ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 4, 2012

DOI: 10.1089/ars.2011.4266

FORUM REVIEW ARTICLE

Thiol-Based Posttranslational Modifications in Parasites

Esther Jortzik, Lihui Wang, and Katja Becker

Abstract

Significance: Cysteine residues of proteins participate in the catalysis of biochemical reactions, are crucial for redox reactions, and influence protein structure by the formation of disulfide bonds. Covalent posttranslational modifications (PTMs) of cysteine residues are important mediators of redox regulation and signaling by coupling protein activity to the cellular redox state, and moreover influence stability, function, and localization of proteins. A diverse group of protozoan and metazoan parasites are a major cause of diseases in humans, such as malaria, African trypanosomiasis, leishmaniasis, toxoplasmosis, filariasis, and schistosomiasis. ***Recent Advances:*** Human parasites undergo dramatic morphological and metabolic changes while they pass complex life cycles and adapt to changing environments in host and vector. These processes are in part regulated by PTMs of parasitic proteins. In human parasites, posttranslational cysteine modifications are involved in crucial cellular events such as signal transduction (*S*-glutathionylation and *S*-nitrosylation), redox regulation of proteins (*S*-glutathionylation and *S*-nitrosylation), protein trafficking and subcellular localization (palmitoylation and prenylation), as well as invasion into and egress from host cells (palmitoylation). This review focuses on the occurrence and mechanisms of these cysteine modifications in parasites. ***Critical Issues:*** Studies on cysteine modifications in human parasites are so far largely based on *in vitro* experiments. ***Future Directions:*** The *in vivo* regulation of cysteine modifications and their role in parasite development will be of great interest in order to understand redox signaling in parasites. *Antioxid. Redox Signal.* 17, 657–673.

Introduction

Tropical diseases caused by *Plasmodium* (malaria), *Trypanosoma* (trypanosomiasis), *Leishmania* (leishmaniasis), *Schistosoma* (schistosomiasis), and others affect not only the health of millions of people mainly in southern countries, but also their social and economic development (25, 93, 177, 192). Parasitic diseases are characterized by a high morbidity and most of them by a high lethality, which is worsened by a lack of effective treatments (93, 97, 156, 177).

Cysteine accounts for 2% of the amino acids in proteins from eukaryotes (168). Due to their chemical characteristics, cysteines contribute to biochemical reactions based on thiol- or thiyl-dependent catalysis, form structurally important disulfide bonds, bind metal ions, and catalyze redox reactions (86, 87, 117). Differential modifications of the oxidation state of sulfur in cysteines mediate redox regulation and signaling in response toward the cellular redox potential (47, 85, 244). Cysteine modifications include reversible oxidation to thiyl radicals and sulfenic acids (Cys-SOH), which are unstable, but highly reactive (174, 178, 195). Further oxidation leads to sulfinic acid (Cys-SO_2H) and irreversibly overoxidized sulfonic acid (Cys-SO_3H) (23, 174, 178) (Fig. 1). Moreover, cysteines form intra- and intermolecular disulfide (Cys-SS-Cys), and mixed disulfides with cysteine (*S*-cysteinylation, Cys-SS-Cys), glutathione (*S*-glutathionylation, Cys-SSG), and nitric oxide (*S*-nitrosylation, Cys-S-NO) (Fig. 2). Besides oxidative modifications, cysteine residues are susceptible to lipidation such as *S*-prenylation, *S*-palmitoylation, and *S*-dolichylation, which subsequently influence subcellular compartmentation, structure, and function of modified proteins (138, 213). This review provides a summary of the current research on oxidative and lipid modifications of cysteines with an emphasis on *S*-nitrosylation, *S*-glutathionylation, palmitoylation, and prenylation in human pathogenic parasites.

Nitric Oxide and *S*-nitrosylation

Nitric oxide (NO) is a ubiquitous signaling molecule that possesses miscellaneous bioactivities (154). Accumulating evidence indicates that many physiological and pathophysiological effects of NO are mediated by protein *S*-nitrosylation, a posttranslational modification (PTM) (104, 216). Protein *S*-nitrosylation occurs via the addition of a nitrosyl group to a reactive cysteine thiol of a protein forming a protein *S*-nitrosothiol, which can affect conformation, stability, localization, activity, protein–protein interaction, and function of the respective protein (98, 104, 125). The homeostasis of

Interdisciplinary Research Center, Justus Liebig University, Giessen, Germany.

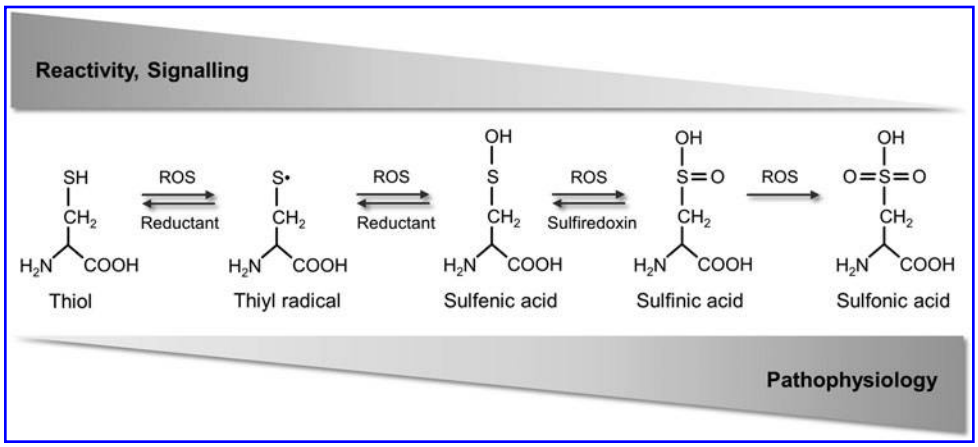

FIG. 1. Different oxidation states of cysteine residues. Cysteines can be oxidized by different reactive oxygen species to sulfenic acids, which can be reduced by several reductants such as glutathione and thioredoxin. Further oxidation leads to sulfinic acid that can be specifically reduced by the sulfinic acid reductase sulfiredoxin, and to irreversibly oxidized sulfonic acid that cannot be reduced.

S-nitrosylation is closely regulated by denitrosylation (the removal of the NO moiety) mediated by enzymes and nonenzymatic factors *in vivo* (Fig. 3) (21), such as *S*-nitrosoglutathione (GSNO) reductase (140), thioredoxin (20), superoxide dismutase (120), protein disulfide isomerase (211), carbonyl reductase (16), and glutathione (Fig. 3). Proteins regulated by *S*-nitrosylation can be found in almost all classes of proteins [reviewed in (104, 146, 201, 216)], and play important regulatory roles in cell signaling, apoptosis, and gene transcription (61, 201), thus attracting interest in discovering the mechanisms of NO redox signaling. The importance of *S*-nitrosylation is underlined by the fact that dysregulation of *S*-nitrosylation is correlated with the pathophysiology of many human diseases, such as sickle cell anemia, diabetes, cancer, and neurodegenerative disorders (75, 158).

Under physiological conditions, the direct formation of *S*-nitrosothiols is achieved by the reaction of a protein cysteine thiolate with NO radicals (*e.g.*, NO^+) (5). Additionally, *S*-nitrosylation occurs via transnitrosylation by transferring an NO equivalent from a nitrosylated thiol to another protein thiol (245). Moreover, auto-*S*-nitrosylation can be catalyzed by protein-bound transition metals or by flavins.

The major part of nonprotein *S*-nitrosothiols *in vivo* is attributed to GSNO, a low-molecular-weight *S*-nitrosothiol derived from the interaction of glutathione with NO derivatives or protein *S*-nitrosothiols (210). GSNO significantly induces the formation of protein *S*-nitrosothiols by transferring the nitrosyl group to protein thiols (transnitrosylation), indicating an important role in the transduction of NO bioactivities (75, 119). In fact, GSNO can be generated from the degradation of protein *S*-nitrosothiols by glutathione, revealing that the metabolism of protein *S*-nitrosothiols via glutathione/GSNO pathway dynamically modulates NO signaling and maintains the homeostasis of protein *S*-nitrosylation (21).

Intracellular levels of GSNO are regulated by the GSNO-reducing activity of glutathione-dependent formaldehyde dehydrogenase, which is conserved throughout evolution

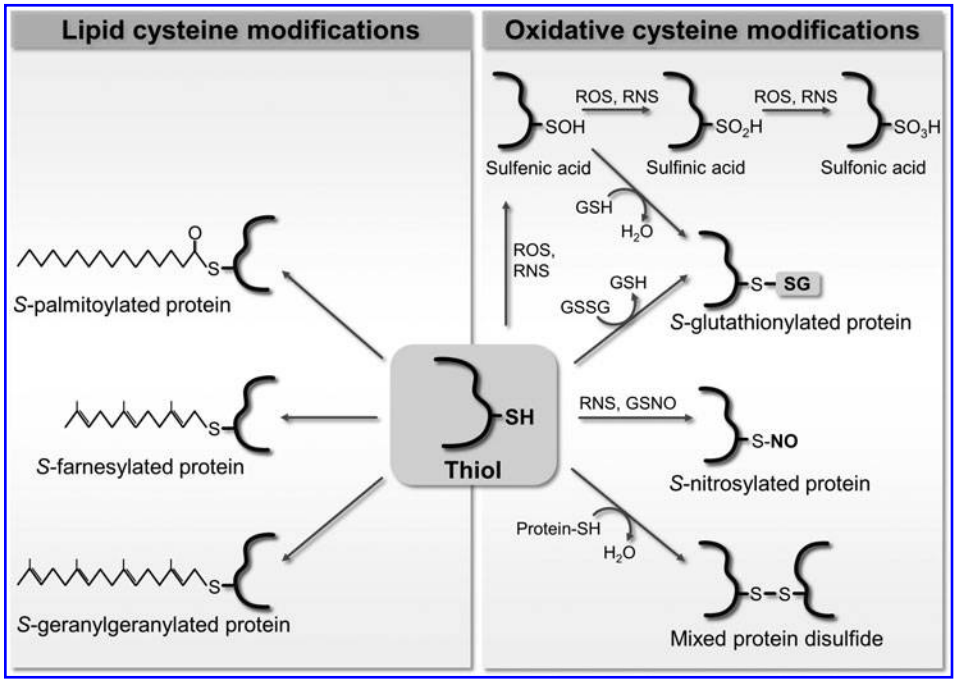

FIG. 2. Summary of the most prevalent posttranslational modifications of cysteine residues. Cysteine can either be oxidatively modified by hydroxylation, *S*-glutathionylation, *S*-nitrosylation, and mixed disulfide formation, or be modified by the attachment of lipid moieties.

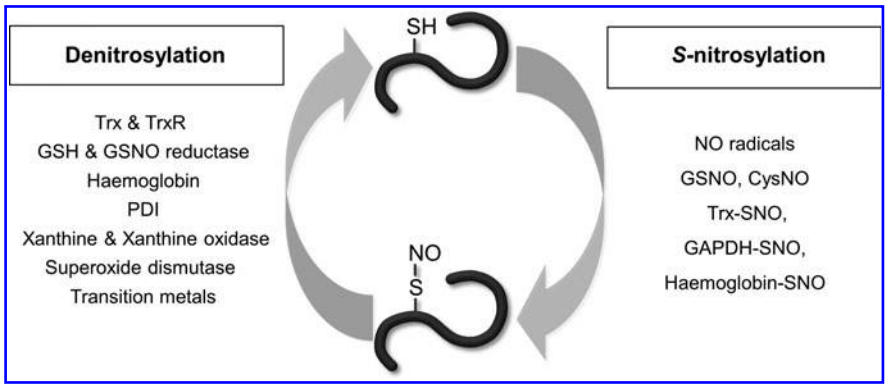

FIG. 3. Catalysis of protein *S*-nitrosylation and denitrosylation. *S*-nitrosylation can be mediated directly by NO radicals, or via transnitrosylation by low-molecular-weight *S*-nitrothiols (GSNO and CysNO) and several transnitrosylases, for example, Trx-SNO, GAPDH-SNO, and haemoglobin-SNO. Trx and glutathione (GSH) denitrosylate protein by transnitrosylation forming Trx-SNO and GSNO, which are then reduced by TrxR and GSNO reductase, respectively. Besides, hemoglobin, protein disulfide isomerase, xanthine/xanthine oxidase, and transition metals (*e.g.*, copper and iron) also catalyze protein denitrosylation. GAPDH-SNO, glyceraldehyde-3-phosphate dehydrogenase-SNO; GSH, reduced glutathione; GSNO, *S*-nitrosoglutathione; TrxR, Trx reductase; Trx-SNO, thioredoxin-SNO.

from bacteria to mammals (140), but has not been identified in parasites so far.

Despite the ubiquitous distribution of cysteines, protein *S*-nitrosylation is considered as a highly specific modification since it selectively targets regulatory or catalytic cysteine residues. The specificity of protein *S*-nitrosylation is determined by the thiol pK_a, local hydrophobicity, and allosteric regulation by ions (104).

In human parasitic diseases, NO functions as an important effector molecule in host–parasite interactions and pharmacological treatments (7, 30). Recent studies have detected NO and reactive nitrogen species (RNS) within several human pathogenic parasites (126, 164, 197). Here, we review NO sources and protein *S*-nitrosylation in human parasites and discuss the role of *S*-nitrosylation in antiparasitic strategies.

NO sources in human parasites

Host–parasite interaction. A burst of NO generation in host cells is a classic paradigm of host immune defense against a wide range of intracellular and extracellular parasites (7, 30). In mammals, endogenous NO is predominantly biosynthesized by the conversion of L-arginine to L-citrulline catalyzed by constitutive and inducible NO synthase (NOS) (218), with the latter producing NO for immunological defense against cancer and microbial infections (30, 228). Similar antiparasitic effects of NO are also essentially involved in defending invertebrates against parasites (*e.g.*, in the mosquito *Anopheles stephensi* against malaria parasites) (171,182), indicating a ubiquitous antiparasitic mechanism of NO in nature. Compelling evidence shows that NO produced by inducible NOS (iNOS) is involved in host immune response against parasites (27, 118, 144, 238, 254). The model of parasite killing by host NO generation is well established in human macrophages (215, 230). Parasite-induced pro-inflammatory cytokines stimulate iNOS expression and NO burst in macrophages during parasite infections (30, 157). Moreover, specific parasite antigens like *Plasmodium* glycosylphosphatidylinositol induce iNOS expression in macrophages (219). Considering that NO easily diffuses through membranes, parasitic proteins are most likely exposed to RNS (44), thus potentially leading to *S*-nitrosylation of cysteine residues.

Besides that the induction of iNOS can kill parasites by direct action of parasiticidal NO, N^{ω}-hydroxy-L-arginine (NOHA), an intermediate in the production of NO by iNOS, can also be an antiparasitic agent by inhibiting parasite arginase and subsequently inducing polyamine starvation in parasites (112). This provides another mechanism of iNOS in parasite elimination, which functions independently from NO.

Although the generation of NO from host immune defense combats parasitic infection, the parasite facilitates its survival in the host by developing strategies to alleviate the insult of nitrosative stress. For example, there is compelling evidence that parasite-encoded arginase attenuates the production of parasiticidal NO from host iNOS via arginine depletion (52, 236). Besides, parasites also manage to possess nonprotein thiols and enzymes for the detoxification of elevated oxidative/nitrosative stress, such as glutathione, 1-N-methyl-4-mercaptohistidine (ovothiol A), thioredoxin/thioredoxin reductase, trypanothione/trypanothione reductase, trypanothione *S*-transferase, and peroxidases (18, 185, 230, 237).

Pharmacologically derived NO. In addition to NO derived from the host immune system, pharmacologically used antiparasitic agents can induce the formation of NO. Enhanced intracellular nitrosative stress by NO donors leads to parasite death (7). Several NO donors, including *S*-nitroso-acetylpenicillamine, *S*-nitroso-N-acetyl-L-cysteine, GSNO, and *S*-nitrosocysteine, have a strong antiparasitic effect on a wide spectrum of parasites (13, 55, 183, 207, 219). Notably, GSNO is an NO carrier present readily and highly abundant *in vivo* (210), and its cytotoxicity is most likely mediated by transnitrosylation (24). The antiparasitic effects of pharmacological NO donors are attributed to inhibition of key parasitic enzymes by *S*-nitrosylation (see below). Compelling evidence that the antiparasitic action of NO-donors is at least in part mediated by *S*-nitrosylation could be shown by the development of oxadiazole compounds as antischistosomal agents (176, 198). Novel NO-releasing agents selectively targeting parasites with

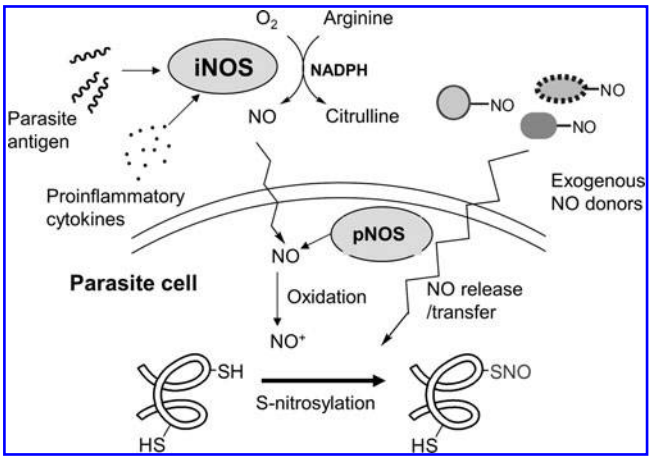

FIG. 4. NO sources and *S*-nitrosylation in human parasites. In response to parasite infection, the release of proinflammatory cytokines (*e.g.,* IFN-γ, TNF-α, and IL-β) and parasite antigen stimulate the host iNOS to release parasiticidal NO via the conversion of arginine to citrulline. The antiparasitic NO readily diffuse into parasite cells and undergo oxidation to form NO radicals (NO^+). Besides, the putative parasite NOS (pNOS) in some parasites may generate inherent NO in parasites. Furthermore, exogenous NO can be derived from pharmacological treatment with NO donors. The presence of NO and NO-derived molecules in parasites may lead to *S*-nitrosylation of redox-sensitive cysteine residues in proteins. NO, nitric oxide; NOS, nitric oxide synthase.

low toxicity to host cells have been developed, such as *trans*-$[RuCl([15]aneN4)NO]^{2+}$ (95, 229).

NO generation in parasites. Apart from exogenous NO, parasites potentially generate endogenous NO. So far, efforts in hunting for parasite NOS led to the discovery of a putative NOS in *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi*, *Entamoeba histolytica*, *Schistosoma japonicum*, and *Schistosoma mansoni* (15, 96, 103, 143, 167). Parasite NOS-like proteins share common features with mammalian NOS, such as NADPH-dependent L-arginine consumption, immunoreactivity against mammalian NOS antibodies, and inhibition by L-NAME, an arginine analog inhibiting NOS (15, 83, 96, 143). Conversely, NOS or NOS-like proteins could not be shown in *Plasmodium falciparum* (68, 164). However, NO production in *P. falciparum* might be attributed to a protein structurally similar to plant nitrate reductases, since it co-localizes with high NO production around the food vacuole (164). Taken together, the formation of NO in the above-mentioned pathways may contribute to the occurrence of *S*-nitrosylation in parasites (Fig. 4).

S-nitrosylation *inhibits key enzymes of parasites*

The antiparasitic properties of NO are supposed to be mediated by *S*-nitrosylation of parasitic proteins, since the effects of NO and NO donors were correlated to inhibition of key parasitic enzymes through *S*-nitrosylation (8, 45). Well-studied *S*-nitrosylation targets in parasites are cysteine proteases (CPs), which utilize a catalytic cysteine residue to hydrolyze peptide bonds (45, 186). CPs are important antiparasitic drug targets, since they play pivotal roles in hemoglobin hydrolysis, host cell invasion, and erythrocyte rupture (186, 193). It has been shown that NO donors potently inhibit the major cysteine protease of *P. falciparum* trophozoites (falcipain), *T. cruzi* epimastigotes (cruzipain), and *Leishmania infantum* promastigotes (6, 8, 24, 233, 234). Inhibition of *P. falciparum* falcipain and *T. cruzi* cruzipain by *S*-nitrosylation results in death of the parasite (233, 234). Transnitrosylation of catalytic cysteine residues of CPs (*e.g.,* Cys25-NO for *T. cruzi* cruzipain) mediated by NO donors has been considered as the underlying inhibitory mechanism (24, 45, 234). Besides, the aspartic protease plasmepsin from *Plasmodium vivax*, which participates in hemoglobin degradation, is inhibited by several NO donors *in vitro* most likely due to *S*-nitrosylation of catalytic cysteine residues, which interrupts the parasite growth (203, 208). Furthermore, alcohol dehydrogenase 2 from *E. histolytica* is inhibited by *S*-nitrosylation (208).

Since the proteomic identification of *S*-nitrosylated proteins is technically possible (74), further studies on the *S*-nitrosoproteome of parasites may shed light on the largely unknown NO targets and the functions of *S*-nitrosylation in parasites.

Protein *S*-glutathionylation

The capacity of glutathione as a signaling molecule is based on its ability to form mixed disulfides with redox-sensitive protein thiols referred to as protein *S*-glutathionylation. This protein modification accounts for 85%–90% of *S*-thiolation and therefore represents the most frequent cysteine modification (37, 199). The susceptibility of cysteine thiols toward *S*-glutathionylation depends on the accessibility and the reactivity of the cysteine (84). Redox-sensitive cysteine residues typically have a low pK_a value as a consequence of neighboring positively charged amino acids (51).

Several reaction mechanisms lead to protein *S*-glutathionylation, including thiol–disulfide exchange between protein thiols and oxidized glutathione (GSSG), direct reaction between reduced glutathione (GSH) and oxidized intermediates such as sulfenic acid, *S*-nitrosyl, or thiyl radicals, and the reaction between protein thiols and *S*-nitrosothiols (51, 80, 84). Thiol–disulfide exchange can occur under high GSSG concentrations *in vitro* [*e.g.* (19, 181, 253)], but is unlikely to mediate *S*-glutathionylation at physiological GSSG concentrations (50, 51, 80). Additionally, glutaredoxin and glutathione *S*-transferase promote *S*-glutathionylation (217, 224).

S-glutathionylation is readily reversible by glutaredoxin, thioredoxin, sulfiredoxin, and glutathione *S*-transferase, or nonenzymatically by GSH (43, 53, 71, 81, 82, 94, 165, 191). Analogous to phosphorylation, *S*-glutathionylation is a regulatory PTM that induces functional changes in target proteins, either activation or in most cases inhibition, and subsequently leads to alteration of cellular pathways in response to oxidative stress [reviewed in (50, 51, 84, 108, 202, 246)]. *S*-glutathionylation protects cysteines from irreversible overoxidation and a persistent loss of protein function during increased oxidative stress and is thus an antioxidant defense mechanism. Changes in *S*-glutathionylation patterns are associated with several pathophysiological processes and human diseases, such as neurodegenerative and cardiovascular diseases (246). Moreover, several anticancer drugs mediate their effect at least in part by increasing protein *S*-glutathionylation (246).

Global *S*-glutathionylation was studied in mammals (76, 77, 137, 180, 199), plants (59, 115), and yeast (205), revealing numerous *S*-glutathionylated proteins involved in versatile cellular pathways.

Glutathione in parasites

In *P. falciparum*, the glutathione system was investigated in detail [reviewed in (17)]. Glutathione is synthesized by γ-glutamyl-cysteine synthetase and glutathione synthetase (148), and kept in a reduced form by glutathione reductase (17, 133). *Plasmodium* depends on reduced glutathione, which has to be either synthesized *de novo* or kept in a reduced state by glutathione reductase (31, 166, 232). In malaria parasites, glutathione is involved in the detoxification of electrophilic compounds by glutathione *S*-transferase (100) and in the degradation of methylglyoxal by glyoxalase (113), but also reduces glutaredoxin (175). Moreover, reduced glutathione might also be involved in the degradation of heme depending on concentrations of oxygen, heme, and glutathione (11).

Trypanosomatids such as *Trypanosoma brucei* and *Leishmania major* conjugate more than 70% of intracellular glutathione with spermidine forming the intermediate glutathionylspermidine and trypanothione, which replaces glutathione in most redox reactions (66, 67). Trypanothione and glutathionylspermidine are reduced by trypanothione reductase (132), a reaction that is essential for *T. brucei* and *L. donovani* (134, 221, 222). Trypanothione reduces tryparedoxin, dehydroascorbate, and glutathione, which in turn deliver reducing equivalents to the respective peroxidases [reviewed in (35, 114, 130, 131)].

An antioxidant system based on glutathione was also demonstrated for *T. gondii* (58). Conversely, *S. mansoni* and *Ecchinococcus granulosus* employ the multifunctional selenoprotein thioredoxin glutathione reductase (TGR) for the reduction of both glutathione and thioredoxin (3, 136).

Interestingly, several parasitic protozoa such as *E. histolytica* (65), *Giardia duodenalis* (28, 29), and *Trichomonas foetus* (28) completely lack glutathione and enzymes of the glutathione metabolism, but rely on cysteine as the major low-molecular-weight thiol to protect from oxidative stress.

S-glutathionylation in parasites

Protein *S*-glutathionylation was studied in Platyhelminths, *Apicomplexa*, and Trypanosomatids. In parasitic Platyhelminths such as *S. mansoni* and *E. granulosus*, protein deglutathionylation and thus homeostasis of *S*-glutathionylation is mediated by TGR (Fig. 5) (26, 107). *E. granulosus* TGR catalyzes protein deglutathionylation via its glutaredoxin domain (26). The disulfide intermediate consisting of TGR and bound glutathione is resolved by selenocysteine in the thioredoxin reductase domain of TGR (26). In TGR from *S. mansoni*, Cys28 of the glutaredoxin domain attacks the *S*-glutathionylated target protein, which is consistent with the mechanism shown for *Echinococcus* TGR, but the mixed disulfide intermediate can be solved either by GSH representing a monothiol deglutathionylation mechanism yielding GSSG, or alternatively by neighboring Cys31 via a dithiol mechanism releasing GSH. In the latter case, the disulfide in the glutaredoxin domain is finally reduced by the selenocysteine of the TrxR domain (107). The difference between *Echinococcus* and *Schistosoma* TGR was

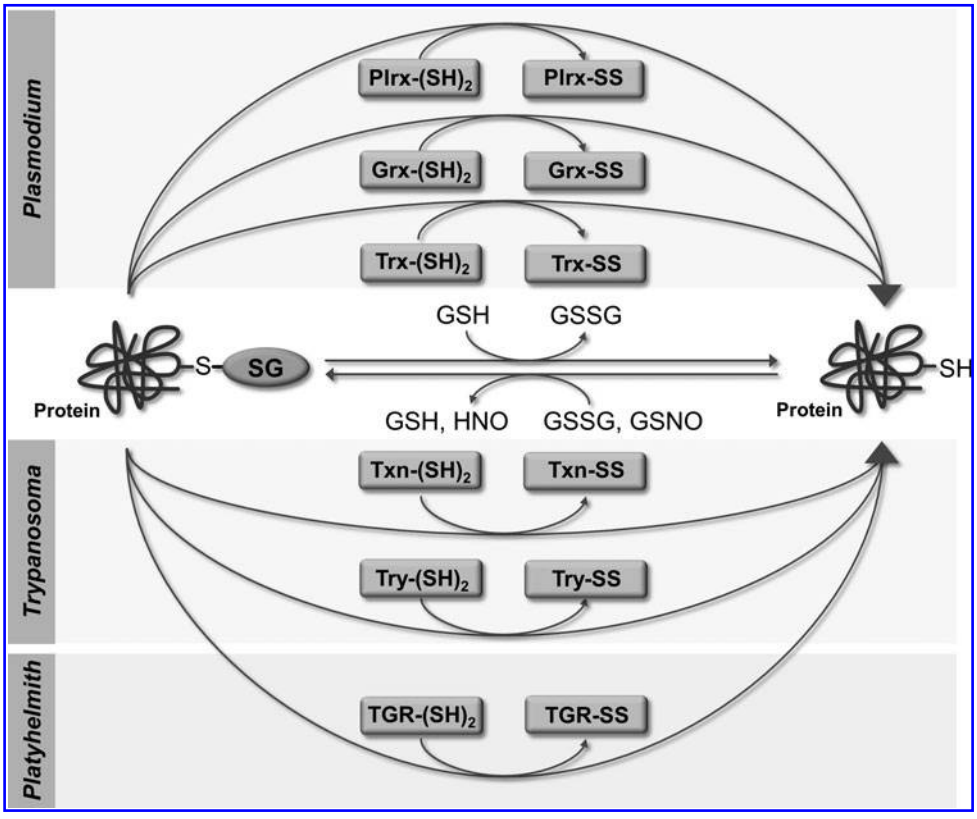

FIG. 5. Enzymes involved in protein deglutathionylation in human parasites. In *Plasmodium falciparum*, thioredoxin, glutaredoxin, and plasmoredoxin are able to deglutathionylate proteins. Trypanothione (Try) and tryparedoxin, but not glutaredoxin from *Trypanosoma*, were shown to deglutathionylate proteins. TGR from *Schistosoma* and *Echinococcus* catalyzes protein deglutathionylation via its glutaredoxin domain. Additionally, deglutathionylation can occur nonenzymatically by reduced glutathione (GSH). TGR, thioredoxin glutathione reductase.

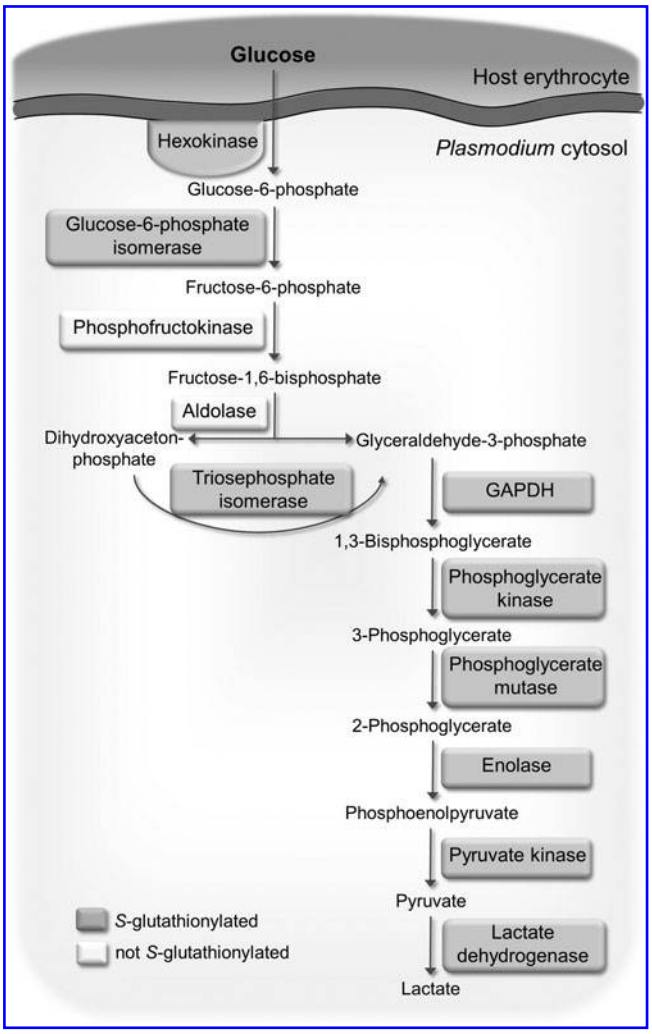

FIG. 6. Most enzymes involved in glycolysis in *Plasmodium falciparum* are *S*-glutathionylated. Except for phosphofructokinase and aldolase, all enzymes of the glycolytic pathway are *S*-glutathionylated. *In vitro*, the glycolytic activities of glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase are inhibited upon *S*-glutathionylation, while the activity of lactate dehydrogenase is not influenced by *S*-glutathionylation.

attributed to a greater distance between the two active site cysteines in the glutaredoxin domain of *Echinococcus* TGR (107), but might also be a result of different experimental design.

Recently, we identified 493 *S*-glutathionylated proteins from *P. falciparum* (124). Three hundred twenty-one of the *S*-glutathionylated proteins have a predicted function and many of them are involved in nitrogen compound metabolism, protein metabolism, and biosynthetic processes (124). Except for aldolase and phosphofructokinase, all enzymes of the glycolytic pathway are *S*-glutathionylated under physiological conditions (Fig. 6), and both *P. falciparum* glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase are inhibited efficiently and reversibly by *S*-glutathionylation *in vitro*. Furthermore, ornithine δ-aminotransferase is regulated by *S*-glutathionylation *in vitro* (124). As shown for other organisms (94, 204), *P. falciparum* glutaredoxin 1 and thioredoxin 1, but interestingly also the *Plasmodium*-specific oxidoreductase plasmoredoxin, exhibit deglutathionylase activity (Fig. 5) (124). Approximately 10% of the proteome from *P. falciparum* is glutathionylated under physiological conditions, thus underlining the importance of *S*-glutathionylation as a central redox-regulatory mechanism. Changes in the *S*-glutathionylation patterns and subsequently in the metabolome during increased oxidative stress, for example, as a consequence of pharmacological treatment, are of great interest in order to understand the function of *S*-glutathionylation as a regulatory process in *Plasmodium*.

In *T. brucei*, monothiol glutaredoxin 1, tryparedoxin peroxidase III, and thioredoxin were shown to be specifically and reversibly *S*-glutathionylated and *S*-glutathionylspermidinylated *in vitro* (149). Tryparedoxin, trypanothione, as well as dithiol glutaredoxin 1 and 2 are able to catalyze deglutathionylation by reducing mixed protein-SSG disulfides *in vitro*, thus demonstrating a functional system in *T. brucei* required for *S*-glutathionylation *in vivo* (Fig. 5) (36, 149). The effect of *S*-glutathionylation on enzyme activity and subsequently cellular function for *T. brucei in vivo* remains to be elucidated. Other Trypanosomatids such as *L. major* and the insect parasite *Crithidia fasciculata* also utilize the GSH conjugate trypanothione as their major redox buffer (46, 48, 67, 102), but *S*-glutathionylation has not yet been studied in these organisms.

In *E. histolytica*, *G. duodenalis*, and *T. foetus*, cysteine replaces glutathione as the major low-molecular-weight reducing agent (28, 29, 65). In *E. histolytica*, cysteine depletion induces metabolic changes, which are not reflected by changes in expression of the respective genes, thus indicating a role of posttranscriptional modifications or PTMs (109, 110). It remains to be studied whether these parasites employ cysteinylation instead of glutathionylation as a PTM for protein regulation in response to oxidative stress.

Studies on the regulation and effect of protein *S*-glutathionylation *in vivo* are missing in the majority of studies, but are essential to enhance the understanding of redox signaling in parasites.

Lipid Modifications of Cysteine Residues

Proteins can be posttranslationally modified by the attachment of lipid moieties to cysteines, such as palmitoylation, prenylation, and dolichylation. In general, lipid modifications increase the hydrophobicity of proteins in order to stabilize membrane localization, but are also crucial for protein trafficking to subcellular compartments and protein–protein interactions. Protein palmitoylation and prenylation were studied in various human parasites with respect to function and mechanism.

Protein palmitoylation

The addition of palmitate, a $C_{16:0}$ fatty acid, to the thiol group of a cysteine via a reversible thioester linkage is termed protein *S*-palmitoylation, sometimes also more general *S*-acylation or thioacylation (138, 212). If the palmitoylated cysteine is located at the protein's N-term, the thioester is rearranged into an irreversible amide bond (*N*-palmitoylation) (170). Proteins can be palmitoylated spontaneously in the presence of palmitoyl-CoA (14), or enzymatically by protein acyltransferases (151, 172). Depalmitoylation reactions are catalyzed by acylprotein thioesterases (106, 248).

The family of palmitoylated proteins is large and diverse with membrane affinity as a common feature. Palmitoylation regulates the localization, stability, and activity of diverse proteins by promoting their membrane association, targeting them to specific membrane domains, mediating protein interactions and degradation (101, 116, 138, 194, 206, 212).

The palmitoyl-proteome was studied by proteomic approaches, identifying 124 (trypanosomes), 300 (rat), and 391 (human) palmitoylated proteins (62, 123, 247), which emphasize the cellular importance of this PTM.

Palmitoylation in parasites

Protein palmitoylation is crucial for the survival of *T. brucei*, since treatment with 2-bromopalmitate, a palmitate analog, results in complete growth inhibition of procyclic and bloodstream forms (62). *T. brucei* possesses 12 putative palmitoyl acyltransferases with redundant functions (62, 63). Recently, 124 palmitoylated proteins were identified in *T. brucei* (62). The palmitoylation of some *Trypanosoma* proteins was studied in more detail: palmitoylation of calcium-binding calflagins specifically induces trafficking of calflagins to the membrane of the flagellum (63). In contrast to a high redundancy of many palmitoyl transferases (62, 188), calflagins can only be palmitoylated by palmitoyl acyltransferase 7 (63). A role of myristate and palmitate for specifically targeting proteins to the flagellar membrane was also shown for *T. brucei* calpain-like proteins (141), *L. major* SMP-1 (226), and *T. cruzi* flagellar calcium binding protein (90). Phosphoinositide-specific phospholipase C from *T. cruzi*, which is involved in parasite differentiation, is targeted to the plasma membrane by the concerted action of *N*-myristoylation and palmitoylation (79, 162). Acylated surface proteins from *L. major* are translocated to and anchored in the membrane by *N*-myristoylation and palmitoylation (56). Furthermore, PPEF-like phosphatases from *L. major* and CAP5.5 from *T. brucei* are targets of palmitoylation *in vivo* (150).

In *Toxoplasma* and *Plasmodium*, cysteine palmitoylation occurs on proteins associated with gliding motility and invasion into host cells. A *T. gondii* myosin light chain, which is associated with myosin D, is attached to the parasite plasma membrane via palmitoylation (173). Moreover, hypoxanthine-xanthine-guanine phosphoribosyltransferase (HXGPRT) II from *T. gondii* is associated with the inner membrane complex (IMC) solely via palmitoylation of a Cys-Cys-Cys motif (Fig. 7) (40), and might supply purine nucleotides for the glideosome complex (40), which is responsible for gliding motility (49).

Dual acylation of *P. falciparum* calcium-dependent protein kinase 1 (PfCDPK1) with myristate and palmitate in combination with a basic cluster is required for targeting PfCDPK1 to the plasma membrane (153), where it phosphorylates components of the acto-myosin motor, such as the 45 kDa gliding-associated protein (GAP45; Fig. 7) (92). *P. falciparum* GAP45 is attached to the IMC by *N*-myristoylation and palmitoylation (Fig. 7), and thereby links the acto-myosin motor to the IMC (179), which might also be the case for *T. gondii* GAP45 (88). Thus, the addition of palmitate mediates the binding of several glideosome-associated proteins to the IMC in *Plasmodium* and *Toxoplasma* and is thus most likely crucial for host cell invasion. A link between palmitoylation and invasion is further supported by the finding that palmitoylation targets proteins to lipid rafts (1, 91, 240), and lipid rafts of the erythrocyte membrane are important for erythrocyte invasion of *P. falciparum* (129, 155).

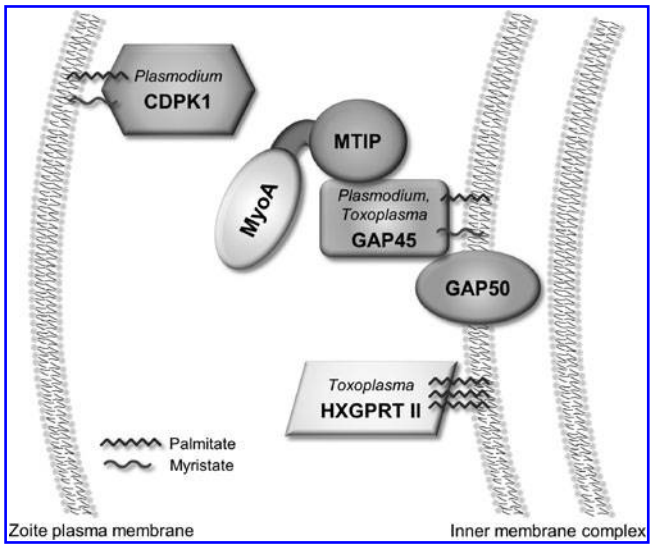

FIG. 7. Palmitoylation of proteins from *Plasmodium falciparum* and *Toxoplasma gondii* involved in gliding motility and invasion. Hypoxanthine-xanthine-guanine phosphoribosyltransferase II (HXGPRTII) from *T. gondii* is attached to the inner membrane complex via palmitoylation of a Cys-Cys-Cys motif, while calcium-dependent protein kinase 1 from *P. falciparum* is located at the parasite plasma membrane by both myristoylation and palmitoylation. The 45 kDa gliding-associated protein (GAP45) is a component of the glideosome complex and mediates the membrane attachment of myosin a tail domain interacting protein (MTIP, myosin light chain) and myosin A. The inner membrane localization of 45 kDa GAP45 from *P. falciparum* and *T. gondii* is mediated by myristoylation and palmitoylation.

Genomic analysis identified 13 potential palmitoyl transferases in *P. falciparum* (4). However, only a study on one DHHC-domain-containing protein, PfAnkDHHC (200), was published so far. Calpain, a cysteine protease essential for cell cycle progression (190), is bound to the plasma membrane by palmitoylation of two cysteines in combination with *N*-myristoylation. Interestingly, upon removal of the palmitoyl moiety, calpain is located in the nucleus, thus suggesting a palmitoylation-dependent regulation of localization (189). Palmitoylation furthermore promotes membrane association of cGMP-dependent protein kinases from *T. gondii* and *Eimeria tenella* (60).

In *Giardia lamblia*, variant specific surface proteins are palmitoylated at cysteines at their C-terminal tail (105, 220). *G. lamblia* codes for three DHHC-containing proteins, but only one was confirmed as a functional palmitoyl transferase *in vivo* (220). Although this palmitoyl transferase is not essential for *G. lamblia*, general palmitoylation inhibitors stop cell growth, indicating that palmitoylation fulfills an essential role for *G. lamblia* (220).

Protein prenylation

Another lipid modification of cysteines is prenylation, in which an isoprenoid group is covalently attached to a cysteine. A typical protein sequence susceptible to prenylation

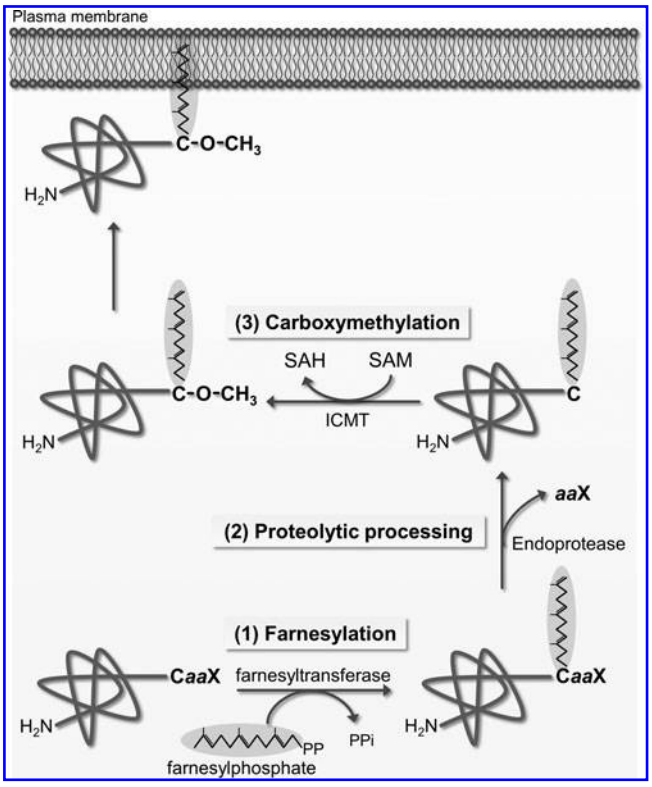

FIG. 8. C*aa*X processing of prenylated proteins. Proteins are prenylated at a C*aa*X motif with *a* representing aliphatic amino acids and X any amino acid. The prenylation (here shown for protein farnesylation) is followed by a proteolytic removal of the *aa*X tripeptide catalyzed by different Cxxx endoproteases such as RceI. Subsequently, the prenylcysteine gets carboxymethylated by isoprenylcysteine carboxyl methyltransferase.

consists of a core prenylation motif including a cysteine (C*aa*X motif, with C denoting cysteine, *a* representing aliphatic residues, and X as any residue) at or close to the C-terminus (147). Proteins can be prenylated by a farnesyl (C_{15}) or a geranylgeranyl lipid (C_{20}) consisting of three and four isoprene moieties, respectively. In eukaryotes, around 0.5% of all proteins are prenylated, with geranylgeranylcysteine being more frequent than farnesylcysteine (64). Whether a protein gets farnesylated or geranygeranylated is determined by the last amino acid of the C*aa*X motif and the specificity of prenyltransferases (34, 69, 152, 187). Prenylated protein are subjected to additional PTM steps referred to as C*aa*X processing: by proteolytic cleavage, the *aa*X tripeptide is removed leaving prenylcysteine as the ultimate residue, which subsequently can be carboxymethylated (Fig. 8) (9, 22). In accordance with palmitoylation, prenylation mediates membrane association, determines the subcellular localization, and is involved in protein–protein interactions (209).

Inhibitors of prenyltransferases and enzymes catalyzing the prenylation-dependent processing steps were intensely studied in order to develop anticancer agents (2, 128, 241, 243).

Protein prenylation in parasites

Protein prenylation has been studied intensely in numerous parasites (32, 39, 41, 70, 111, 135, 145, 250), with a special emphasis on drug discovery strategies. Interestingly, various prenyltransferase inhibitors were found to inhibit the differentiation and growth of *Plasmodium* (121, 161, 242), *Trypanosoma* (251, 252), *Toxoplasma* (111), *Schistosoma* (163), and *Leishmania* (251), thus suggesting that prenyltransferases are promising targets for novel antiparasitic drugs development.

So far, there are only a few reports on prenylated proteins in *Plasmodium* (39), and the extent and function of cysteine prenylation in malaria parasites remains unclear. A database search for C*aa*X motifs revealed 14 potentially farnesylated proteins in *P. falciparum* (169). One of these proteins is a PRL protein tyrosine phosphatase that is indeed farnesylated *in vitro* (169). The *P. falciparum* SNARE protein Ykt6 is prenylated at its C-terminal C*aa*X motif, but also geranylgeranylated. Prenylation is required to target *P. falciparum* Ykt6 to the membrane (12). *P. falciparum* farnesyltransferase and type I geranylgeranyltransferase were characterized as antimalarial drug tragets (38, 39). A range of highly selective *Plasmodium* farnesyltransferase inhibitors such as compounds based on a benzophenone scaffold, terpenes inhibiting isoprenoid synthesis, tetrahydroquinoline-based compounds, ethylenediamine-based inhibitors, and the nitrogen-containing biphosphonate risedronate were intensely studied and inhibit the growth of *P. falciparum in vitro* and *Plasmodium berghei* or *Plasmodium vinckei* infection in mice (38, 72, 89, 121, 127, 161, 184, 231, 242). Together, these findings support the inhibition of *Plasmodium* farnesyltransferase as a valuable concept for the development of antimalarial drugs and emphasize crucial functions of protein prenylation in *Plasmodium*.

At least 14 prenylated proteins were detected in *T. brucei*, with geranylgeranylated proteins being more abundant than farnesylated proteins (70), and distinct patterns of protein farnesylation and geranylgeranylation (250). Farnesyltransferases from *T. brucei*, *T. cruzi*, and *Leishmania* spp. show a different substrate specificity toward the C*aa*X motif compared to mammalian farnesyltransferases due to distinct amino acids in the substrate binding pocket, and have several insertions with unknown function (32, 33, 250). Moreover, *T. cruzi* employs a type I geranylgeranyltransferase with a distinct C*aa*X specificity that is exploited for the development of selective inhibitors (249, 250). Imidazole-containing C*aa*X peptidomimetics inhibit the growth of *T. brucei*, with the effect being attributed to inhibition of *T. brucei* farnesyltransferase (160, 161).

Experiments based on metabolic labeling and polyclonal antibodies confirmed the presence of prenylated proteins and a farnesyltransferase in *T. gondii*, which can be inhibited by known peptidomimetic farnesyltransferase inhibitors (111). Interestingly, *T. gondii* synthesizes the substrates for prenylation, farnesylphosphate, and geranylgeranylphosphate, by a bifunctional farnesyl-diphosphate/geranylgeranyl-diphosphate synthase (139).

A farnesyltransferase was also identified in *E. histolytica*, which has narrow substrate specificity and is not inhibited by known C*aa*X peptidomimetic inhibitors of mammalian farnesyltransferase (135).

By measuring the incorporation of labeled mevalonate into proteins as farnesyl or geranylgeranyl isoprenoids, several prenylated proteins were detected but not identified in *G. lamblia* (145). However, no prenyltransferase in *Giardia* has been described to our knowledge.

General protein prenylation was demonstrated in *S. mansoni* (41). A rab-related GTP-binding protein gets geranylgeranylated at its CCXXX motif (142), a Ras protein is farnesylated at a CCIQ motif (163), and Rho1 is geranylgeranylated, but not farnesylated (235). All three prenylated proteins are GTP-binding membrane-associated proteins involved in signaling pathways, thus suggesting a role for prenylation in signal transduction in *S. mansoni*.

Pathophysiological implications of posttranslational cysteine modifications

In eukaryotes, oxidative cysteine modifications such as *S*-glutathionylation and *S*-nitrosylation have been connected to pathophysiological processes and several diseases. Accumulation of *S*-glutathionylated proteins was observed in the context of type 2 diabetes (196), Friedreich's ataxia (214), cystic fibrosis (239), and Alzheimer's disease (57, 159). Moreover, *S*-glutathionylation was suggested to be involved in drug resistance in cancer (54). Similarly, altered protein *S*-nitrosylation was detected in neurodegenerative diseases such as Parkinson's and Alzheimer's disease (42, 225, 227), cardiovascular diseases (99, 122), and cancer (78). Remarkably, some anti-tumor agents such as adriamycin and NOV-002, a mix of GSSG and cisplatin, act at least in part by affecting *S*-glutathionylation (10, 223), while other anticancer drugs such as carmustine, bischloroethylnitroso-urea (BCNU) and auranofin affect protein *S*-nitrosylation (20, 73). Even though interpretation of cause and consequence of cysteine modifications in pathophysiological processes is difficult, potential pathophysiological roles of cysteine modifications in parasitic diseases and their implications in drug resistance is an important field of future studies. Furthermore, it might be interesting to investigate whether *S*-glutathionylation is involved in the action of antiparasitic drugs acting by increasing oxidative stress, as it has been suggested for anticancer agents (10, 223).

Conclusion

Cysteine modifications regulate the activity and function of diverse proteins in protozoan and metazoan parasites. *S*-glutathionylation is employed as a redox-regulatory modification of proteins from versatile cellular pathways. *S*-nitrosylation is especially interesting with respect to drug development, since parasites react vulnerably toward increased NO concentrations and increased *S*-nitrosylation. To which extent and on the basis of which mechanisms *S*-nitrosylation is employed as a way of redox regulation in parasites remains to be studied in detail. Cysteine palmitoylation and prenylation target proteins to subcellular membrane compartments and are thus crucial for the function of numerous membrane-bound proteins. However, many aspects of regulation and function of cysteine modifications remain unknown and the current knowledge is largely based on *in vitro* data. Further studies are needed to understand the complex interplay between the cellular redox state in health and disease, cysteine modifications, their influence on protein function, and subsequently their (patho-) physiological role in the cell in response to various stimuli. Studying the role of cysteine modifications in parasite metabolism and development will be of great interest in order to gain more insight into the redox biology of human parasites.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft (BE 1540/11-1).

References

1. Abrami L, Kunz B, Iacovache I, and van der Goot FG. Palmitoylation and ubiquitination regulate exit of the Wnt signaling protein LRP6 from the endoplasmic reticulum. *Proc Natl Acad Sci U S A* 105: 5384–5389, 2008.
2. Agrawal AG and Somani RR. Farnesyltransferase inhibitor as anticancer agent. *Mini Rev Med Chem* 9: 638–652, 2009.
3. 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.
4. Aravind L, Iyer LM, Wellems TE, and Miller LH. Plasmodium biology: genomic gleanings. *Cell* 115: 771–785, 2003.
5. Arnelle DR and Stamler JS. NO+, NO, and NO- donation by S-nitrosothiols: implications for regulation of physiological functions by S-nitrosylation and acceleration of disulfide formation. *Arch Biochem Biophys* 318: 279–285, 1995.
6. Ascenzi P, Bocedi A, Gentile M, Visca P, and Gradoni L. Inactivation of parasite cysteine proteinases by the NO-donor 4-(phenylsulfonyl)-3-((2-(dimethylamino)ethyl)thio)-furoxan oxalate. *Biochim Biophys Acta* 1703: 69–77, 2004.
7. Ascenzi P, Bocedi A, and Gradoni L. The anti-parasitic effects of nitric oxide. *IUBMB Life* 55: 573–578, 2003.
8. Ascenzi P, Salvati L, Bolognesi M, Colasanti M, Polticelli F, and Venturini G. Inhibition of cysteine protease activity by NO-donors. *Curr Protein Pept Sci* 2: 137–153, 2001.
9. Ashby MN. CaaX converting enzymes. *Curr Opin Lipidol* 9: 99–102, 1998.
10. Asmis R, Wang Y, Xu L, Kisgati M, Begley JG, and Mieyal JJ. A novel thiol oxidation-based mechanism for adriamycin-induced cell injury in human macrophages. *FASEB J* 19: 1866–18668, 2005.
11. Atamna H and Ginsburg H. 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, 1995.
12. Ayong L, DaSilva T, Mauser J, Allen CM, and Chakrabarti D. Evidence for prenylation-dependent targeting of a Ykt6 SNARE in Plasmodium falciparum. *Mol Biochem Parasitol* 175: 162–168, 2011.
13. Balmer P, Phillips HM, Maestre AE, McMonagle FA, and Phillips RS. The effect of nitric oxide on the growth of Plasmodium falciparum, P. chabaudi and P. berghei *in vitro*. *Parasite Immunol* 22: 97–106, 2000.
14. Bano MC, Jackson CS, and Magee AI. Pseudo-enzymatic S-acylation of a myristoylated yes protein tyrosine kinase peptide *in vitro* may reflect non-enzymatic S-acylation *in vivo*. *Biochem J* 330 (Pt 2): 723–731, 1998.
15. Basu NK, Kole L, Ghosh A, Das PK. Isolation of a nitric oxide synthase from the protozoan parasite, Leishmania donovani. *FEMS Microbiol Lett* 156: 43–47, 1997.
16. Bateman RL, Rauh D, Tavshanjian B, Shokat KM. Human carbonyl reductase 1 is an S-nitrosoglutathione reductase. *J Biol Chem* 283: 35756–35762, 2008.
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. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, and Ginsburg H. Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. *Int J Parasitol* 34: 163–189, 2004.
19. Beer SM, Taylor ER, Brown SE, Dahm CC, Costa NJ, Runswick MJ, and Murphy MP. Glutaredoxin 2 catalyzes the reversible oxidation and glutathionylation of mitochondrial membrane thiol proteins: implications for mitochondrial redox regulation and antioxidant DEFENSE. *J Biol Chem* 279: 47939–47951, 2004.
20. Benhar M, Forrester MT, Hess DT, and Stamler JS. Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. *Science* 320: 1050–1054, 2008.
21. Benhar M, Forrester MT, and Stamler JS. Protein denitrosylation: enzymatic mechanisms and cellular functions. *Nat Rev Mol Cell Biol* 10: 721–732, 2009.
22. Bergo MO, Leung GK, Ambroziak P, Otto JC, Casey PJ, Gomes AQ, Seabra MC, and Young SG. Isoprenylcysteine carboxyl methyltransferase deficiency in mice. *J Biol Chem* 276: 5841–5845, 2001.
23. Biteau B, Labarre J, and Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. *Nature* 425: 980–984, 2003.
24. Bocedi A, Gradoni L, Menegatti E, and Ascenzi P. Kinetics of parasite cysteine proteinase inactivation by NO-donors. *Biochem Biophys Res Commun* 315: 710–718, 2004.
25. Boelaert M, Meheus F, Robays J, and Lutumba P. Socio-economic aspects of neglected diseases: sleeping sickness and visceral leishmaniasis. *Ann Trop Med Parasitol* 104: 535–542, 2010.
26. Bonilla M, Denicola A, Marino SM, Gladyshev VN, and Salinas G. Linked thioredoxin-glutathione systems in platyhelminth parasites: alternative pathways for glutathione reduction and deglutathionylation. *J Biol Chem* 286: 4959–4967, 2011.
27. Brandonisio O, Panaro MA, Sisto M, Acquafredda A, Fumarola L, Leogrande D, and Mitolo V. Nitric oxide production by Leishmania-infected macrophages and modulation by cytokines and prostaglandins. *Parassitologia* 43 Suppl 1: 1–6, 2001.
28. 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.
29. Brown DM, Upcroft JA, and Upcroft P. Free radical detoxification in Giardia duodenalis. *Mol Biochem Parasitol* 72: 47–56, 1995.
30. Brunet LR. Nitric oxide in parasitic infections. *Int Immunopharmacol* 1: 1457–1467, 2001.
31. 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.
32. Buckner FS, Eastman RT, Nepomuceno-Silva JL, Speelmon EC, Myler PJ, Van Voorhis WC, and Yokoyama K. Cloning, heterologous expression, and substrate specificities of protein farnesyltransferases from Trypanosoma cruzi and Leishmania major. *Mol Biochem Parasitol* 122: 181–188, 2002.
33. Buckner FS, Yokoyama K, Nguyen L, Grewal A, Erdjument-Bromage H, Tempst P, Strickland CL, Xiao L, Van Voorhis WC, and Gelb MH. Cloning, heterologous expression, and distinct substrate specificity of protein farnesyltransferase from Trypanosoma brucei. *J Biol Chem* 275: 21870–21876, 2000.
34. Caplin BE, Hettich LA, and Marshall MS. Substrate characterization of the Saccharomyces cerevisiae protein farnesyltransferase and type-I protein geranylgeranyltransferase. *Biochim Biophys Acta* 1205: 39–48, 1994.
35. Castro H and Tomas AM. Peroxidases of trypanosomatids. *Antioxid Redox Signal* 10: 1593–1606, 2008.
36. 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.
37. Chai YC, Hendrich S, and Thomas JA. Protein S-thiolation in hepatocytes stimulated by t-butyl hydroperoxide, menadione, and neutrophils. *Arch Biochem Biophys* 310: 264–272, 1994.
38. Chakrabarti D, Azam T, DelVecchio C, Qiu L, Park YI, and Allen CM. Protein prenyl transferase activities of Plasmodium falciparum. *Mol Biochem Parasitol* 94: 175–184, 1998.
39. Chakrabarti D, Da Silva T, Barger J, Paquette S, Patel H, Patterson S, and Allen CM. Protein farnesyltransferase and protein prenylation in Plasmodium falciparum. *J Biol Chem* 277: 42066–42073, 2002.
40. Chaudhary K, Donald RG, Nishi M, Carter D, Ullman B, and Roos DS. Differential localization of alternatively spliced hypoxanthine-xanthine-guanine phosphoribosyltransferase isoforms in Toxoplasma gondii. *J Biol Chem* 280: 22053–22059, 2005.
41. Chen GZ and Bennett JL. Characterization of mevalonate-labeled lipids isolated from parasite proteins in Schistosoma mansoni. *Mol Biochem Parasitol* 59: 287–292, 1993.
42. Chinta SJ and Andersen JK. Nitrosylation and nitration of mitochondrial complex I in Parkinson's disease. *Free Radic Res* 45: 53–58, 2011.
43. Chrestensen CA, Starke DW, and Mieyal JJ. Acute cadmium exposure inactivates thioltransferase (Glutaredoxin), inhibits intracellular reduction of protein-glutathionyl-mixed disulfides, and initiates apoptosis. *J Biol Chem* 275: 26556–26565, 2000.
44. Colasanti M, Gradoni L, Mattu M, Persichini T, Salvati L, Venturini G, and Ascenzi P. Molecular bases for the antiparasitic effect of NO (Review). *Int J Mol Med* 9: 131–134, 2002.
45. Colasanti M, Salvati L, Venturini G, Ascenzi P, and Gradoni L. Cysteine protease as a target for nitric oxide in parasitic organisms. *Trends Parasitol* 17: 575, 2001.
46. Comini M, Menge U, Wissing J, and Flohe L. Trypanothione synthesis in crithidia revisited. *J Biol Chem* 280: 6850–6860, 2005.
47. Cross JV and Templeton DJ. Regulation of signal transduction through protein cysteine oxidation. *Antioxid Redox Signal* 8: 1819–1827, 2006.
48. Cunningham ML and Fairlamb AH. Trypanothione reductase from Leishmania donovani. Purification, characterisation and inhibition by trivalent antimonials. *Eur J Biochem* 230: 460–468, 1995.
49. Daher W and Soldati-Favre D. Mechanisms controlling glideosome function in apicomplexans. *Curr Opin Microbiol* 12: 408–414, 2009.
50. Dalle-Donne I, Rossi R, Colombo G, Giustarini D, and Milzani A. Protein S-glutathionylation: a regulatory device from bacteria to humans. *Trends Biochem Sci* 34: 85–96, 2009.
51. Dalle-Donne I, Rossi R, Giustarini D, Colombo R, and Milzani A. S-glutathionylation in protein redox regulation. *Free Radic Biol Med* 43: 883–898, 2007.

52. Das P, Lahiri A, and Chakravortty D. Modulation of the arginase pathway in the context of microbial pathogenesis: a metabolic enzyme moonlighting as an immune modulator. *PLoS Pathog* 6: e1000899, 2010.
53. Davis DA, Newcomb FM, Starke DW, Ott DE, Mieyal JJ, and Yarchoan R. Thioltransferase (glutaredoxin) is detected within HIV-1 and can regulate the activity of glutathionylated HIV-1 protease *in vitro*. *J Biol Chem* 272: 25935–25940, 1997.
54. De Luca A, Moroni N, Serafino A, Primavera A, Pastore A, Pedersen JZ, Petruzzelli R, Farrace MG, Pierimarchi P, Moroni G, Federici G, Sinibaldi Vallebona P, and Lo Bello M. Treatment of doxorubicin resistant MCF7/Dx cells with nitric oxide causes histone glutathionylation and reversal of drug resistance. *Biochem J* 440: 175–183, 2011.
55. de Souza GF, Yokoyama-Yasunaka JK, Seabra AB, Miguel DC, de Oliveira MG, and Uliana SR. Leishmanicidal activity of primary S-nitrosothiols against Leishmania major and Leishmania amazonensis: implications for the treatment of cutaneous leishmaniasis. *Nitric Oxide* 15: 209–216, 2006.
56. Denny PW, Gokool S, Russell DG, Field MC, and Smith DF. Acylation-dependent protein export in Leishmania. *J Biol Chem* 275: 11017–11025, 2000.
57. Di Domenico F, Cenini G, Sultana R, Perluigi M, Uberti D, Memo M, and Butterfield DA. Glutathionylation of the pro-apoptotic protein p53 in Alzheimer's disease brain: implications for AD pathogenesis. *Neurochem Res* 34: 727–733, 2009.
58. Ding M, Kwok LY, Schluter D, Clayton C, and Soldati D. The antioxidant systems in Toxoplasma gondii and the role of cytosolic catalase in defence against oxidative injury. *Mol Microbiol* 51: 47–61, 2004.
59. Dixon DP, Skipsey M, Grundy NM, and Edwards R. Stress-induced protein S-glutathionylation in Arabidopsis. *Plant Physiol* 138: 2233–2244, 2005.
60. Donald RG and Liberator PA. Molecular characterization of a coccidian parasite cGMP dependent protein kinase. *Mol Biochem Parasitol* 120: 165–175, 2002.
61. Duan S and Chen C. S-nitrosylation/denitrosylation and apoptosis of immune cells. *Cell Mol Immunol* 4: 353–358, 2007.
62. Emmer BT, Nakayasu ES, Souther C, Choi H, Sobreira TJ, Epting CL, Nesvizhskii AI, Almeida IC, and Engman DM. Global analysis of protein palmitoylation in African trypanosomes. *Eukaryot Cell* 10: 455–463, 2011.
63. Emmer BT, Souther C, Toriello KM, Olson CL, Epting CL, and Engman DM. Identification of a palmitoyl acyltransferase required for protein sorting to the flagellar membrane. *J Cell Sci* 122: 867–874, 2009.
64. Epstein WW, Lever D, Leining LM, Bruenger E, and Rilling HC. Quantitation of prenylcysteines by a selective cleavage reaction. *Proc Natl Acad Sci U S A* 88: 9668–9670, 1991.
65. Fahey RC, Newton GL, Arrick B, Overdank-Bogart T, and Aley SB. Entamoeba histolytica: a eukaryote without glutathione metabolism. *Science* 224: 70–72, 1984.
66. 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.
67. Fairlamb AH and Cerami A. Metabolism and functions of trypanothione in the Kinetoplastida. *Annu Rev Microbiol* 46: 695–729, 1992.
68. Fan Z, Lv G, Zhang L, Gan X, Wu Q, Zhong S, Yan G, and Lin G. Bioinformatics analysis and prediction for structure and function of nitric oxide synthase and similar proteins from Plasmodium berghei. *Asian Pac J Trop Med* 4: 1–4, 2011.
69. Farnsworth CC, Seabra MC, Ericsson LH, Gelb MH, and Glomset JA. Rab geranylgeranyl transferase catalyzes the geranylgeranylation of adjacent cysteines in the small GTPases Rab1A, Rab3A, and Rab5A. *Proc Natl Acad Sci U S A* 91: 11963–11967, 1994.
70. Field H, Blench I, Croft S, Field MC. Characterisation of protein isoprenylation in procyclic form Trypanosoma brucei. *Mol Biochem Parasitol* 82: 67–80, 1996.
71. Findlay VJ, Townsend DM, Morris TE, Fraser JP, He L, and Tew KD. A novel role for human sulfiredoxin in the reversal of glutathionylation. *Cancer Res* 66: 6800–6806, 2006.
72. Fletcher S, Cummings CG, Rivas K, Katt WP, Horney C, Buckner FS, Chakrabarti D, Sebti SM, Gelb MH, Van Voorhis WC, and Hamilton AD. Potent, Plasmodium-selective farnesyltransferase inhibitors that arrest the growth of malaria parasites: structure-activity relationships of ethylenediamine-analogue scaffolds and homology model validation. *J Med Chem* 51: 5176–5197, 2008.
73. Forrester MT, Foster MW, and Stamler JS. Assessment and application of the biotin switch technique for examining protein S-nitrosylation under conditions of pharmacologically induced oxidative stress. *J Biol Chem* 282: 13977–13983, 2007.
74. Foster MW. Methodologies for the characterization, identification and quantification of S-nitrosylated proteins. *Biochim Biophys Acta* 2011 [Epub ahead of print]; DOI: 10.1016/j.bbagen.2011.03.013
75. Foster MW, Hess DT, and Stamler JS. Protein S-nitrosylation in health and disease: a current perspective. *Trends Mol Med* 15: 391–404, 2009.
76. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, Bonetto V, Mengozzi M, Duffieux F, Miclet E, Bachi A, Vandekerckhove J, Gianazza E, and Ghezzi P. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc Natl Acad Sci U S A* 99: 3505–3510, 2002.
77. Fratelli M, Demol H, Puype M, Casagrande S, Villa P, Eberini I, Vandekerckhove J, Gianazza E, and Ghezzi P. Identification of proteins undergoing glutathionylation in oxidatively stressed hepatocytes and hepatoma cells. *Proteomics* 3: 1154–1161, 2003.
78. Fukumura D, Kashiwagi S, and Jain RK. The role of nitric oxide in tumour progression. *Nat Rev Cancer* 6: 521–534, 2006.
79. Furuya T, Kashuba C, Docampo R, Moreno SN. A novel phosphatidylinositol-phospholipase C of Trypanosoma cruzi that is lipid modified and activated during trypomastigote to amastigote differentiation. *J Biol Chem* 275: 6428–6438, 2000.
80. Gallogly MM and Mieyal JJ. Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress. *Curr Opin Pharmacol* 7: 381–391, 2007.
81. Gao XH, Zaffagnini M, Bedhomme M, Michelet L, Cassier-Chauvat C, Decottignies P, and Lemaire SD. Biochemical characterization of glutaredoxins from Chlamydomonas reinhardtii: kinetics and specificity in deglutathionylation reactions. *FEBS Lett* 584: 2242–2248, 2010.
82. Garcera A, Barreto L, Piedrafita L, Tamarit J, and Herrero E. Saccharomyces cerevisiae cells have three Omega class glutathione S-transferases acting as 1-Cys thiol transferases. *Biochem J* 398: 187–196, 2006.

83. Genestra M, Guedes-Silva D, Souza WJ, Cysne-Finkelstein L, Soares-Bezerra RJ, Monteiro FP, and Leon LL. Nitric oxide synthase (NOS) characterization in Leishmania amazonensis axenic amastigotes. *Arch Med Res* 37: 328–333, 2006.
84. Ghezzi P. Regulation of protein function by glutathionylation. *Free Radic Res* 39: 573–580, 2005.
85. Ghezzi P, Bonetto V, and Fratelli M. Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. *Antioxid Redox Signal* 7: 964–972, 2005.
86. Giles NM, Giles GI, and Jacob C. Multiple roles of cysteine in biocatalysis. *Biochem Biophys Res Commun* 300: 1–4, 2003.
87. Giles NM, Watts AB, Giles GI, Fry FH, Littlechild JA, and Jacob C. Metal and redox modulation of cysteine protein function. *Chem Biol* 10: 677–693, 2003.
88. Gilk SD, Gaskins E, Ward GE, and Beckers CJ. GAP45 phosphorylation controls assembly of the Toxoplasma myosin XIV complex. *Eukaryot Cell* 8: 190–196, 2009.
89. Glenn MP, Chang SY, Horney C, Rivas K, Yokoyama K, Pusateri EE, Fletcher S, Cummings CG, Buckner FS, Pendyala PR, Chakrabarti D, Sebti SM, Gelb M, Van Voorhis WC, and Hamilton AD. Structurally simple, potent, Plasmodium selective farnesyltransferase inhibitors that arrest the growth of malaria parasites. *J Med Chem* 49: 5710–5727, 2006.
90. Godsel LM and Engman DM. Flagellar protein localization mediated by a calcium-myristoyl/palmitoyl switch mechanism. *EMBO J* 18: 2057–2065, 1999.
91. Gonnord P, Delarasse C, Auger R, Benihoud K, Prigent M, Cuif MH, Lamaze C, and Kanellopoulos JM. Palmitoylation of the P2X7 receptor, an ATP-gated channel, controls its expression and association with lipid rafts. *FASEB J* 23: 795–805, 2009.
92. Green JL, Rees-Channer RR, Howell SA, Martin SR, Knuepfer E, Taylor HM, Grainger M, and Holder AA. The motor complex of Plasmodium falciparum: phosphorylation by a calcium-dependent protein kinase. *J Biol Chem* 283: 30980–30989, 2008.
93. Greenwood BM, Bojang K, Whitty CJ, and Targett GA. Malaria. *Lancet* 365: 1487–1498, 2005.
94. Greetham D, Vickerstaff J, Shenton D, Perrone GG, Dawes IW, and Grant CM. Thioredoxins function as deglutathionylase enzymes in the yeast Saccharomyces cerevisiae. *BMC Biochem* 11: 3, 2010.
95. Guedes PM, Oliveira FS, Gutierrez FR, da Silva GK, Rodrigues GJ, Bendhack LM, Franco DW, Do Valle Matta MA, Zamboni DS, da Silva RS, and Silva JS. Nitric oxide donor trans-[RuCl([15]aneN)NO] as a possible therapeutic approach for Chagas' disease. *Br J Pharmacol* 160: 270–282, 2010.
96. Gutierrez-Escobar AJ, Arenas AF, Villoria-Guerrero Y, Padilla-Londono JM, and Gomez-Marin JE. Toxoplasma gondii: molecular cloning and characterization of a nitric oxide synthase-like protein. *Exp Parasitol* 119: 358–363, 2008.
97. Hagan P. Schistosomiasis—a rich vein of research. *Parasitology* 136: 1611–1619, 2009.
98. Hara MR, Agrawal N, Kim SF, Cascio MB, Fujimuro M, Ozeki Y, Takahashi M, Cheah JH, Tankou SK, Hester LD, Ferris CD, Hayward SD, Snyder SH, and Sawa A. S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. *Nat Cell Biol* 7: 665–674, 2005.
99. Hare JM and Stamler JS. NO/redox disequilibrium in the failing heart and cardiovascular system. *J Clin Invest* 115: 509–517, 2005.
100. Harwaldt P, Rahlfs S, and Becker K. Glutathione S-transferase of the malarial parasite Plasmodium falciparum: characterization of a potential drug target. *Biol Chem* 383: 821–830, 2002.
101. Hemsley PA and Grierson CS. Multiple roles for protein palmitoylation in plants. *Trends Plant Sci* 13: 295–302, 2008.
102. Henderson GB, Fairlamb AH, and Cerami A. Trypanothione dependent peroxide metabolism in Crithidia fasciculata and Trypanosoma brucei. *Mol Biochem Parasitol* 24: 39–45, 1987.
103. Hernandez-Campos ME, Campos-Rodriguez R, Tsutsumi V, Shibayama M, Garcia-Latorre E, Castillo-Henkel C, and Valencia-Hernandez I. Nitric oxide synthase in Entamoeba histolytica: its effect on rat aortic rings. *Exp Parasitol* 104: 87–95, 2003.
104. Hess DT, Matsumoto A, Kim SO, Marshall HE, and Stamler JS. Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* 6: 150–166, 2005.
105. Hiltpold A, Frey M, Hulsmeier A, and Kohler P. Glycosylation and palmitoylation are common modifications of giardia variant surface proteins. *Mol Biochem Parasitol* 109: 61–65, 2000.
106. Hirano T, Kishi M, Sugimoto H, Taguchi R, Obinata H, Ohshima N, Tatei K, and Izumi T. Thioesterase activity and subcellular localization of acylprotein thioesterase 1/lysophospholipase 1. *Biochim Biophys Acta* 1791: 797–805, 2009.
107. Huang HH, Day L, Cass CL, Ballou DP, Williams CH, and Williams DL. Investigations on the Catalytic Mechanism of Thioredoxin Glutathione Reductase (TGR) from *Schistosoma mansoni. Biochemistry* 50: 5870–5882, 2011.
108. Hurd TR, Costa NJ, Dahm CC, Beer SM, Brown SE, Filipovska A, and Murphy MP. Glutathionylation of mitochondrial proteins. *Antioxid Redox Signal* 7: 999–1010, 2005.
109. 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.
110. 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.
111. Ibrahim M, Azzouz N, Gerold P, and Schwarz RT. Identification and characterisation of Toxoplasma gondii protein farnesyltransferase. *Int J Parasitol* 31: 1489–1497, 2001.
112. Iniesta V, Gomez-Nieto LC, and Corraliza I. The inhibition of arginase by N(omega)-hydroxy-l-arginine controls the growth of Leishmania inside macrophages. *J Exp Med* 193: 777–784, 2001.
113. Iozef R, Rahlfs S, Chang T, Schirmer H, and Becker K. Glyoxalase I of the malarial parasite Plasmodium falciparum: evidence for subunit fusion. *FEBS Lett* 554: 284–288, 2003.
114. Irigoin F, Cibils L, Comini MA, Wilkinson SR, Flohe L, and Radi R. Insights into the redox biology of Trypanosoma cruzi: Trypanothione metabolism and oxidant detoxification. *Free Radic Biol Med* 45: 733–742, 2008.
115. Ito H, Iwabuchi M, and Ogawa K. The sugar-metabolic enzymes aldolase and triose-phosphate isomerase are

targets of glutathionylation in Arabidopsis thaliana: detection using biotinylated glutathione. *Plant Cell Physiol* 44: 655–660, 2003.
116. Iwanaga T, Tsutsumi R, Noritake J, Fukata Y, and Fukata M. Dynamic protein palmitoylation in cellular signaling. *Prog Lipid Res* 48: 117–127, 2009.
117. Jacob C, Giles GI, Giles NM, and Sies H. Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew Chem Int Ed Engl* 42: 4742–4758, 2003.
118. James SL, Cheever AW, Caspar P, and Wynn TA. Inducible nitric oxide synthase-deficient mice develop enhanced type 1 cytokine-associated cellular and humoral immune responses after vaccination with attenuated Schistosoma mansoni cercariae but display partially reduced resistance. *Infect Immun* 66: 3510–3518, 1998.
119. Ji Y, Akerboom TP, Sies H, and Thomas JA. S-nitrosylation and S-glutathiolation of protein sulfhydryls by S-nitroso glutathione. *Arch Biochem Biophys* 362: 67–78, 1999.
120. Johnson MA, Macdonald TL, Mannick JB, Conaway MR, and Gaston B. Accelerated s-nitrosothiol breakdown by amyotrophic lateral sclerosis mutant copper,zinc-superoxide dismutase. *J Biol Chem* 276: 39872–39878, 2001.
121. Jordao FM, Saito AY, Miguel DC, de Jesus Peres V, Kimura EA, and Katzin AM. In vitro and in vivo antiplasmodial activities of risedronate and its interference with protein prenylation in plasmodium falciparum. *Antimicrob Agents Chemother* 55: 2026–2031, 2011.
122. Kaneki M, Shimizu N, Yamada D, and Chang K. Nitrosative stress and pathogenesis of insulin resistance. *Antioxid Redox Signal* 9: 319–329, 2007.
123. Kang R, Wan J, Arstikaitis P, Takahashi H, Huang K, Bailey AO, Thompson JX, Roth AF, Drisdel RC, Mastro R, Green WN, Yates JR, 3rd, Davis NG, and El-Husseini A. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. *Nature* 456: 904–909, 2008.
124. Kehr S, Jortzik E, Delahunty C, Yates Iii JR, Rahlfs S, and Becker K. Protein S-glutathionylation in malaria parasites. *Antioxid Redox Signal* 15: 2855–2865, 2011.
125. Kim S, Wing SS, and Ponka P. S-nitrosylation of IRP2 regulates its stability via the ubiquitin-proteasome pathway. *Mol Cell Biol* 24: 330–337, 2004.
126. Kohn AB, Lea JM, Moroz LL, and Greenberg RM. Schistosoma mansoni: use of a fluorescent indicator to detect nitric oxide and related species in living parasites. *Exp Parasitol* 113: 130–133, 2006.
127. Kohring K, Wiesner J, Altenkamper M, Sakowski J, Silber K, Hillebrecht A, Haebel P, Dahse HM, Ortmann R, Jomaa H, Klebe G, and Schlitzer M. Development of benzophenone-based farnesyltransferase inhibitors as novel antimalarials. *ChemMedChem* 3: 1217–1231, 2008.
128. Konstantinopoulos PA, Karamouzis MV, and Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* 6: 541–555, 2007.
129. Koshino I and Takakuwa Y. Disruption of lipid rafts by lidocaine inhibits erythrocyte invasion by Plasmodium falciparum. *Exp Parasitol* 123: 381–383, 2009.
130. 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.
131. Krauth-Siegel RL and Comini MA. Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. *Biochim Biophys Acta* 1780: 1236–1248, 2008.
132. 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.
133. Krauth-Siegel RL, Muller 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.
134. 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.
135. Kumagai M, Makioka A, Takeuchi T, and Nozaki T. Molecular cloning and characterization of a protein farnesyltransferase from the enteric protozoan parasite Entamoeba histolytica. *J Biol Chem* 279: 2316–2323, 2004.
136. 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.
137. Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, von Lowenhielm HB, Holmgren A, and Cotgreave IA. Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. *Arch Biochem Biophys* 406: 229–240, 2002.
138. Linder ME and Deschenes RJ. Palmitoylation: policing protein stability and traffic. *Nat Rev Mol Cell Biol* 8: 74–84, 2007.
139. Ling Y, Li ZH, Miranda K, Oldfield E, and Moreno SN. The farnesyl-diphosphate/geranylgeranyl-diphosphate synthase of Toxoplasma gondii is a bifunctional enzyme and a molecular target of bisphosphonates. *J Biol Chem* 282: 30804–30816, 2007.
140. Liu L, Hausladen A, Zeng M, Que L, Heitman J, and Stamler JS. A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. *Nature* 410: 490–494, 2001.
141. Liu W, Apagyi K, McLeavy L, and Ersfeld K. Expression and cellular localisation of calpain-like proteins in Trypanosoma brucei. *Mol Biochem Parasitol* 169: 20–26, 2010.
142. Loeffler IK and Bennett JL. A rab-related GTP-binding protein in Schistosoma mansoni. *Mol Biochem Parasitol* 77: 31–40, 1996.
143. Long XC, Bahgat M, Chlichlia K, Ruppel A, and Li YL. Detection of inducible nitric oxide synthase in Schistosoma japonicum and S. mansoni. *J Helminthol* 78: 47–50, 2004.
144. Lu W, Wei G, Pan W, and Tabel H. Trypanosoma congolense infections: induced nitric oxide inhibits parasite growth in vivo. *J Parasitol Res* 2011: 316067, 2011.
145. Lujan HD, Mowatt MR, Chen GZ, and Nash TE. Isoprenylation of proteins in the protozoan Giardia lamblia. *Mol Biochem Parasitol* 72: 121–127, 1995.
146. Marozkina NV and Gaston B. S-Nitrosylation signaling regulates cellular protein interactions. *Biochim Biophys Acta* 2011 [Epub ahead of print]; DOI: 10.1016/j.bbagen.2011.06.017
147. Maurer-Stroh S and Eisenhaber F. Refinement and prediction of protein prenylation motifs. *Genome Biol* 6: R55, 2005.
148. Meierjohann S, Walter RD, and Muller S. Glutathione synthetase from Plasmodium falciparum. *Biochem J* 363: 833–838, 2002.

149. Melchers J, Dirdjaja N, Ruppert T, and Krauth-Siegel RL. Glutathionylation of trypanosomal thiol redox proteins. *J Biol Chem* 282: 8678–8694, 2007.
150. Mills E, Price HP, Johner A, Emerson JE, and Smith DF. Kinetoplastid PPEF phosphatases: dual acylated proteins expressed in the endomembrane system of Leishmania. *Mol Biochem Parasitol* 152: 22–34, 2007.
151. Mitchell DA, Vasudevan A, Linder ME, and Deschenes RJ. Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J Lipid Res* 47: 1118–1127, 2006.
152. Moores SL, Schaber MD, Mosser SD, Rands E, O'Hara MB, Garsky VM, Marshall MS, Pompliano DL, and Gibbs JB. Sequence dependence of protein isoprenylation. *J Biol Chem* 266: 14603–14610, 1991.
153. Moskes C, Burghaus PA, Wernli B, Sauder U, Durrenberger M, and Kappes B. Export of Plasmodium falciparum calcium-dependent protein kinase 1 to the parasitophorous vacuole is dependent on three N-terminal membrane anchor motifs. *Mol Microbiol* 54: 676–691, 2004.
154. Murad F. Discovery of some of the biological effects of nitric oxide and its role in cell signaling. *Biosci Rep* 24: 452–474, 2004.
155. Murphy SC, Hiller NL, Harrison T, Lomasney JW, Mohandas N, and Haldar K. Lipid rafts and malaria parasite infection of erythrocytes. *Mol Membr Biol* 23: 81–88, 2006.
156. Murray HW, Berman JD, Davies CR, and Saravia NG. Advances in leishmaniasis. *Lancet* 366: 1561–1577, 2005.
157. Nahrevanian H. Involvement of nitric oxide and its up/down stream molecules in the immunity against parasitic infections. *Braz J Infect Dis* 13: 440–448, 2009.
158. Nakamura T and Lipton SA. Emerging roles of S-nitrosylation in protein misfolding and neurodegenerative diseases. *Antioxid Redox Signal* 10: 87–101, 2008.
159. Newman SF, Sultana R, Perluigi M, Coccia R, Cai J, Pierce WM, Klein JB, Turner DM, and Butterfield DA. An increase in S-glutathionylated proteins in the Alzheimer's disease inferior parietal lobule, a proteomics approach. *J Neurosci Res* 85: 1506–1514, 2007.
160. Ohkanda J, Buckner FS, Lockman JW, Yokoyama K, Carrico D, Eastman R, de Luca-Fradley K, Davies W, Croft SL, Van Voorhis WC, Gelb MH, Sebti SM, and Hamilton AD. Design and synthesis of peptidomimetic protein farnesyltransferase inhibitors as anti-Trypanosoma brucei agents. *J Med Chem* 47: 432–445, 2004.
161. Ohkanda J, Lockman JW, Yokoyama K, Gelb MH, Croft SL, Kendrick H, Harrell MI, Feagin JE, Blaskovich MA, Sebti SM, and Hamilton AD. Peptidomimetic inhibitors of protein farnesyltransferase show potent antimalarial activity. *Bioorg Med Chem Lett* 11: 761–764, 2001.
162. Okura M, Fang J, Salto ML, Singer RS, Docampo R, and Moreno SN. A lipid-modified phosphoinositide-specific phospholipase C (TcPI-PLC) is involved in differentiation of trypomastigotes to amastigotes of Trypanosoma cruzi. *J Biol Chem* 280: 16235–16243, 2005.
163. Osman A, Niles EG, and LoVerde PT. Characterization of the Ras homologue of Schistosoma mansoni. *Mol Biochem Parasitol* 100: 27–41, 1999.
164. Ostera G, Tokumasu F, Oliveira F, Sa J, Furuya T, Teixeira C, and Dvorak J. Plasmodium falciparum: food vacuole localization of nitric oxide-derived species in intraerythrocytic stages of the malaria parasite. *Exp Parasitol* 120: 29–38, 2008.
165. Park JW, Mieyal JJ, Rhee SG, and Chock PB. Deglutathionylation of 2-Cys peroxiredoxin is specifically catalyzed by sulfiredoxin. *J Biol Chem* 284: 23364–23374, 2009.
166. 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.
167. Paveto C, Pereira C, Espinosa J, Montagna AE, Farber M, Esteva M, Flawia MM, and Torres HN. The nitric oxide transduction pathway in Trypanosoma cruzi. *J Biol Chem* 270: 16576–16579, 1995.
168. Pe'er I, Felder CE, Man O, Silman I, Sussman JL, and Beckmann JS. Proteomic signatures: amino acid and oligopeptide compositions differentiate among phyla. *Proteins* 54: 20–40, 2004.
169. Pendyala PR, Ayong L, Eatrides J, Schreiber M, Pham C, Chakrabarti R, Fidock DA, Allen CM, and Chakrabarti D. Characterization of a PRL protein tyrosine phosphatase from Plasmodium falciparum. *Mol Biochem Parasitol* 158: 1–10, 2008.
170. Pepinsky RB, Zeng C, Wen D, Rayhorn P, Baker DP, Williams KP, Bixler SA, Ambrose CM, Garber EA, Miatkowski K, Taylor FR, Wang EA, and Galdes A. Identification of a palmitic acid-modified form of human Sonic hedgehog. *J Biol Chem* 273: 14037–14045, 1998.
171. Peterson TM, Gow AJ, and Luckhart S. Nitric oxide metabolites induced in Anopheles stephensi control malaria parasite infection. *Free Radic Biol Med* 42: 132–142, 2007.
172. Planey SL and Zacharias DA. Palmitoyl acyltransferases, their substrates, and novel assays to connect them (Review). *Mol Membr Biol* 26: 14–31, 2009.
173. Polonais V, Javier Foth B, Chinthalapudi K, Marq JB, Manstein DJ, Soldati-Favre D, and Frenal K. Unusual anchor of a motor complex (MyoD-MLC2) to the plasma membrane of Toxoplasma gondii. *Traffic* 12: 287–300, 2011.
174. Poole LB and Nelson KJ. Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr Opin Chem Biol* 12: 18–24, 2008.
175. 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.
176. Rai G, Sayed AA, Lea WA, Luecke HF, Chakrapani H, Prast-Nielsen S, Jadhav A, Leister W, Shen M, Inglese J, Austin CP, Keefer L, Arner ES, Simeonov A, Maloney DJ, Williams DL, and Thomas CJ. Structure mechanism insights and the role of nitric oxide donation guide the development of oxadiazole-2-oxides as therapeutic agents against schistosomiasis. *J Med Chem* 52: 6474–6483, 2009.
177. Rassi A, Jr., Rassi A, and Marin-Neto JA. Chagas disease. *Lancet* 375: 1388–1402, 2010.
178. Reddie KG and Carroll KS. Expanding the functional diversity of proteins through cysteine oxidation. *Curr Opin Chem Biol* 12: 746–754, 2008.
179. Rees-Channer RR, Martin SR, Green JL, Bowyer PW, Grainger M, Molloy JE, and Holder AA. Dual acylation of the 45 kDa gliding-associated protein (GAP45) in Plasmodium falciparum merozoites. *Mol Biochem Parasitol* 149: 113–116, 2006.
180. Reynaert NL, Ckless K, Guala AS, Wouters EF, van der Vliet A, and Janssen-Heininger YM. *In situ* detection of S-glutathionylated proteins following glutaredoxin-1 catalyzed cysteine derivatization. *Biochim Biophys Acta* 1760: 380–387, 2006.
181. Reynaert NL, van der Vliet A, Guala AS, McGovern T, Hristova M, Pantano C, Heintz NH, Heim J, Ho YS,

Matthews DE, Wouters EF, and Janssen-Heininger YM. Dynamic redox control of NF-kappaB through glutaredoxin-regulated S-glutathionylation of inhibitory kappaB kinase beta. *Proc Natl Acad Sci U S A* 103: 13086–13091, 2006.
182. Rivero A. Nitric oxide: an antiparasitic molecule of invertebrates. *Trends Parasitol* 22: 219–225, 2006.
183. Rockett KA, Awburn MM, Cowden WB, and Clark IA. Killing of Plasmodium falciparum *in vitro* by nitric oxide derivatives. *Infect Immun* 59: 3280–3283, 1991.
184. Rodrigues Goulart H, Kimura EA, Peres VJ, Couto AS, Aquino Duarte FA, and Katzin AM. Terpenes arrest parasite development and inhibit biosynthesis of isoprenoids in Plasmodium falciparum. *Antimicrob Agents Chemother* 48: 2502–2509, 2004.
185. Romao PR, Tovar J, Fonseca SG, Moraes RH, Cruz AK, Hothersall JS, Noronha-Dutra AA, Ferreira SH, and Cunha FQ. Glutathione and the redox control system trypanothione/trypanothione reductase are involved in the protection of Leishmania spp. against nitrosothiol-induced cytotoxicity. *Braz J Med Biol Res* 39: 355–363, 2006.
186. Rosenthal PJ. Cysteine proteases of malaria parasites. *Int J Parasitol* 34: 1489–1499, 2004.
187. Roskoski R, Jr. and Ritchie P. Role of the carboxyterminal residue in peptide binding to protein farnesyltransferase and protein geranylgeranyltransferase. *Arch Biochem Biophys* 356: 167–176, 1998.
188. Roth AF, Wan J, Bailey AO, Sun B, Kuchar JA, Green WN, Phinney BS, Yates JR, 3rd, and Davis NG. Global analysis of protein palmitoylation in yeast. *Cell* 125: 1003–1013, 2006.
189. Russo I, Oksman A, and Goldberg DE. Fatty acid acylation regulates trafficking of the unusual Plasmodium falciparum calpain to the nucleolus. *Mol Microbiol* 72: 229–245, 2009.
190. Russo I, Oksman A, Vaupel B, and Goldberg DE. A calpain unique to alveolates is essential in Plasmodium falciparum and its knockdown reveals an involvement in pre-S-phase development. *Proc Natl Acad Sci U S A* 106: 1554–1559, 2009.
191. Saaranen MJ, Salo KE, Latva-Ranta MK, Kinnula VL, and Ruddock LW. The C-terminal active site cysteine of *Escherichia coli* glutaredoxin 1 determines the glutathione specificity of the second step of peptide deglutathionylation. *Antioxid Redox Signal* 11: 1819–1828, 2009.
192. Sachs J and Malaney P. The economic and social burden of malaria. *Nature* 415: 680–685, 2002.
193. Sajid M and McKerrow JH. Cysteine proteases of parasitic organisms. *Mol Biochem Parasitol* 120: 1–21, 2002.
194. Salaun C, Greaves J, and Chamberlain LH. The intracellular dynamic of protein palmitoylation. *J Cell Biol* 191: 1229–1238, 2010.
195. Salsbury FR, Jr., Knutson ST, Poole LB, and Fetrow JS. Functional site profiling and electrostatic analysis of cysteines modifiable to cysteine sulfenic acid. *Protein Sci* 17: 299–312, 2008.
196. Sampathkumar R, Balasubramanyam M, Sudarslal S, Rema M, Mohan V, and Balaram P. Increased glutathionylated hemoglobin (HbSSG) in type 2 diabetes subjects with microangiopathy. *Clin Biochem* 38: 892–899, 2005.
197. Sarkar A, Saha P, Mandal G, Mukhopadhyay D, Roy S, Singh SK, Das S, Goswami RP, Saha B, Kumar D, Das P, and Chatterjee M. Monitoring of intracellular nitric oxide in leishmaniasis: its applicability in patients with visceral leishmaniasis. *Cytometry A* 79: 35–45, 2011.
198. Sayed AA, Simeonov A, Thomas CJ, Inglese J, Austin CP, and Williams DL. Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nat Med* 14: 407–412, 2008.
199. Schuppe-Koistinen I, Gerdes R, Moldeus P, and Cotgreave IA. Studies on the reversibility of protein S-thiolation in human endothelial cells. *Arch Biochem Biophys* 315: 226–234, 1994.
200. Seydel KB, Gaur D, Aravind L, Subramanian G, and Miller LH. Plasmodium falciparum: characterization of a late asexual stage golgi protein containing both ankyrin and DHHC domains. *Exp Parasitol* 110: 389–393, 2005.
201. Sha Y and Marshall HE. S-nitrosylation in the regulation of gene transcription. *Biochim Biophys Acta* 2011 [Epub ahead of print]; DOI: 10.1016/j.bbagen.2011.05.008
202. Shackelford RE, Heinloth AN, Heard SC, and Paules RS. Cellular and molecular targets of protein S-glutathiolation. *Antioxid Redox Signal* 7: 940–950, 2005.
203. Sharma A, Eapen A, and Subbarao SK. Parasite killing in Plasmodium vivax malaria by nitric oxide: implication of aspartic protease inhibition. *J Biochem* 136: 329–334, 2004.
204. Shelton MD, Chock PB, and Mieyal JJ. Glutaredoxin: role in reversible protein s-glutathionylation and regulation of redox signal transduction and protein translocation. *Antioxid Redox Signal* 7: 348–366, 2005.
205. Shenton D and Grant CM. Protein S-thiolation targets glycolysis and protein synthesis in response to oxidative stress in the yeast Saccharomyces cerevisiae. *Biochem J* 374: 513–519, 2003.
206. Shipston MJ. Ion channel regulation by protein palmitoylation. *J Biol Chem* 286: 8709–8716, 2011.
207. Silva JJ, Pavanelli WR, Pereira JC, Silva JS, and Franco DW. Experimental chemotherapy against Trypanosoma cruzi infection using ruthenium nitric oxide donors. *Antimicrob Agents Chemother* 53: 4414–4421, 2009.
208. Siman-Tov R and Ankri S. Nitric oxide inhibits cysteine proteinases and alcohol dehydrogenase 2 of Entamoeba histolytica. *Parasitol Res* 89: 146–149, 2003.
209. Sinensky M. Functional aspects of polyisoprenoid protein substituents: roles in protein-protein interaction and trafficking. *Biochim Biophys Acta* 1529: 203–209, 2000.
210. Singh SP, Wishnok JS, Keshive M, Deen WM, and Tannenbaum SR. The chemistry of the S-nitrosoglutathione/glutathione system. *Proc Natl Acad Sci U S A* 93: 14428–14433, 1996.
211. Sliskovic I, Raturi A, and Mutus B. Characterization of the S-denitrosation activity of protein disulfide isomerase. *J Biol Chem* 280: 8733–8741, 2005.
212. Smotrys JE and Linder ME. Palmitoylation of intracellular signaling proteins: regulation and function. *Annu Rev Biochem* 73: 559–587, 2004.
213. Sorek N, Bloch D, and Yalovsky S. Protein lipid modifications in signaling and subcellular targeting. *Curr Opin Plant Biol* 12: 714–720, 2009.
214. Sparaco M, Gaeta LM, Santorelli FM, Passarelli C, Tozzi G, Bertini E, Simonati A, Scaravilli F, Taroni F, Duyckaerts C, Feleppa M, and Piemonte F. Friedreich's ataxia: oxidative stress and cytoskeletal abnormalities. *J Neurol Sci* 287: 111–118, 2009.
215. Stafford JL, Neumann NF, and Belosevic M. Macrophage-mediated innate host defense against protozoan parasites. *Crit Rev Microbiol* 28: 187–248, 2002.
216. Stamler JS, Lamas S, and Fang FC. Nitrosylation. the prototypic redox-based signaling mechanism. *Cell* 106: 675–683, 2001.

217. Starke DW, Chock PB, and Mieyal JJ. Glutathione-thiyl radical scavenging and transferase properties of human glutaredoxin (thioltransferase). Potential role in redox signal transduction. *J Biol Chem* 278: 14607–14613, 2003.
218. Stuehr DJ. Mammalian nitric oxide synthases. *Biochim Biophys Acta* 1411: 217–230, 1999.
219. Totino PR, Daniel-Ribeiro CT, Corte-Real S, and de Fatima Ferreira-da-Cruz M. Plasmodium falciparum: erythrocytic stages die by autophagic-like cell death under drug pressure. *Exp Parasitol* 118: 478–486, 2008.
220. Touz MC, Conrad JT, and Nash TE. A novel palmitoyl acyl transferase controls surface protein palmitoylation and cytotoxicity in Giardia lamblia. *Mol Microbiol* 58: 999–1011, 2005.
221. 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.
222. Tovar J, Wilkinson S, Mottram JC, and Fairlamb AH. Evidence that trypanothione reductase is an essential enzyme in Leishmania by targeted replacement of the tryA gene locus. *Mol Microbiol* 29: 653–660, 1998.
223. Townsend DM, He L, Hutchens S, Garrett TE, Pazoles CJ, and Tew KD. NOV-002, a glutathione disulfide mimetic, as a modulator of cellular redox balance. *Cancer Res* 68: 2870–2877, 2008.
224. Townsend DM, Manevich Y, He L, Hutchens S, Pazoles CJ, and Tew KD. Novel role for glutathione S-transferase pi. Regulator of protein S-Glutathionylation following oxidative and nitrosative stress. *J Biol Chem* 284: 436–445, 2009.
225. Tsang AH, Lee YI, Ko HS, Savitt JM, Pletnikova O, Troncoso JC, Dawson VL, Dawson TM, and Chung KK. S-nitrosylation of XIAP compromises neuronal survival in Parkinson's disease. *Proc Natl Acad Sci U S A* 106: 4900–4905, 2009.
226. Tull D, Vince JE, Callaghan JM, Naderer T, Spurck T, McFadden GI, Currie G, Ferguson K, Bacic A, and McConville MJ. SMP-1, a member of a new family of small myristoylated proteins in kinetoplastid parasites, is targeted to the flagellum membrane in Leishmania. *Mol Biol Cell* 15: 4775–4786, 2004.
227. Uehara T, Nakamura T, Yao D, Shi ZQ, Gu Z, Ma Y, Masliah E, Nomura Y, and Lipton SA. S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441: 513–517, 2006.
228. Umansky V and Schirrmacher V. Nitric oxide-induced apoptosis in tumor cells. *Adv Cancer Res* 82: 107–131, 2001.
229. Valdez CA, Saavedra JE, Showalter BM, Davies KM, Wilde TC, Citro ML, Barchi JJ, Jr., Deschamps JR, Parrish D, El-Gayar S, Schleicher U, Bogdan C, and Keefer LK. Hydrolytic reactivity trends among potential prodrugs of the O2-glycosylated diazeniumdiolate family. Targeting nitric oxide to macrophages for antileishmanial activity. *J Med Chem* 51: 3961–3970, 2008.
230. Van Assche T, Deschacht M, da Luz RA, Maes L, and Cos P. Leishmania-macrophage interactions: Insights into the redox biology. *Free Radic Biol Med* 51: 337–351, 2011.
231. Van Voorhis WC, Rivas KL, Bendale P, Nallan L, Horney C, Barrett LK, Bauer KD, Smart BP, Ankala S, Hucke O, Verlinde CL, Chakrabarti D, Strickland C, Yokoyama K, Buckner FS, Hamilton AD, Williams DK, Lombardo LJ, Floyd D, and Gelb MH. Efficacy, pharmacokinetics, and metabolism of tetrahydroquinoline inhibitors of Plasmodium falciparum protein farnesyltransferase. *Antimicrob Agents Chemother* 51: 3659–3671, 2007.
232. 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.
233. Venturini G, Colasanti M, Salvati L, Gradoni L, and Ascenzi P. Nitric oxide inhibits falcipain, the Plasmodium falciparum trophozoite cysteine protease. *Biochem Biophys Res Commun* 267: 190–193, 2000.
234. Venturini G, Salvati L, Muolo M, Colasanti M, Gradoni L, and Ascenzi P. Nitric oxide inhibits cruzipain, the major papain-like cysteine proteinase from Trypanosoma cruzi. *Biochem Biophys Res Commun* 270: 437–441, 2000.
235. Vermeire JJ, Osman A, LoVerde PT, and Williams DL. Characterisation of a Rho homologue of Schistosoma mansoni. *Int J Parasitol* 33: 721–731, 2003.
236. Vincendeau P, Gobert AP, Daulouede S, Moynet D, and Mossalayi MD. Arginases in parasitic diseases. *Trends Parasitol* 19: 9–12, 2003.
237. 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.
238. Vouldoukis I, Mazier D, Moynet D, Thiolat D, Malvy D, and Mossalayi MD. IgE mediates killing of intracellular Toxoplasma gondii by human macrophages through CD23-dependent, interleukin-10 sensitive pathway. *PLoS One* 6: e18289, 2011.
239. Wang W, Oliva C, Li G, Holmgren A, Lillig CH, and Kirk KL. Reversible silencing of CFTR chloride channels by glutathionylation. *J Gen Physiol* 125: 127–141, 2005.
240. Webb Y, Hermida-Matsumoto L, and Resh MD. Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. *J Biol Chem* 275: 261–270, 2000.
241. Wiemer AJ, Hohl RJ, and Wiemer DF. The intermediate enzymes of isoprenoid metabolism as anticancer targets. *Anticancer Agents Med Chem* 9: 526–542, 2009.
242. Wiesner J, Kettler K, Sakowski J, Ortmann R, Katzin AM, Kimura EA, Silber K, Klebe G, Jomaa H, and Schlitzer M. Farnesyltransferase inhibitors inhibit the growth of malaria parasites *in vitro* and *in vivo*. *Angew Chem Int Ed Engl* 43: 251–254, 2004.
243. Winter-Vann AM and Casey PJ. Post-prenylation-processing enzymes as new targets in oncogenesis. *Nat Rev Cancer* 5: 405–412, 2005.
244. Wouters MA, Fan SW, and Haworth NL. Disulfides as redox switches: from molecular mechanisms to functional significance. *Antioxid Redox Signal* 12: 53–91, 2010.
245. Wu C, Liu T, Chen W, Oka S, Fu C, Jain MR, Parrott AM, Baykal AT, Sadoshima J, and Li H. Redox regulatory mechanism of transnitrosylation by thioredoxin. *Mol Cell Proteomics* 9: 2262–2275, 2010.
246. Xiong Y, Uys JD, Tew KD, and Townsend DM. S-Glutathionylation: From Molecular Mechanisms to Health Outcomes. *Antioxid Redox Signal* 15:233–270, 2011.
247. Yang W, Di Vizio D, Kirchner M, Steen H, and Freeman MR. Proteome scale characterization of human S-acylated

proteins in lipid raft-enriched and non-raft membranes. *Mol Cell Proteomics* 9: 54–70, 2010.
248. Yeh DC, Duncan JA, Yamashita S, and Michel T. Depalmitoylation of endothelial nitric-oxide synthase by acyl-protein thioesterase 1 is potentiated by Ca(2+)-calmodulin. *J Biol Chem* 274: 33148–33154, 1999.
249. Yokoyama K, Gillespie JR, Van Voorhis WC, Buckner FS, and Gelb MH. Protein geranylgeranyltransferase-I of Trypanosoma cruzi. *Mol Biochem Parasitol* 157: 32–43, 2008.
250. Yokoyama K, Lin Y, Stuart KD, and Gelb MH. Prenylation of proteins in Trypanosoma brucei. *Mol Biochem Parasitol* 87: 61–69, 1997.
251. Yokoyama K, Trobridge P, Buckner FS, Scholten J, Stuart KD, Van Voorhis WC, and Gelb MH. The effects of protein farnesyltransferase inhibitors on trypanosomatids: inhibition of protein farnesylation and cell growth. *Mol Biochem Parasitol* 94: 87–97, 1998.
252. Yokoyama K, Trobridge P, Buckner FS, Van Voorhis WC, Stuart KD, and Gelb MH. Protein farnesyltransferase from Trypanosoma brucei. A heterodimer of 61- and 65-kda subunits as a new target for antiparasite therapeutics. *J Biol Chem* 273: 26497–26505, 1998.
253. Zaffagnini M, Michelet L, Marchand C, Sparla F, Decottignies P, Le Marechal P, Miginiac-Maslow M, Noctor G, Trost P, and Lemaire SD. The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-phosphate dehydrogenase is selectively regulated by glutathionylation. *FEBS J* 274: 212–226, 2007.
254. Zhu J, Krishnegowda G, and Gowda DC. Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: the requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-kappaB pathways for the expression of proinflammatory cytokines and nitric oxide. *J Biol Chem* 280: 8617–8627, 2005.

Address correspondence to:
Dr. Katja Becker
Interdisciplinary Research Center
Justus Liebig University
Heinrich-Buff-Ring 26-32
D-35392 Giessen
Germany

E-mail: katja.becker@ernaehrung.uni-giessen.de

Date of first submission to ARS Central, September 5, 2011; date of acceptance, November 15, 2011.

Abbreviations Used

CDPK = calcium-dependent protein kinase
CP = cysteine protease
Cys-SS-Cys = intra- or intermolecular disulfide or cysteinylation
Cys-SSG = *S*-glutathionylation
Cys-S-NO = *S*-nitrosylation
GAP45 = 45kDa gliding-associated protein
GSH = reduced glutathione
GSNO = *S*-nitrosoglutathione
GSSG = oxidized glutathione
HXGPRT = hypoxanthine-xanthine-guanine phosphoribosyltransferase
IMC = inner membrane complex
NO = nitric oxide
NOS = nitric oxide synthase
PfCDPK1 = *Plasmodium falciparum* calcium-dependent protein kinase 1
PTM = posttranslational modification
RNS = reactive nitrogen species
TGR = thioredoxin glutathione reductase