. The dithiol trypanothione has been shown to be involved in the detoxification of hydroperoxides [39], homeostasis of ascorbate [98] as well as the synthesis of deoxyribonucleotides catalysed by ribonucleotide reductase [99]. Trypanothione reduces a major disulfide protein of trypanosomatids, namely tryparedoxin (M_r 16 kDa; intracellular concentration 5% or >100 µM). The cascade of reducing equivalents from NADPH via trypanothione reductase, trypanothione, tryparedoxin and tryparedoxin peroxidase was first discovered in the insect pathogen *Crithidia fasciculata* [100], but it is also present in the human pathogens *Trypanosoma cruzi* [101], *T. brucei* [99], *Leishmania major* [102] and – at least partially – in *L. donovani* (AAK00633). Enzymes of the trypanothione metabolism are attractive target molecules for the rational development of new antiparasitic drugs (for a recent review see [97]). The uniqueness of trypanothione metabolism and the failure to detect TrxR in trypanosomatids have led to the suggestion that these protozoa lack a Trx system [96]. Recently, the genome sequencing project of *L. major* revealed a sequence that probably codes for a Trx [103]. Based on this observation a gene encoding Trx from *T. brucei* was cloned, overexpressed and characterized as a classical Trx [104] – the first Trx of an organism belonging to the order Kinetoplastida. Phylogenetically, the Trxs of *T. brucei* and *L. major* form a new branch distinct from all other eukaryotic lineages, whereby the parasite proteins are more closely related to mammalian Trxs than those of yeasts and plants. In *L. major*, a second Trx-like protein (AAG10802) with assumed cytoplasmatic localization has been identified but not further characterized.

The Trxs mentioned above as well as *T. brucei, T. cruzi* and *C. fasciculata* I and II tryparedoxins with their typical active site motif – WCPPC – have been included in the Trx alignment (fig. 1).

Both *T. brucei* Trx and tryparedoxin [99, 104] catalyse the reduction of *T. brucei* ribonucleotide reductase by dithioerythrol (DTE) as efficiently as *E. coli* Trx does. Thus, most probably trypanosomes have developed two systems that provide electrons for the synthesis of DNA precursors, as it is the case in other organisms [9].

African trypanosomes change between three main life stages. In the blood of the mammalian host the parasites occur as dividing long slender and nondividing short stumpy forms. Upon a blood meal on an infected animal, the tsetse fly takes up parasites, and the short stumpy cells differentiate to procyclics, which multiply in the insect vector. The Trx gene is expressed in all three developmental stages of *T. brucei*. The occurrence of Trx mRNA in the nondividing short stumpy parasites may indicate that the protein is not only involved in deoxyribonucleotide synthesis but also serves additional purposes.

In trypanosomatids, a unique cascade composed of trypanothione reductase/trypanothione/tryparedoxin/tryparedoxin peroxidase has been shown to detoxify hydroperoxides [39, 41, 100, 105, 106]. The parasite peroxidase is a member of the Prx family of proteins (fig. 4). Thus future work will show whether tryparedoxin perox-

[1] The 'cysteine-containing GPx' of *T. cruzi* described in [94] has a very low specific activity, which might indicate that GSH itself is not the major reducing substrate [95].

idase also accepts electrons from the parasite Trx and how the dithiol form of Trx is subsequently regenerated [104]. *T. brucei* Trx is rather unique in having a calculated pI value as high as 8.5. The protein contains several arginine residues resulting in an overall positive charge. The protein is a substrate of human TrxR, but it is not reduced by trypanothione reductase, which suggests the existence of a TrxR or another reducing system [104]. Disruption of the *trx* gene in *T. brucei* is in progress and will reveal whether Trx is essential for the viability and virulence of the parasite[1].

Recently, the coexistence of a trypanothione reductase and a GR was reported in the non-trypanosomatid Euglenozoa *Euglena gracilis* [108], indicating a wider distribution of the trypanothione system. Furthermore, a novel tryparedoxin peroxidase with a mitochondrial target sequence was identified in *T. cruzi* [94], *T. brucei* [109] and in *L. major* (CAB58299) [110]. Interestingly, these mitochondrial tryparedoxin peroxidases – together with the Prx of *Giardia intestinalis* – show a deviation at the second active site. Instead of the typical VCP motif, they possess a V(A)IPC motif.

Coccidea

Cryptosporidium parvum

In this parasite a partial Trx cDNA sequence with a WCGPC active-site motif can be found in the databases (AQ003781). Furthermore, a partial disulfide reductase cDNA sequence has been entered (AA555349). Since this enzyme contains a C-terminally located putative redox center which consists of two cysteine residues interrupted by four additional amino acids, as described for *Plasmodium falciparum* TrxR [31], it is likely to represent a TrxR.

Toxoplasma gondii

A putative Trx sequence (fig. 1) with a fully conserved WCGPC active-site sequence as well as an (incomplete) TPx sequence were found using a tachyzoite cDNA library of a *Toxoplasma* EST-sequencing project. Although the sequence homologies around the first VCP motif indicate the protein to be a member of the 2-Cys family of Prxs, a clear assignment is not yet possible since the 3′-terminal part of the sequence with the second conserved cysteine is still missing.

[1] The methodology has been established for trypanothione reductase, which was shown to be an essential protein for *T. brucei* [107].

Haematozoa

Oxidative stress and antioxidant defense in *P. falciparum*

Tropical malaria is caused by infection with the protozoon *P. falciparum*. Currently, more than 2 million people die of malaria each year, and more than 300 million people become infected. These worrying numbers as well as the increasing resistance of the parasites against the currently available drugs explain the urgent demand for novel antimalarial drugs [111–113].

Apart from the parasites' high metabolic rate and the oxidative stress imposed on infected red cells by the host's immune system, hemoglobin degradation – a crucial process in intraerythrocytic parasite development – is an additional source of reactive oxygen species. During proteolytic degradation of up to 80% of the host cell's hemoglobin, released heme is normally oxidized to hemin and sequestered as the inert polymer hemozoin which is related to β-hematin, a polymer chemically prepared in vitro. By this process the oxidative toxicity of heme iron is masked. Two classes of drugs are likely to interact with heme to exert their antimalarial effect: peroxide antimalarials such as artemisinin and its derivatives are believed to be activated by heme resulting in the formation of alkylating free radicals [114]. Quinoline antimalarials such as quinine, chloroquine and mefloquine have been shown to inhibit β-hematin formation in vivo and in vitro [115–118]. It has been suggested, that a quinoline-heme complex is added to the growing polymer, terminating further chain extension [119].

Apart from heme polymerization, heme degradation either by glutathione [120, 121] or by a peroxidative process [122] has been described. Chloroquine and other quinolines were shown to inhibit both processes in vitro [122, 123].

Furthermore, individuals with inherited glucose-6-phosphate dehydrogenase deficiency are protected from severe malaria. Since G6PDH represents the major source of reducing equivalents for red cells, evolution also appears to have exploited oxidative stress as an important antimalarial principle. Taken together, parasite and host cell redox metabolism represent most promising targets for antiparasitic drug development.

P. falciparum possesses at least two NADPH-dependent disulfide-reducing systems, both of which are well characterized (fig. 5). The Trx system consists of NADPH, TrxR [30, 124–126], Trx [31, 127] and at least four TPxs. Three of these peroxidases belong to the Prx family, including one 1-Cys Prx and two 2-Cys Prxs [46, 128–130]. The fourth peroxidase belongs according to sequence alignments to the family of GPxs [11]; biochemical characterization, however, revealed a dependency on Trx [95]. A second member of this novel class of enzymes – GPx-like Tpxs – has been identified in

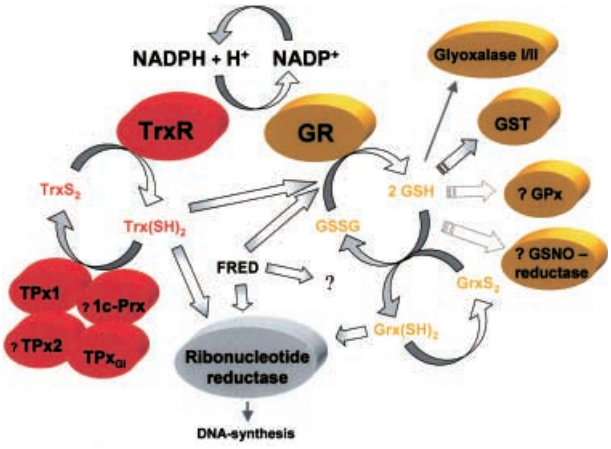

Figure 5. The Trx and the glutathione system of *P. falciparum*. The Trx system is shown in red, the glutathione/Grx system in orange. The Trx dependency of TPx2 as well as the presence of a GPx have yet to be verified. FRED (plasmoredoxin) is a Trx-like protein which is capable of reducing glutathione disulfide as well as ribonucleotide reductase [S. M. Kanzok et al., personal communication].

Drosophila melanogaster [F. Missirlis et al., personal communication].

In parallel, a functional glutathione system has been described in *P. falciparum*. It comprises NADPH, an FAD-dependent homodimeric GR [6–8, 131], glutathione, Grx [12], a glutathione-*S*-transferase with peroxidase activity (P. Harwaldt et al., unpublished), glyoxalase [10] and possibly GSNO-reductase (= formaldehyde dehydrogenase).

TrxR of *P. falciparum*

The *P. falciparum* TrxR gene was first described in 1995 [132]. Since, however, the high molecular weight TrxRs had not yet been recognized as a novel class of oxidoreductases [27], the sequence was originally interpreted as a GR, although striking differences at the substrate binding site were already noticed [132, 133]. One year later, heterologous expression and enzymatic characterization clearly demonstrated the Trx-reducing capacity of the protein [30]. The enzyme is a homodimeric, FAD-dependent oxidoreductase of 55-kDa subunit molecular mass. The functional amino acids at the active site of the enzyme including Cys88, Cys93 and His509 have been characterized by site-directed mutagenesis [124]; and, as for mammalian TrxRs [16, 27, 34, 134], the role of the C-terminal redox center in catalysis has been proven [125], and its interaction with the active site disulfide-dithiol has been studied in detail [27, 126, 135].

Further members of the high M_r TrxR family have been described in *C. elegans* [29], *D. melanogaster* and the malarial vector *A. gambiae* [28]. Interestingly, the enzymes of this family differ in their C-terminal sequences. Mammalian TrxRs, the TGR of *S. mansoni* mentioned above as well as one out of two *C. elegans* TrxRs [29], contain a Cys-Sec pair as an additional redox center. If this selenocysteine is mutated to a cysteine – as demonstrated for the mammalian enzyme – only 1% of the original activity is left [16]. *D. melanogaster* and a second TrxR from *C. elegans* (CAA77459, annotated as a GR!) have a Cys-Cys pair and – as shown for DmTrxR – exhibit rather high specific activities [28]. *P. falciparum* TrxR is characterized by a CGGGKC motif, which has also been detected in a sequence fragment of a putative TrxR from *Cryptosporidium parvum*. The different structural and functional properties of the three types of C-terminal redox motifs in large TrxRs (fig. 3) will have to be studied in further detail. However, the fact that PfTrxR – and probably also other parasitic TrxRs – is non-selenium dependent, in contrast to mammalian TrxRs, represents a good starting point for the development of antiparasitic drugs directed against this redox active enzyme [5, 22, 27, 32, 34].

The heterologous expression of PfTrxR in bacteria has been optimized by multiple silent mutagenesis of the gene [31]. The recombinant protein shows activity with DTNB (K_M = 465 µM), NADPH (K_M = 2.8 µM) and PfTrx-1 (K_M = 10.4 µM). Glutathione disulfide is not accepted as substrate. For high-throughput screening of inhibitors directed against PfTrxR, 5,5′-dithiobis(2-nitrobenzamides) have been synthesized and established as alternative substrates of the enzymes [136].

S-nitrosoglutathione represents an important transport form of nitric oxide (NO) in biological systems, and NO is likely to be involved in the pathophysiology of cerebral malaria. GSNO has furthermore been shown to be an inhibitor of GR [137] and a substrate of the mammalian selenocysteine-containing Trx system [138]. GSNO was also found to be a substrate of PfTrxR and is furthermore reduced by PfTrx$(SH)_2$ in a chemical reaction. The reduction of GSSG by Trx will be described in the next section [31].

Trx of *P. falciparum*

The complete Trx system of *P. falciparum* including also Trx has recently been described [31]. The thioredoxin gene was cloned and heterologously expressed in *E. coli*. The recombinant protein has a molecular mass of 11.7 kDa, possesses the classical active site motif WCGPCK and – in addition to both these active-site cysteines – one more cysteine at position 43. Homology modelling resulted in a characteristic thioredoxin fold. The highest degree of protein sequence identity was found with thioredoxin of *Schizosaccharomyces pombe* (51%), 38% and 31% identity could be found with human Trx1 and *E. coli* Trx1, respectively. Biochemical characterization of PfTrx-1 with PfTrxR revealed a K_M of 10.4 µM and a V_{max} of 50.8 U/mg enzyme [31]. This resulted in a k_{cat}/K_M ratio

of 5.0×10^6 M^{-1} s^{-1}. Interestingly, this catalytic efficacy is about five times higher with the host enzyme, hTrxR, which suggests that *P. falciparum* may use host TrxR for parasite-related functions at a certain stage of its life cycle.

As shown by site-directed mutagenesis [127], Cys43 might contribute to dimer formation of PfTrx. Cys43 as well as the active-site residues Cys30 and Cys33 were shown to be accessible to modification by DTNB. Interestingly, the N-terminal active site cysteine (Cys30) is less reactive than Cys33, suggesting a reaction mechanism of PfTrx that differs from other known thioredoxins.

Over the last months the genes of three additional Trx-like proteins have been detected in *P. falciparum* and are presently under investigation [C. Nickel, S. Rahlfs and K. Becker, unpublished].

TPxs of *P. falciparum*

Based on its sequence homologies with GPxs, the first peroxidase identified in *P. falciparum* was initially classified as a GPx [11] with a putative selenium dependence [139]. As recently demonstrated, PfTrx-1 is a better substrate than GSH. However, the enzyme is not a typical member of the Prx family [95]. To distinguish between this TPx and other peroxidases found in *P. falciparum*, we suggest to name it TPx_{Gl} – GPx-like TPx (for our suggestions on the nomenclature of *P. falciparum* peroxidases see table 1). The monomeric enzyme of 19.7 kDa has been recombinantly produced in *E. coli*. As a GPx homologue in which selenocysteine is replaced by cysteine, its reactions with hydroperoxides and GSH are three orders of magnitude lower than those of typical selenoperoxidases. With PfTrx it is reacting much faster and displays ping-pong kinetics with the tested substrates H_2O_2, cumene and *t*-butyl hydroperoxide [95]. The existence of a genuine GPx in *P. falciparum* remains to be verified.

The first Prx described for *P. falciparum* – and for protozoan parasites in general – is a 1-Cys Prx that exhibits the typical active-site motif PVCT (fig. 4) [46]. The respective gene was recombinantly expressed in *E. coli,* which yielded a 25.2-kDa protein. This size is in good agreement with other 1-Cys Prxs which are slightly larger in size than 2-Cys Prx (~22 kDa). The enzyme is reduced by low M_r thiols such as DTT or GSH when studied in the Prx assay. With immunochemical methods, a 25-kDa band was detected for *P. falciparum* late trophozoites (~0.5% of total protein) but also for ring stages. A highly homologous 1-Cys Prx gene (92% amino acid identity) was cloned and expressed by another group [129]. As indicated by that study, the enzyme is active with reduced Trx and H_2O_2 as substrates, but activity with alkyl hydroperoxides was not observed. As indicated by the differing results of the two groups, the specific thiol dependency of this 1-Cys-Prx remains to be studied in further detail. By Western blotting the protein was identified in all stages of *P. falciparum,* with strongest signals in old trophozoites and schizonts.

Table 1. Suggested nomenclature for *P. falciparum* peroxidases.

Nomenclature suggested in this review	$PfTPx_{Gl}$	Pf1-Cys-Prx	PfTPx-1	PfTPx-2
Nomenclature ref.				
11	PfGPx	–	–	–
95	PfTPx	–	–	–
46	–	PfTPx1	–	–
130	–	–	–	–
128	–	–	PfTPx1	PfTPx2
129	–	1-Cys Px	PfPrx2	–
130	–	Pf-Px2	PfPrx1	–

The first-characterized Prx of the 2-Cys family [128] has the two typical conserved VCP regions [36] and a molecular mass of 21.8 kDa. It was recombinantly expressed in *E. coli* and has antioxidant activity in the MCO assay. The enzyme was clearly shown to be Trx dependent, with a K_M of 4 µM for PfTrx-1. It accepts H_2O_2, *t*-butylhydroperoxide and cumene hydroperoxide as substrate, with k_{cat} values of 67, 56 and 41 min^{-1}, respectively, in the presence of 10 µM Trx and 200 µM peroxide substrate. As described for many other Prxs, PfTPx1 does not follow saturation kinetics. Furthermore, in oxidizing milieu the protein is converted to another protein species that migrates faster in SDS gel electrophoresis [128].

In parallel, PfTPx-1 was studied by two other groups and named PfPrx2 [129] and PfPrx1, respectively [130]. In this paper we will continue using TPx1. The enzyme as well as the 1-Cys Prx are reported to possess the peroxisomal targeting sequences SKL and SSL, respectively, at their C-terminus [129]. This fact might point to the existence of peroxisome-like organelles in *Plasmodium* – a matter that has been intensively discussed in recent years. The Trx dependency of TPx-1 was confirmed by Müller's group using H_2O_2 as substrate; however, in contrast to other studies, no activity with alkyl hydroperoxides was observed. A third group showed by Southern blot analysis that the PfTPx-1 gene is a single-copy gene [130]. Under nonreducing conditions, the recombinantly produced protein as well as the authentic protein in parasite extracts occur in dimeric form and dissociate into monomers when dithiothreitol is added. Furthermore, PfTPx1 was identified in *P. falciparum* ring and late trophozoite stages by Western blotting [130].

A second member of the 2-Cys Prx family, PfTPx-2, was identified in *P. falciparum* [128]. The molecular mass of this protein is 24.7 kDa, and recombinantly produced protein shows only slight activity in the MCO assay and in coupled Trx-dependent peroxidase assays. The protein

which contains a putative mitochondrial targeting sequence and, in addition to the active site residues, several cysteine residues will be studied in further detail.

Interactions between the Trx and the glutathione system

When characterizing the Trx system of *P. falciparum*, we observed that glutathione disulfide can be reduced in the presence of NADPH, PfTrxR and PfTrx. Since PfTrxR does not accept GSSG as a substrate, it seemed likely that Trx acted as an electron shuttle between reduced TrxR and GSSG. This hypothesis was proven, and the PfTrx system was shown to support GSSG fluxes up to 200 µM/min even at the low temperature of 25 °C [31]. This activity is based on a chemical reaction between Trx and GSSG and might be relevant for stages of *P. falciparum*, such as the merozoites, which contain glutathione but practically no GR [7]. The GSSG reduction by Trx is not restricted to *P. falciparum* but was also demonstrated for the Trx systems of humans, *E. coli*, *D. melanogaster,* and *A. gambiae* [28]. The functional importance of this reaction is supported by the fact that not all glutathione-containing organisms have a genuine GR, a case in point being the fruit fly *D. melanogaster* [28].

As mentioned above, very recently enzymes – namely the TGR of mouse and *S. mansoni* – have been detected that reduce both Trx and glutathione [66]. Furthermore, the presence of a TPx_{GI} which operates with GSH but has a preference for Trx [95] and a 1-Cys Prx with GPx activity [45], point to multiple and complex interactions between the glutathione and the Trx system (fig. 5).

Both Trx and Grx can reduce ribonucleotide reductase. A typical Grx as well as a second Grx-like protein have been found in *P. falciparum* [12]. This is the first report on a Grx in parasites. Until now, only incomplete Grx sequences were described for *O. volvulus* (AI096116), *T. brucei* (CAB95453; this protein lacks the classical active site CPYC and has a CQFC motif instead), *T. cruzi* (AI043269; sequence related to Trx or Grx, without a CxxC motif) and *L. major* (CAB89595 and AAC24623; active sites are missing). Furthermore, in *T. cruzi* a 52-kDa protein has been studied which contains the Grx active-site motif CPYC and is reported to catalyse thiol-disulfide exchange [140]. Like *E. coli* Grx2, which was shown to possess structural similarities with mammalian glutathione *S*-transferases [141], this *T. cruzi* 52-kDa protein has sequence homologies with glutathione *S*-transferases [142].

Conclusions

In most parasites studied so far, antioxidant defence and ribonucleotide reduction are essentially supported by the Trx system, a notable exception being the trypanosomatids. Trx and Trx-dependent metabolism are most completely understood in *P. falciparum*. However, for many other parasites Trx, TrxR, and Prxs have been described. The importance of the Trx system is underlined by the fact that some organisms lack GR, catalase or GPx. TPx has been shown to be expressed at levels comparable to those of other antioxidant proteins in *S. mansoni* [64]. In other parasites, TPx has been found in secretory and excretory products [58, 143], located to the surface of parasites [91, 144], and reported as a target of host immune responses [83, 145–147]. A therapeutic and prophylactic potential of targeting the Trx system can be deduced from the fact that immunization with TPx has conferred protective immune responses against *L. major* [143] and *E. histolytica* [148], and that selective disruption of the TPx gene in *Saccharomyces cerevisae* resulted in a mutant strain with enhanced sensitivity to oxidative stress [149]. Furthermore, helminthic parasites are likely to adapt to oxidative stress by synthesizing high levels of antioxidant enzymes and by expressing them at the host-parasite interface [77, 150, 151].

The most promising intervention targets are obviously the parasitic TrxRs because they differ from the human isofunctional enzymes. *P. falciparum*, for instance, has a high M_r TrxR like the human selenoenzyme, but its C-terminal redox center contains a dithiol instead of the selenol/thiol ensemble. Among other distinctions, the difference between sulfur and selenium chemistry can be exploited for developing parasite-enzyme specific inhibitors as potential antimalarial drugs [32]. Other parasites like amoeba and *Giardia* species possess bacterial-type TrxRs that differ completely in M_r, domain structure and catalytic mechanism from human TrxRs. These enzymes indeed fulfil most criteria of ideal drug targets [27, 32]. Our further understanding of the redox metabolism of parasites and their hosts will open additional avenues for the development of antiparasite strategies.

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1 Halliwell B. (1999) Antioxidant defence mechanisms: from the beginning to the end (of the beginning). Free Radic. Res. **31:** 261–272
2 Atamna H. and Ginsburg H. (1993) Origin of reactive oxygen species in erythrocytes infected with *Plasmodium falciparum*. Mol. Biochem. Parasitol. **61:** 231–241
3 Francis S. E., Sullivan D. J. Jr and Goldberg D. E. (1997) Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. Annu. Rev. Microbiol. **51:** 97–123
4 Famin O., Krugliak M. and Ginsburg H. (1999) Kinetics of inhibition of glutathione-mediated degradation of ferriprotoporphyrin IX by antimalarial drugs. Biochem. Pharmacol. **58:** 59–68
5 Schirmer R. H., Müller J. G. and Krauth-Siegel R. L. (1995) Disulfide-reductase inhibitors as chemotherapeutic agents:

the design of drugs for trypanosomiasis and malaria. Angew. Chem. Int. Ed. Engl. **34:** 141–154
6 Färber P. M., Becker K., Müller S., Schirmer R. H. and Franklin R. M. (1996) Molecular cloning and characterization of a putative glutathione reductase gene, the PfGR2 gene, from *Plasmodium falciparum*. Eur. J. Biochem. **239:** 655–661
7 Krauth-Siegel R. L., Müller J. G., Lottspeich F. and Schirmer R. H. (1996) Glutathione reductase and glutamate dehydrogenase of *Plasmodium falciparum*, the causative agent of tropical malaria. Eur. J. Biochem. **235:** 345–350
8 Färber P. M., Arscott L. D., Williams C. H. Jr, Becker K. and Schirmer R. H. (1998) Recombinant *Plasmodium falciparum* glutathione reductase is inhibited by the antimalarial dye methylene blue. FEBS Lett. **422:** 311–314
9 Holmgren A. (2000) Antioxidant function of thioredoxin and glutaredoxin. Antioxid. Redox. Signal. **2:** 811–820
10 Thornalley P. J., Strath M. and Wilson R. J. (1994) Antimalarial activity in vitro of the glyoxalase I inhibitor diester, S-p-bromobenzylglutathione diethyl ester. Biochem. Pharmacol. **47:** 418–420
11 Gamain B., Langsley G., Fourmaux M. N., Touzel J. P., Camus D., Dive D. et al. (1996) Molecular characterization of the glutathione peroxidase gene of the human malaria parasite *Plasmodium falciparum*. Mol. Biochem. Parasitol. **78:** 237–248
12 Rahlfs S., Fischer M. and Becker K. (2001) *Plasmodium falciparum* possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. J. Biol. Chem. **276:** 37133–37140
13 Srivastava P., Puri S. K., Kamboj K. K. and Pandey V. C. (1999) Glutathione-*S*-transferase activity in malarial parasites. Trop. Med. Int. Health **4:** 251–254
14 O'Brien E., Kurdi-Haidar B., Wanachiwanawin W., Carvajal J. L., Vulliamy T. J., Cappadoro M. et al. (1994) Cloning of the glucose 6-phosphate dehydrogenase gene from *Plasmodium falciparum*. Mol. Biochem. Parasitol. **64:** 313–326
15 Draculic T., Dawes I. W. and Grant C. M. (2000) A single glutaredoxin or thioredoxin gene is essential for viability in the yeast *Saccharomyces cerevisiae*. Mol. Microbiol. **36:** 1167–1174
16 Arner E. S. and Holmgren A. (2000) Physiological functions of thioredoxin and thioredoxin reductase. Eur. J. Biochem. **267:** 6102–6109
17 Miranda-Vizuete A., Damdimopoulos A. E. and Spyrou G. (2000) The mitochondrial thioredoxin system. Antioxid. Redox. Signal. **2:** 801–810
18 Powis G. and Montfort W. R. (2001) Properties and biological activities of thioredoxins. Annu. Rev. Biophys. Biomol. Struct. **41:** 421–455
19 Chae H. Z., Chung S. J. and Rhee S. G. (1994) Thioredoxin-dependent peroxide reductase from yeast. J. Biol. Chem. **269:** 27670–27678
20 Baker A., Payne C. M., Briehl M. M. and Powis G. (1997) Thioredoxin, a gene found overexpressed in human cancer, inhibits apoptosis in vitro and in vivo. Cancer Res. **57:** 5162–5167
21 Gasdaska J. R., Berggren M. and Powis G. (1995) Cell growth stimulation by the redox protein thioredoxin occurs by a novel helper mechanism. Cell Growth Differ. **6:** 1643–1650
22 Gromer S., Schirmer R. H. and Becker K. (1999) News and views on thioredoxin reductases. Redox Rep. **4:** 221–228
23 Nakamura H., Nakamura K. and Yodoi J. (1997) Redox regulation of cellular activation. Annu. Rev. Immunol. **15:** 351–369
24 Powis G., Mustacich D. and Coon A. (2000) The role of the redox protein thioredoxin in cell growth and cancer. Free Radic. Biol. Med. **29:** 312–322
25 Chalar C., Martinez C., Agorio A., Salinas G., Soto J. and Ehrlich R. (1999) Molecular cloning and characterization of a thioredoxin gene from *Echinococcus granulosus*. Biochem. Biophys. Res. Commun. **262:** 302–307
26 Williams C. H. Jr. (1992) Lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase and mercuric ion reductase – a family of flavoenzyme transhydrogenases. In: Chemistry and Biochemistry of Flavoenzymes vol. 3, pp. 121–211, Miller F. (ed.), CRC Press, Boca Raton, FL
27 Williams C. H., Arscott L. D., Müller S., Lennon B. W., Ludwig M. L., Wang P. F. et al. (2000) Thioredoxin reductase: two modes of catalysis have evolved. Eur. J. Biochem. **267:** 6110–6117
28 Kanzok S. M., Fechner A., Bauer H., Ulschmid J. K., Müller H. M., Botella-Munoz J. et al. (2001) Substitution of the thioredoxin system for glutathione reductase in *Drosophila melanogaster*. Science **291:** 643–646
29 Gladyshev V. N., Krause M., Xu X. M., Korotkov K. V., Kryukov G. V., Sun Q. A. et al. (1999) Selenocysteine-containing thioredoxin reductase in *C. elegans*. Biochem. Biophys. Res. Commun. **259:** 244–249
30 Müller S., Gilberger T. W., Färber P. M., Becker K., Schirmer R. H. and Walter R. D. (1996) Recombinant putative glutathione reductase of *Plasmodium falciparum* exhibits thioredoxin reductase activity. Mol. Biochem. Parasitol. **80:** 215–219
31 Kanzok S. M., Schirmer R. H., Turbachova I., Iozef R. and Becker K. (2000) The thioredoxin system of the malaria parasite *Plasmodium falciparum*. Glutathione reduction revisited. J. Biol. Chem. **275:** 40180–40186
32 Becker K., Gromer S., Schirmer R. H. and Müller S. (2000) Thioredoxin reductase as a pathophysiological factor and drug target. Eur. J. Biochem. **267:** 6118–6125
33 Lennon B. W., Williams C. H. Jr and Ludwig M. L. (2000) Twists in catalysis: alternating conformations of *Escherichia coli* thioredoxin reductase. Science **289:** 1190–1194
34 Mustacich D. and Powis G. (2000) Thioredoxin reductase. Biochem. J. **346:** 1–8
35 Tamura T. and Stadtman T. C. (1996) A new selenoprotein from human lung adenocarcinoma cells: Purification, properties, and thioredoxin reductase activity. Proc. Natl. Acad. Sci. USA **93:** 1006–1011
36 McGonigle S., Dalton J. P. and James E. R. (1998) Peroxiredoxins: a new antioxidant family. Parasitol. Today **14:** 139–145
37 Schröder E. and Ponting C. P. (1998) Evidence that peroxiredoxins are novel members of the thioredoxin fold superfamily. Protein Sci. **7:** 2465–2468
38 Kim K., Kim I. H., Lee K. Y., Rhee S. G. and Stadtman E. R. (1988) The isolation and purification of a specific 'protector' protein which inhibits enzyme inactivation by a thiol/Fe(III)/O_2 mixed-function oxidation system. J. Biol. Chem. **263:** 4704–4711
39 Nogoceke E., Gommel D. U., Kiess M., Kalisz H. M. and Flohé L. (1997) A unique cascade of oxidoreductases catalyses trypanothione-mediated peroxide metabolism in *Crithidia fasciculata*. Biol. Chem. **378:** 827–836
40 Kim I. H., Kim K. and Rhee S. G. (1989) Induction of an antioxidant protein of *Saccharomyces cerevisiae* by O_2, Fe^{3+}, or 2-mercaptoethanol. Proc. Natl. Acad. Sci. USA **86:** 6018–6022
41 Montemartini M., Nogoceke E., Singh M., Steinert P., Flohé L. and Kalisz H. M. (1998) Sequence analysis of the tryparedoxin peroxidase gene from *Crithidia fasciculata* and its functional expression in *Escherichia coli*. J. Biol. Chem. **273:** 4864–4871
42 Kang S. W., Baines I. C. and Rhee S. G. (1998) Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. J. Biol. Chem. **273:** 6303–6311
43 Pedrajas J. R., Miranda-Vizuete A., Javanmardy N., Gustafsson J. A. and Spyrou G. (2000) Mitochondria of *Saccha-*

romyces cerevisiae contain one-conserved cysteine type peroxiredoxin with thioredoxin peroxidase activity. J. Biol. Chem. **275:** 16296–16301
44 Fisher A. B., Dodia C., Manevich Y., Chen J. W. and Feinstein S. I. (1999) Phospholipid hydroperoxides are substrates for non-selenium glutathione peroxidase. J. Biol. Chem. **274:** 21326–21334
45 Chen J. W., Dodia C., Feinstein S. I., Jain M. K. and Fisher A. B. (2000) 1-Cys peroxiredoxin, a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities. J. Biol. Chem. **275:** 28421–28427
46 Kawazu S., Tsuji N., Hatabu T., Kawai S., Matsumoto Y. and Kano S. (2000) Molecular cloning and characterization of a peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. Mol. Biochem. Parasitol. **109:** 165–169
47 Hofmann B., Hecht H. J. and Flohé L. (2002) Peroxiredoxins. Biol. Chem., in press
48 Brown D. M., Upcroft J. A. and Upcroft P. (1996) A thioredoxin reductase-class of disulphide reductase in the protozoan parasite *Giardia duodenalis*. Mol. Biochem. Parasitol. **83:** 211–220
49 Cohen G., Yanko M., Mislovati M., Argaman A., Schreiber R., Av-Gay Y. et al. (1993) Thioredoxin-thioredoxin reductase system of *Streptomyces clavuligerus*: sequences, expression, and organization of the genes. J. Bacteriol. **175:** 5159–5167
50 Cohen G., Argaman A., Schreiber R., Mislovati M. and Aharonowitz Y. (1994) The thioredoxin system of *Penicillium chrysogenum* and its possible role in penicillin biosynthesis. J. Bacteriol. **176:** 973–984
51 Cavalier-Smith T. (1987) The origin of eukaryotic and archaebacterial cells. Ann. N. Y. Acad. Sci. **503:** 17–54
52 Cavalier-Smith T. (1993) Kingdom protozoa and its 18 phyla. Microbiol. Rev. **57:** 953–994
53 Brown D. M., Upcroft J. A., Edwards M. R. and Upcroft P. (1998) Anaerobic bacterial metabolism in the ancient eukaryote *Giardia duodenalis*. Int. J. Parasitol. **28:** 149–164
54 Bruchhaus I. and Tannich E. (1995) Identification of an *Entamoeba histolytica* gene encoding a protein homologous to prokaryotic disulphide oxidoreductases. Mol. Biochem. Parasitol. **70:** 187–191
55 Poole L. B., Chae H. Z., Flores B. M., Reed S. L., Rhee S. G. and Torian B. E. (1997) Peroxidase activity of a TSA-like antioxidant protein from a pathogenic amoeba. Free Radic. Biol. Med. **23:** 955–959
56 Mirelman D., Monheit D. and Varon S. (1987) Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle of garlic extract (*Allium sativum*). J. Infect. Dis. **156:** 243–244
57 Ankri S. and Mirelman D. (1999) Antimicrobial properties of allicin from garlic. Microbes Infect. **1:** 125–129
58 McGonigle S., Curley G. P. and Dalton J. P. (1997) Cloning of peroxiredoxin, a novel antioxidant enzyme, from the helminth parasite *Fasciola hepatica*. Parasitology **115:** 101–104
59 Salazar-Calderon M., Martin-Alonso J. M., Ruiz de Eguino A. D., Casais R., Marin M. S. and Parra F. (2000) *Fasciola hepatica*: heterologous expression and functional characterization of a thioredoxin peroxidase. Exp. Parasitol. **95:** 63–70
60 Salazar-Calderon M., Martin-Alonso J. M., Ruiz de Eguino A. D. and Parra F. (2001) Heterologous expression and functional characterization of thioredoxin from *Fasciola hepatica*. Parasitol. Res. **87:** 390–395
61 Shoda L. K., Rice-Ficht A. C., Zhu D., McKown R. D. and Brown W. C. (1999) Bovine T cell responses to recombinant thioredoxin of *Fasciola hepatica*. Vet. Parasitol. **82:** 35–47
62 Barrett, J. (1980) Peroxide metabolism in the liver fluke, *Fasciola hepatica*. J. Parasitol. **66:** 697
63 Sanchez-Moreno M., Leon P., Garcia-Ruiz M. A. and Monteoliva M. (1987) Superoxide dismutase activity in nematodes. J. Helminthol. **61:** 229–232
64 Kwatia M. A., Botkin D. J. and Williams D. L. (2000) Molecular and enzymatic characterization of *Schistosoma mansoni* thioredoxin peroxidase. J. Parasitol. **86:** 908–915
65 Williams D. L., Asahi H., Botkin D. J. and Stadecker M. J. (2001) Schistosome infection stimulates host CD4(+) T helper cell and B-cell responses against a novel egg antigen, thioredoxin peroxidase. Infect. Immun. **69:** 1134–1141
66 Sun Q.-A., Kirnarsky L., Sherman S. and Gladyshev V. N. (2001) Selenoprotein oxidoreductase with specificity for thioredoxin and glutathione systems. Proc. Natl. Acad. Sci. USA **98:** 3673–3678
67 Williams D. L, Pierce R. J., Cookson E. and Capron A. (1992) Molecular cloning and sequencing of glutathione peroxidase from *Schistosoma mansoni*. Mol. Biochem. Parasitol. **52:** 127–130
68 Roche C., Williams D. L., Khalife J., LePresle T., Capron A. and Pierce R. J. (1994) Cloning and characterization of the gene encoding *Schistosoma mansoni* glutathione peroxidase. Gene **138:** 149–152
69 Mei H., Thakur A., Schwartz J. and Lo Verde P. T. (1996) Expression and characterization of glutathione peroxidase activity in the human blood fluke *Schistosoma mansoni*. Infect. Immun. **64:** 4299–4306
70 Maiorino M., Pierce R. and Flohé L. (1997) 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
71 Gladyshev V. N. and Hatfield D. L. (1999) Selenocysteine-containing proteins in mammals. J. Biomed. Sci. **6:** 151–160
72 Stadtman T. C. (1980) Selenium-dependent enzymes. Annu. Rev. Biochem. **49:** 93–110
73 Vorholt J. A., Vaupel M. and Thauer R. K. (1997) A selenium-dependent and a selenium-independent formylmethanofuran dehydrogenase and their transcriptional regulation in the hyperthermophilic *Methanopyrus kandleri*. Mol. Microbiol. **23:** 1033–1042
74 Guimaraes M. J., Peterson D., Vicari A., Cocks B. G., Copeland N. G., Gilbert D. J. et al. (1996) Identification of a novel selD homolog from eukaryotes, bacteria, and archaea: is there an autoregulatory mechanism in selenocysteine metabolism? Proc. Natl. Acad. Sci. USA. **93:** 15086–15091
75 Buettner C., Harney J. W. and Berry M. J. (1999) The *Caenorhabditis elegans* homologue of thioredoxin reductase contains a selenocysteine insertion sequence (SECIS) element that differs from mammalian SECIS elements but directs selenocysteine incorporation. J. Biol. Chem. **274:** 21598–21602
76 Feng J. J., Guo H. F., Yao M. Y. and Xiao S. H. (1995) Effects of mebendazole, albendazole and praziquantel on glutathione *S*-transferase and superoxide dismutase of *Echinococcus granulosus* cyst wall harbored in mice. Zhongguo Yao Li Xue Bao. **16:** 297–300
77 Salinas G., Fernandez V., Fernandez C. and Selkirk M. E. (1998) *Echinococcus granulosus*: cloning of a thioredoxin peroxidase. Exp. Parasitol. **90:** 298–301
78 Selkirk M. E., Smith V. P., Thomas G. R. and Gounaris K. (1998) Resistance of filarial nematode parasites to oxidative stress. Int. J. Parasitol. **28:** 1315–1332
79 Henkle-Dührsen K. and Kampkotter A. (2001) Antioxidant enzyme families in parasitic nematodes. Mol. Biochem. Parasitol. **114:** 129–142
80 Tsuji N., Kasuga-Aoki H., Isobe T. and Yoshihara S. (2000) Cloning and characterisation of a peroxiredoxin from the swine roundworm *Ascaris suum*. Int. J. Parasitol. **30:** 125–128
81 Ghosh I., Eisinger S. W., Raghavan N. and Scott A. L. (1998) Thioredoxin peroxidases from *Brugia malayi*. Mol. Biochem. Parasitol. **91:** 207–220

82 Blaxter M. L., Raghavan N., Ghosh I., Guiliano D., Lu W., Williams S. A. et al. (1996) Genes expressed in *Brugia malayi* infective third stage larvae. Mol. Biochem. Parasitol. **77:** 77–93

83 Klimowski L., Chandrashekar R. and Tripp C. A. (1997) Molecular cloning, expression and enzymatic activity of a thioredoxin peroxidase from *Dirofilaria immitis*. Mol. Biochem. Parasitol. **90:** 297–306

84 Chandrashekar R., Tsuji N., Morales T. H., Carmody A. B, Ozols V. O., Welton J. et al. (2000) Removal of hydrogen peroxide by a 1-cysteine peroxiredoxin enzyme of the filarial parasite *Dirofilaria immitis*. Parasitol. Res. **86:** 200–206

85 Lund P. A. (1995) The roles of molecular chaperones in vivo. Essays Biochem. **29:** 113–123

86 Burston S. G. and Clarke A. R. (1995) Molecular chaperones: physical and mechanistic properties. Essays Biochem. **29:** 125–136

87 Kuchler K. and Thorner J. (1992) Secretion of peptides and proteins lacking hydrophobic signal sequences: the role of adenosine triphosphate-driven membrane translocators. Endocr. Rev. **13:** 499–514

88 Tripp C., Frank R. S., Selkirk M. E., Tang L., Grieve M. M., Frank G. R. et al. (1988) *Dirofilaria immitis*: molecular cloning and expression of a cDNA encoding a selenium-independent secreted glutathione peroxidase. Exp. Parasitol. **88:** 43–50

89 Callahan H. L., Crouch R. K. and James E. R. (1991) *Dirofilaria immitis* superoxide dismutase: purification and characterization. Mol. Biochem. Parasitol. **49:** 245–251

90 Jaffe J. J. and Lambert R. A. (1986) Glutathione *S*-transferase in adult *Dirofilaria immitis* and *Brugia pahangi*. Mol. Biochem. Parasitol. **20:** 199–206

91 Lu W., Egerton G. L., Bianco A. E. and Williams S. A. (1998) Thioredoxin peroxidase from *Onchocerca volvulus*: a major hydrogen peroxide detoxifying enzyme in filarial parasites. Mol. Biochem. Parasitol. **91:** 221–235

92 Chandrashekar R., Curtis K. C., Lu W. and Weil G. J. (1998) Molecular cloning of an enzymatically active thioredoxin peroxidase from *Onchocerca volvulus*. Mol. Biochem. Parasitol. **93:** 309–312

93 Schrum S., Bialonski A., Marti T. and Zipfel P. F. (1998) Identification of a peroxidoxin protein (OvPXN-2) of the human parasitic nematode *Onchocerca volvulus* by sequential protein fractionation. Mol. Biochem. Parasitol. **94:** 131–135

94 Wilkinson S. R, Temperton N. J., Mondragon A. and Kelly J. M. (2000) Distinct mitochondrial and cytosolic enzymes mediate trypanothione-dependent peroxide metabolism in *Trypanosoma cruzi*. J. Biol. Chem. **275:** 8220–8225

95 Sztajer H., Gamain B., Aumann K. D., Slomianny C., Becker K., Brigelius-Flohé R. et al. (2001) The putative glutathione peroxidase gene of *Plasmodium falciparum* codes for a thioredoxin peroxidase. J. Biol. Chem. **276:** 7397–7403

96 Fairlamb A. H. and Cerami A. (1992) Metabolism and functions of trypanothione in the Kinetoplastida. Ann. Rev. Microbiol. **46:** 695–729

97 Krauth-Siegel R. L. and Coombs G. H. (1999) Enzymes of parasite thiol metabolism as drug targets. Parasitol. Today **15:** 404–409

98 Krauth-Siegel R. L. and Lüdemann H. (1996) Reduction of dehydroascorbate by trypanothione. Mol. Biochem. Parasitol. **80:** 203–208

99 Lüdemann H., Dormeyer M., Sticherling C., Stallmann D., Follmann H. and Krauth-Siegel R. L. (1998) *Trypanosoma brucei* tryparedoxin, a thioredoxin-like protein in African trypanosomes. FEBS Lett. **431:** 381–385

100 Gommel D. U., Nogoceke E., Morr M., Kiess M., Kalisz H. M. and Flohé L. (1997) Catalytic characteristics of tryparedoxin. Eur. J. Biochem. **248:** 913–918

101 Lopez J. A., Carvalho T. U., de Souza W., Flohé L., Guerrero S. A., Montemartini M. et al. (2000) Evidence for a trypanothione-dependent peroxidase system in *Trypanosoma cruzi*. Free Radic. Biol. Med. **28:** 767–772

102 Levick M. P., Tetaud E., Fairlamb A. H. and Blackwell J. M. (1998) Identification and characterisation of a functional peroxidoxin from *Leishmania major*. Mol. Biochem. Parasitol. **96:** 125–137

103 Myler P. J., Audleman L., deVos T., Hixson G., Kiser P., Lemley C. et al. (1999) *Leishmania major* Friedlin chromosome 1 has an unusual distribution of protein-coding genes. Proc. Natl. Acad. Sci. USA **96:** 2902–2906

104 Reckenfelderbäumer N., Lüdemann H., Schmidt H., Steverding D. and Krauth-Siegel R. L. (2000) Identification and functional characterization of thioredoxin from *Trypanosoma brucei brucei*. J. Biol. Chem. **275:** 7547–7552

105 Tetaud E. and Fairlamb A. H. (1998) Cloning, expression and reconstitution of the trypanothione-dependent peroxidase system of *Crithidia fasciculata*. Mol. Biochem. Parasitol. **96:** 111–123

106 Steinert P., Dittmar K., Kalisz H. M., Montemartini M., Nogoceke E., Rohde M. et al. (1999) Cytoplasmic localization of the trypanothione peroxidase system in *Crithidia fasciculata*. Free Radic. Biol. Med. **26:** 844–849

107 Krieger S., Schwarz W., Ariyanayagam M. R., Fairlamb A. H., Krauth-Siegel R. L. and Clayton C. (2000) Trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. Mol. Microbiol. **35:** 542–552

108 Montrichard F., Le Guen F., Laval-Martin D. L. and Davioud-Charvet E. (1999) Evidence for the co-existence of glutathione reductase and trypanothione reductase in the non-trypanosomatid Euglenozoa: *Euglena gracilis Z.* FEBS Lett. **442:** 29–33

109 Tetaud E., Giroud C., Prescott A. R., Parkin D. W., Baltz D., Biteau N. et al. (2001) Molecular characterisation of mitochondrial and cytosolic trypanothione-dependent tryparedoxin peroxidases in *Trypanosoma brucei*. Mol. Biochem. Parasitol. **116:** 171–83

110 Ivens A. C., Lewis S. M., Bagherzadeh A., Zhang L., Chan H. M. and Smith D. F. (1998) A physical map of the *Leishmania major* Friedlin genome. Genome Res. **8:** 135–145

111 Butler D., Maurice J. and O'Brien C. (1997) Time to put malaria control on the global agenda. Nature **386:** 535–536

112 Olliaro P. (2001) Mode of action and mechanisms of resistance for antimalarial drugs. Pharmacol. Ther. **89:** 207–219

113 Yamey G. (2001) Global campaign to eradicate malaria. Brit. Med. J. **322:** 1191–1192

114 Meshnick S. R. (1996) Is haemozoin a target for antimalarial drugs? Ann. Trop. Med. Parasitol. **90:** 367–372

115 Slater F. G. and Cerami A. (1992) Inhibition by chloroquine of a novel heme polymerase enzyme activity in malaria trophozoites. Nature **355:** 167–169

116 Egan T., Ross D. C., and Adams P. A. (1994) Quinoline antimalarial drugs inhibit spontaneous formation of β-haematin (malaria pigment). FEBS Lett. **352:** 54–57

117 Blauer G. and Akkawi M. (1997) Investigations of B- and β-hematin. J. Inorg. Biochem. **66:** 145–152

118 Zhang J., Krugliak M. and Ginsburg H. (1999) The fate of ferriprotoporphyrin IX in malaria infected erythrocytes in conjugation with the mode of action of antimalarial drugs. Mol. Biochem. Parasitol. **99:** 129–141

119 Sullivan D. S. Jr, Matile H., Ridley R. G. and Goldberg D. E. (1998) A common mechanism for blockade of heme polymerization by antimalarial drugs. J. Biol. Chem. **47:** 31103–31107

120 Atamna H. and Ginsburg H. (1995) Heme degradation in the presence of glutathione. A proposed mechanism to account for the high levels of non-heme iron found in the membranes of hemoglobinopathic red blood cells. J. Biol. Chem. **270:** 24876–24883

121 Platel D. F. N., Mangou F. and Tribouley-Duret J. (1999) Role of glutathione in the detoxification of ferriprotoporphyrin IX in chloroquine resistant *Plasmodium berghei*. Mol. Biochem. Parasitol. **98:** 215–223

122 Loria P., Miller S., Foley M. and Tilley L. (1999) Inhibition of the peroxidative degradation of heme as the basis of action of chloroquine and other quinoline antimalarials. Biochem. J. **339:** 363–370

123 Ginsburg H., Famin O., Zhang J. and Krugliak M. (1998) Inhibition of glutathione-dependent degradation of heme by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. Biochem. Pharmacol. **56:** 1305–1313

124 Gilberger T. W., Walter R. D. and Muller S. (1997) Identification and characterization of the functional amino acids at the active site of the large thioredoxin reductase from *Plasmodium falciparum*. J. Biol. Chem. **272:** 29584–29589

125 Gilberger T. W., Bergmann B., Walter R. D. and Muller S. (1998) The role of the C-terminus for catalysis of the large thioredoxin reductase from *Plasmodium falciparum*. FEBS Lett. **425:** 407–410

126 Wang P. F., Arscott L. D., Gilberger T. W., Muller S. and Williams C. H. Jr (1999) Thioredoxin reductase from *Plasmodium falciparum*: evidence for interaction between the C-terminal cysteine residues and the active site disulfide-dithiol. Biochemistry **38:** 3187–3196

127 Krnajski Z., Gilberger T. W., Walter R. D. and Muller S. (2001) The malaria parasite *Plasmodium falciparum* possesses a functional thioredoxin system. Mol. Biochem. Parasitol. **112:** 219–228

128 Rahlfs S. and Becker K. (2001) Thioredoxin peroxidases of the malarial parasite *Plasmodium falciparum*. Eur. J. Biochem. **268:** 1404–1409

129 Krnajski Z., Walter R. D. and Muller S. (2001) Isolation and functional analysis of two thioredoxin peroxidases (peroxiredoxins) from *Plasmodium falciparum*. Mol. Biochem. Parasitol. **113:** 303–308

130 Kawazu S., Komaki K., Tsuji N., Kawai S., Ikenoue N., Hatabu T. et al. (2001) Molecular characterization of a 2-Cys peroxiredoxin from the human malaria parasite *Plasmodium falciparum*. Mol. Biochem. Parasitol. **116:** 73–79

131 Boehme C. C., Arscott L. D., Becker K., Schirmer R. H. and Williams C. H. (2000) Kinetic characterization of glutathione reductase from the malarial parasite *Plasmodium falciparum.* Comparison with the human enzyme. J. Biol. Chem. **275:** 37317–37323

132 Müller S., Becker K., Bergmann B., Schirmer R. H. and Walter R. D. (1995) *Plasmodium falciparum* glutathione reductase exhibits sequence similarities with the human host enzyme in the core structure but differs at the ligand-binding sites. Mol. Biochem. Parasitol. **74:** 11–18

133 Becker K., Müller S., Keese M. A., Walter R. D. and Schirmer R. H. (1996) A glutathione reductase-like flavoenzyme of the malaria parasite *Plasmodium falciparum*: structural considerations based on the DNA sequence. Biochem. Soc. Trans. **24:** 67–72

134 Arscott L. D., Gromer S., Schirmer R. H., Becker K. and Williams C. H. Jr (1997) The mechanism of thioredoxin reductase from human placenta is similar to the mechanisms of lipoamide dehydrogenases and glutathione reductases and is distinct from the mechanism of thioredoxin reductase from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **94:** 3621–3626

135 Krnajski Z., Gilberger T. W., Walter R. D. and Müller S. (2000) Intersubunit interactions in *Plasmodium falciparum* thioredoxin reductase. J. Biol. Chem. **275:** 40874–40878

136 Davioud-Charvet E., Becker K., Landry V., Gromer S., Loge C. and Sergheraert C. (1999) Synthesis of 5,5′-dithiobis(2-nitrobenzamides) as alternative substrates for trypanothione reductase and thioredoxin reductase: a microtiter colorimetric assay for inhibitor screening. Anal. Biochem. **268:** 1–8

137 Becker K., Savvides S. N., Keese M., Schirmer R. H. and Karplus P. A. (1998) Enzyme inactivation through sulfhydryl oxidation by physiologic NO-carriers. Nat. Struct. Biol. **5:** 267–271

138 Nikitovic D. and Holmgren A. (1996) *S*-nitrosoglutathione is cleaved by the thioredoxin system with liberation of glutathione and redox regulating nitric oxide. J. Biol. Chem. **271:** 19180–19185

139 Gamain B., Arnaud J., Favier A., Camus D., Dive D. and Slomianny C. (1996) Increase in glutathione peroxidase activity in malaria parasites after selenium supplementation. Free Radic. Biol. Med. **21:** 559–565

140 Moutiez M., Aumercier M., Schoneck R., Meziane-Cherif D., Lucas V., Aumercier P. et al. (1995) Purification and characterization of a trypanothione-glutathione thioltransferase from *Trypanosoma cruzi*. Biochem. J. **310:** 433–437

141 Xia B., Vlamis-Gardikas A., Holmgren A., Wright P. E. and Dyson H. J. (2001) Solution structure of *Escherichia coli* glutaredoxin-2 shows similarity to mammalian glutathione-*S*-transferases. J. Mol. Biol. **310:** 907–918

142 Ouaissi A., Vergnes B., Borges M. and Guilvard E. (2000) Identification and molecular characterization of two novel *Trypanosoma cruzi* genes encoding polypeptides sharing sequence motifs found in proteins involved in RNA editing reactions. Gene **253:** 271–280

143 Webb J. R., Campos-Neto A., Ovendale P. J., Martin T. I., Stromberg E. J., Badaro R. et al. (1998) Human and murine immune responses to a novel *Leishmania major* recombinant protein encoded by members of a multicopy gene family. Infect. Immun. **66:** 3279–3289

144 Zipfel P. F., Schrum S., Bialonski A. and Buttner D. W. (1998) The peroxidoxin 2 protein of the human parasite *Onchocerca volvulus*: recombinant expression, immunolocalization, and demonstration of homologous molecules in other species. Parasitol. Res. **84:** 623–631

145 Flores B. M., Reed S. L., Ravdin J. I. and Torian B. E. (1993) Serologic reactivity to purified recombinant and native 29-kilodalton peripheral membrane protein of pathogenic *Entamoeba histolytica*. J. Clin. Microbiol. **31:** 1403–1407

146 Soong C. J., Torian B. E., Abd-Alla M. D., Jackson T. F., Gatharim V. and Ravdin J. I. (1995) Protection of gerbils from amebic liver abscess by immunization with recombinant *Entamoeba histolytica* 29-kD antigen. Infect. Immun. **63:** 472–477

147 Torian B. E., Flores B. M., Stroeher V. L., Hagen F. S. and Stamm W. E. (1990) cDNA sequence analysis of a 29-kDa cysteine-rich surface antigen of pathogenic *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA **87:** 6358–6362

148 Stadecker M. J. (1999) The development of granulomas in schistosomiasis: genetic backgrounds, regulatory pathways, and specific egg antigen responses that influence the magnitude of disease. Microbes Infect. **1:** 505–510

149 Chae H. Z., Kim I. H., Kim K. and Rhee S. G. (1993) Cloning, sequencing, and mutation of thiol-specific antioxidant gene of *Saccharomyces cerevisiae*. J. Biol. Chem. **268:** 16815–16821

150 Cookson E., Blaxter M. L. and Selkirk M. E. (1992) Identification of the major soluble cuticular glycoprotein of lymphatic filarial nematode parasites (gp29) as a secretory homolog of glutathione peroxidase. Proc. Natl. Acad. Sci. USA. **89:** 5837–5841

151 James E. R., McLean D. C. and Perler F. (1994) Molecular cloning of an *Onchocerca volvulus* extracellular Cu-Zn superoxide dismutase. Infect. Immun. **62:** 713–716