Depletion of *Plasmodium berghei* Plasmoredoxin Reveals a Non-Essential Role for Life Cycle Progression of the Malaria Parasite

**Kathrin Buchholz**1,2,3, **Stefan Rahlfs**1, **R. Heiner Schirmer**2, **Katja Becker**1*, **Kai Matuschewski**2*

1 Interdisciplinary Research Centre, Justus-Liebig University, Giessen, Germany, 2 Biochemistry Centre, Ruprecht-Karls University, Heidelberg, Germany, 3 Department of Parasitology, School of Medicine, Heidelberg University, Heidelberg, Germany

**Abstract**

Proliferation of the pathogenic *Plasmodium* asexual blood stages in host erythrocytes requires an exquisite capacity to protect the malaria parasite against oxidative stress. This function is achieved by a complex antioxidant defence system composed of redox-active proteins and low MW antioxidants. Here, we disrupted the *P. berghei* plasmoredoxin gene that encodes a parasite-specific 22 kDa member of the thioredoxin superfamily. The successful generation of plasmoredoxin knockout mutants in the rodent model malaria parasite and phenotypic analysis during life cycle progression revealed a non-vital role in *vivo*. Our findings suggest that plasmoredoxin fulfils a specialized and dispensable role for *Plasmodium* and highlights the need for target validation to inform drug development strategies.

---

**Introduction**

*Plasmodium*, the etiologic agent of malaria, is a unicellular facultative intracellular parasite of the phylum *Apicomplexa*. A hallmark of the malaria parasite is its remarkable capacity to specifically invade and replicate inside red blood cells. This intracellular proliferation phase ultimately leads to the disease known as malaria. Due to the high metabolic rates of the rapidly growing and multiplying parasite, large quantities of toxic redox-active by-products are generated. Additional reactive oxygen and nitrogen species are generated by immune effector cells of the host in response to parasite infection and during hemoglobin degradation in the food vacuole of the parasite. Therefore, inside erythrocytes, the ability of *Plasmodium* to defend itself against oxidative damage is of vital importance for parasite survival [1,2], and it appears to be highly effective in this respect [3]. *Plasmodium* employs multiple biochemical pathways that mediate antioxidant defense and redox-regulation and play a central role in pathogenesis [4–9].

In most eukaryotic organisms, redox-active enzymes, such as catalase, superoxide dismutase, and peroxidases as well as an enzymatic cascade that generates relaxed electron donors, i.e. glutathione (GSH) and thioredoxin (Trx), sustain the cellular redox homeostasis [10]. This redox network is split into two major arms, the GSH and the Trx system, that serve complementary functions in antioxidant defense and DNA synthesis. Interestingly, the malarial parasite *Plasmodium* lacks two central antioxidant enzymes: (i) catalase that typically detoxifies hydrogen peroxide and (ii) a classical glutathione peroxidase, a selenoenzyme that reduces lipid hydroperoxides to their alcohols [9]. This apparent deficiency further underscores the central importance of the thioredoxin system in the parasite. In good agreement, Trx reductase, which transfers electrons from NADPH to Trx, appears to perform vital functions for asexual development of the malaria parasite *in vitro* and is considered an attractive target for antimalarial drug development [11].

Intriguingly, malaria parasites possess-in addition to the classical thioredoxins-a *Plasmodium*-specific member of the thioredoxin superfamily termed plasmoredoxin (Plrx) (*P. falciparum* GenBank AAF87222) [12]. Plrx is a 22 kDa dithiol protein with the unique active site sequence WCKYC. Plrx is not reduced by thioredoxin reductase but can react with glutaredoxin and glutathione. Although a non-enzymatic reaction between reduced Trx and glutathione disulfide (GSSG) has been described in insects, which lack glutathione reductase [13], the physiologic electron donor for *P. falciparum* plasmoredoxin still remains to be identified. As described for certain thioredoxins and glutaredoxins, Plrx has been shown to serve as electron donor for ribonucleotide reductase. Furthermore, the protein is capable of reducing disulfide bonds in general and in particular *P. falciparum* thioredoxin peroxidase 1, the major cytosolic peroxiredoxin of the parasite [12,14].

In this study, we addressed the cellular role of Plrx in the rodent malaria model parasite *Plasmodium berghei*. Targeted gene disruption permits drug target validation or elucidation of the *in vivo* role of the gene product. The corresponding predicted experimental outcomes are refractoriness to gene targeting, which would correlate with a vital role in asexual blood stage development, or a detectable phenotype during life cycle progression, respectively. Because of the potential to design tailor-made inhibitors of the *Plasmodium* redox network as innovative antimalarial drugs [4,5], target validation of individual redox-active enzymes paves the way for future drug discovery approaches. Our findings suggest that loss of Plrx...
function does not affect parasite development under normal growth conditions exemplifies the important role of reverse genetics in guiding drug development against malaria.

**Results**

**Generation of Plrx(-) parasites**

To test whether plasmoredoxin is important for asexual replication of *P. berghei*, we first targeted the *PbPlrx* gene with an integration vector that disrupts the gene locus via a single cross over event. After a single transfection we successfully integrated the disruption plasmid (data not shown). To confirm our unexpected finding that *PbPlrx* can be disrupted we constructed a replacement vector containing the *PbPlrx* 5’ and 3’ UTRs that flank the positive selectable marker, which confers resistance to the antifolate pyrimethamine (Fig. 1A). Upon a double cross over event this vector is predicted to disrupt the entire *PbPlrx* open reading frame (ORF). After continuous selection with oral pyrimethamine a resistant population was obtained and genotyped (data not shown). This parental parasite population contained the correct integration mixed with WT parasites and was used for cloning of four independent parasite *Plrx*-deficient lines, named *Plrx(-)Rep*. Replacement-specific PCR analysis verified the correct replacement event after homologous recombination (Fig. 1B). To confirm the absence of *Plrx* transcripts in *Plrx(-) parasites*, RT-PCR and subsequent cDNA synthesis was performed with poly(A)+ RNA from mixed blood stages (Fig. 1C). In good agreement with the previous expression profiling of *PfPlrx* [12], we detected the *PbPlrx* transcript in asexual blood stage parasites of the WT. As predicted, no *Plrx* transcripts were detected in the knockout parasite lines. Moreover, Western blot analysis of *Plrx(-) blood stages* with a *PbPlrx*-specific anti-peptide antiserum confirmed complete absence of the protein in *Plrx(-) parasites* (Fig. 1D). Together, the successful generation of *Plrx*-deficient parasites demonstrates that this gene is not essential for proliferation of the asexual blood stages.

*Plrx* is dispensable for *in vivo* and *in vitro* growth of asexual blood stages

To test whether *Plrx* serves an auxiliary role during blood stage growth we first performed an *in vivo* growth assay by intravenous injection of 1,000 asexual parasites, followed by parasitemia counts every 12 hours post infection (Fig. 2A). Notably, proliferation of

![Figure 1. Targeted deletion of the *P. berghei* plasmoredoxin gene.](https://www.plosone.org/article/image?doi=10.1371/journal.pone.0002474.g001)
parasites. Five and six naïve animals were injected intravenously with 1,000 WT and Plrx(-) parasites, respectively. Treatment started immediately after infection with 1,000 WT and Plrx(-) parasites, respectively. Experiments revealed rapid elimination of WT parasites with 100 mg/kg body weight MB (data not shown). Even with enhanced oxidative stress in vivo Plrx-deficient parasites grow indistinguishable from WT parasites (Fig. 2B), excluding a central role of P. berghei Plrx in antioxidant defense.

Depletion of Plrx induces only weak changes of transcript levels of selected redox proteins

Since our in vitro and in vivo data exclude an essential function of Plrx to maintain the parasite's redox equilibrium, we extended our analysis of the Plrx-deficient strain to expression profiling of selected redox proteins. This analysis was expected to further reveal the modulation of intracellular redox networks. We studied the effects of the Plrx-deletion on mRNA levels of genes related to cellular redox metabolism with a focus on the cytosolic components because Plrx is a cytosolic member of the antioxidant network [12]. Gene transcript levels were measured by quantitative real-time RT-PCR and the effect on a target gene is reported as differences in comparison to a WT control population (Fig. 3). Transcript levels of mRNA for the two major sustainers of redox homeostasis, thioredoxin reductase (TrxR) and glutathione reductase (GrR) increased only slightly. Plrx was previously shown to directly interact with these two systems [12], thereby potentially acting as an additional antioxidant defence line of Plasmodium. The prediction was that deletion of Plrx may be accompanied by a compensatory upregulation of functional paralogues that balance the reducing capacity of Plrx. Such a function can most likely be fulfilled by thioredoxin (Trx) and/or glutathione (GSH). While this assumption is supported by the weak increase of Trx mRNA levels, GSH cannot be tested directly because it is only a tripeptide.

Another member of the thioredoxin superfamily, glutaredoxin (Grx) did not change significantly. Moreover, Plrx-deficient parasites show a slight decrease in mRNA levels of thioredoxin peroxidase 1 (TPx1), the major cytosolic peroxiredoxin of the parasite, and ribonucleotide reductase (RiboR). Collectively, these data show that depletion of PlPhbx caused only weak alterations in gene expression of selected members of the cytosolic redox network compared to wild type parasites.
Plasmodium parasites (Table 2). When tested in vivo infected cells and produced high numbers of mature liver stage infected with Plrx(-) sporozoites were indistinguishable from WT parasites in sexual development, which is a prerequisite for completion of the Plasmodium life cycle (Table 2). Together these data exclude a vital role for Plrx in Plasmodium life cycle progression under standard conditions.

**Discussion**

We initiated this study to test the potential of plasmoredoxin (Plrx), a Plasmodium specific member of the thioredoxin superfamily [12], as a novel antimalarial drug target. Using classical reverse genetics we could demonstrate that Plrx is dispensable for Plasmodium development inside its host cells. This finding rejects future drug discovery efforts that aim at specifically targeting Plrx, most likely even in combination with existing antimalarial drugs. Successful generation of Plrx(-) mutants permitted a detailed observation of the in vivo function of Plrx during life cycle progression of the malaria parasite. Again, no vital role at any stage of the parasite life cycle was revealed. Therefore, specific targeting of plasmoredoxin is not suitable either for transmission-blocking or causal-prophylactic malaria intervention strategies.

The redox-active proteins thioredoxin and glutaredoxin are founding members of the thioredoxin superfamily. Additional members include tryparedoxin of Trypanosomes, the protein disulfide isomerase and a few bacterial disulfide bondforming proteins. This group of proteins carries out oxidation and reduction reactions based on the chemistry of the catalytic cysteine residues. The unifying features of all family members are (i) the typical active site motif C-X-X-C and (ii) similarity in the overall tertiary protein structure, the so-called thioredoxin fold, despite low overall amino acid sequence similarity [18]. Intriguingly, homozygous deletions of the mouse cytoplasmic Trx1 or the mitochondrial isoform Trx2 resulted in early embryonic lethality indicating key roles of the thioredoxin system in the development of multicellular organisms [19,20]. In analogy, the recent characterization of the Plasmodium-specific dithiol-disulfide oxidoreductase Plrx raised the attractive possibility that the protein performs an important function for the intracellular life style of the malaria parasite.

Thus far, five thioredoxin related proteins have been identified in Plasmodium falciparum, in addition to plasmoredoxin [21]. Cytosolic Trx1 is the major substrate of thioredoxin reductase. It reduces thioredoxin-dependent peroxidases and is also capable of reacting with peroxides, dehydroascorbate, lipoic acid, and lipoyamide directly. Trx2 (and Tpx2) were shown to be mitochondrial [22]. Trx2 also displays general disulfide reducing activity and serves as electron donor for thioredoxin peroxidases and GSSG. Trx3, which also carries a targeting sequence, has been shown to be redox active and reducible by PfTrxR. In addition, two thioredoxin-like proteins, Tlp1, which might
represent a small dynel subunit, and Tlp2 have been reported in *Plasmodium falciparum* and might exhibit partially overlapping functions with classical thioredoxins.

An explanation for the non-vital role of *Plrx* in malarial parasites might thus be redundancy in the function of the multiple members of the thioredoxin superfamily. There is precedence from other systems. For instance, the bacterial cytoplasm typically contains triplets of the thioredoxin superfamily. There is precedence from other Plasmodium falciparum functions with classical thioredoxins.

Drugs

Experimental animals

Sprague-Dawley rats, NMRI mice and C57bl/6 mice were obtained from Charles Rivers Laboratories. All animal experiments were conducted in accordance with European regulations and approved by the state authorities (Regierungsspräsidium Karlsruhe).

Drugs

The following drugs were used: chloroquine diphosphate and amodiaquine (Sigma-Aldrich, Steinheim, Germany), melloquine-HCl (Roche, Mannheim, Germany), artemisinin (Aldrich Chemical Co., Milwaukee, Wis.) as well as methylene blue (Roth, Karlsruhe, Germany).

Plasmoredoxin targeting vectors and *P.berghei* transfection

Two independent strategies, gene disruption and gene replacement, were used to disrupt the *P.berghei* plasmoredoxin gene using a standard *P.berghei* transfection vector, which contains the mutated *Toxoplasma gondii* dhfr/tgs gene as a marker for positive selection with the antibiotic pyrimethamine [32]. The *Plrx* integration vector was generated by combining two PCR fragments that were amplified using the following primer pairs and *P.berghei* genomic DNA as template: *PlPlrx*for1 (5'-CCGGGATCCATTACCTACGAA- AATGAAGACC-GCC-3'; BamHI site is underlined and *PlPlrx*rev1 (5'-GGACTAGTATATATATATATTCTAAAAAAGGG-3'; SpeI site is underlined), and *PlPlrx*for2 (5'-GGGATCTGTGATGCAAATACAGATTATATCAATT-3'; *Pb*3 site is underlined) and *PlPlrx*rev2 (5'-TCCGGGGCGGATTATTTTGAAT- GTTGTCAATACAGATTATCAATT-3'; *SacII* site is underlined). For integration of the targeting vector via single cross-over the introduced cleavage site (*SpeI*) was used. The *Plrx* replacement vector was generated using the primers *PlPlrx*for2 (5'-GGGATCTGTGATGCAAATACAGATTATCAATT-3'; *Pb*3 site is underlined) and *PlPlrx*rev2 (5'-TCCGGGGCGGATTATTTTGAATGCTTAATACAGATTATCAAT-3'; *SacII* site is underlined). Sequence analyses confirmed the correct sequence of the different plasmids. *P.berghei* transfection and positive selection was done by the Nucleofector technology [33]. Clonal parasites were obtained by limited dilution of single parasites generated by combining two PCR fragments that were amplified from genomic DNA as a marker for positive selection with 5'-CGCGGACGAGCCCGGAT-GCCAGATCC-TCC-3' and Plrxtestrev (5'-GGACGTGTTTGGCTA- CTTCC-3', as well as 5'-testfor (5'-GCCAATGTACCATGTA- CACAGC-3') and Plrg (5'-CCAACCTCAATTTAATAGTG- GTTATGTG-3') were used. The resulting PCR products were sequenced to confirm the correct gene replacement and the accurate generation of the recombinant *Plrx(-)locus*. To test for the presence of residual WT parasites a *Plrx*-specific primer pair, Plrxstart (5'-ATGGCAATGAAAGTGATAAAACG-3') and Plrxend (5'- TCTTTAATGAAATGCAAATAAGTCCC-3') and Plrg (5'- ATGGCAATGAAAGTGATAAAACG-3') were used. For the control amplification a glutathione reductase-specific primer pair, *PlGrfor* (5'-TTGGCTATAGGTGTTGTTGATGCC-3') and *PlGrrev* (5'-AGTCGAATGCTAAAATCCTG-3') was used. We obtained four independent *Plrx(-)REP* clonal parasite populations that were phenotypically identical. Detailed analysis was performed with one representative clone.

Immunoblotting

Three rats were infected with WT and *Plrx(-)* blood stage parasites, respectively, and cytosolic parasite proteins were extracted using 2M Urea buffer. For immunoblot analysis, proteins were separated on 15% polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad) by electrophoretic blotting. Plrx (21.5 KDA) was detected by incubation of membranes with a polyclonal anti-*P.berghei* Plrx antiserum (dilution 1:1,500). This antiserum was obtained by immunization of rabbits with synthetic peptides of the N-terminal region of Plrx (Plrx1: CYYKNNLKKIDSSYFQSK) and Plrx2: CKVDKALEHSTQNEAPSK) (Eurogentec, Seraing, Belgium). Bound antibodies were detected using peroxidase coupled anti-rabbit and anti-mouse antibodies, to detect *Pb*Plrx and actin, respectively. Immunostained proteins were visualized with enhanced chemiluminescence detection (Pierce). Recombinant *P.berghei* Plrx was used as a positive control (obtained according to [12]). The anti-Dicyostelium discoidum actin antiserum, which cross-reacts with

Materials and Methods

Experimental animals

Sprague-Dawley rats, NMRI mice and C57bl/6 mice were obtained from Charles Rivers Laboratories. All animal experiments were conducted in accordance with European regulations and approved by the state authorities (Regierungsspräsidium Karlsruhe).
apicomplexan actins, was kindly provided by Dr. Markus Meissner (Heidelberg).

**Determination of IC₅₀ values**

NMRI mice were infected with *P. berghei* WT and Plrx(-) parasites. Collection of infected erythrocytes was done by cardiac puncture. Infected erythrocytes were cultured in an isotopic drug sensitivity assay based on incorporation of radioactive [³H] hypoxanthine (protocol kindly provided by Sergio Wittlin, Basel, Switzerland). Briefly, serial dilutions of various drugs were prepared in 96-well microtiter plates (Nunc). 100 µl infected red blood cells, resulting in 5% hematocrit and 1-5% parasitemia, were added per well and incubated in hypoxanthine-free medium at 37°C in a gas mixture consisting of 94% N₂, 3% O₂ and 3% CO₂. After 16 h incubation, 0.5 µCi of [³H] hypoxanthine (Amersham Pharmacia) in 50 µl medium was added and plates were incubated for an additional 8 h. Parasites were harvested onto glass fiber filters (Perkin-Elmer, Rodgau-Jugelsheim, Germany), washed and dried. Radioactivity was counted using a β-counter (Matrix 9600; Packard). The results were recorded as counts per minute (cpm) per well at each drug concentration and growth inhibition was expressed as percent 3H incorporation compared with untreated controls. Fifty percent inhibitory concentrations (IC₅₀) were calculated from plotted data. All experiments were done in duplicate from at least three independent mouse infections each.

**Expression profiles of Plasmodium WT and Plrx(-) parasites using quantitative real-time RT-PCR**

For qRT-PCR analyses poly(A)⁺ RNA was isolated using oligo(dT) columns (Macherey-Nagel, Düren, Germany) from mixed blood stage of *Plasmodium berghei* WT and Plrx(-) lines. After a DNase treatment, aliquots of 800 ng of each sample were reversely transcribed to cDNA using a Abgene cDNA synthesis kit and oligo(dT) primers (Ambigen, Hamburg, Germany). The SYBR Green Jumpstart Taq Ready Mix (Sigma, Steinheim, Germany) was utilized for quantitative real-time PCR on Rotor-Gene 3000 Real-Time PCR system (Corbett Research, Sydney, Australia) using the primers listed in Table 3. Specificity of the amplification products was confirmed by melting curve analysis; a no-template control was calculated from plotted data. All experiments were done in duplicate from at least three independent mouse infections each.

**Table 3. Oligonucleotide primers used for the quantitative real time PCR.**

| Primer name | Sequence | Product Size (bp) |
|-------------|----------|-------------------|
| Pb stRNA a  | TAGTGGCTGACATAGAGGTT | 158 bp |
| Pb stRNA a as | CTCTGACACTTCTCCATTCA | 138 bp |
| Pb GR b as | CTTCCTCTTACTAATGGTG | 125 bp |
| Pb TxnR c as | ATGGAATGACAAATGAAAAAT | 125 bp |
| Pb Gnx d as | GTGAAATGTAGTTAGTACTGCC | 149 bp |
| Pb Gnx d | GTGAAATGTAGTTAGTACTGCC | 197 bp |
| Pb TxnR c | TAAAGCCCTTCCTATGGCTTC | 160 bp |
| Pb Tpx-1 d as | TCGGATGGTTCTGATGCCTCC | 165 bp |
| Pb RibOR e as | GCGATAATCTACTAGAATTTTAGG | 165 bp |

| Primer name | Sequence | Product Size (bp) |
|-------------|----------|-------------------|
| Pb GR b | TGGACCTTGCAAGAAGATAGCTC | 197 bp |
| Pb TxnR c | TCAAAAGCTCCCTATGGCTCC | 160 bp |
| Pb Tpx-1 d as | TCGGATGGTTCTGATGCCTCC | 165 bp |
| Pb RibOR e as | GCGATAATCTACTAGAATTTTAGG | 165 bp |

*Pb* stRNA: seryl-tRNA synthetase, *GR*: Glutathione Reductase, *TxnR*: Thioredoxin Reductase, *Gnx*: Glutaredoxin, *Trx*: Thioredoxin, *Tpx*: Peroxiredoxin, *RibOR*: Ribonucleotide reductase; s: sense primer, a: antisense primer

doi:10.1371/journal.pone.0002474.t003

checked for gametocyte formation and exflagellation of microgametes prior to mosquito feeding. For mosquito infection, *A. stephensi* mosquitoes were allowed to bloodfeed on anesthetized mice for 15 minutes. Dissection of mosquitoes was conducted at days 10, 14 and 17 in order to determine infectivity and sporozoite numbers in midguts and salivary glands, respectively. Gliding motility was assessed by deposition of sporozoites onto precoated glass coverslips and visualisation by indirect immunofluorescence using a primary antibody against the *P. berghei* circumsporozoite protein (CSP) [35] followed by detection with an Alexa Fluor 488-conjugated anti-mouse antibody. To analyse liver stage development sporozoites were deposited onto a semi-confluent monolayer of hepatoma cells (HuH7) and incubated for 2 h, followed by washing and incubation in cell culture medium. Liver stages were detected after 48 h with a primary antibody directed against the *P. berghei* heat shock protein 70 (HSP70) [36], followed by an Alexa Fluor 488-conjugated anti-mouse antibody. To analyse sporozoite infectivity in vivo, Sprague-Dawley rats were infected intravenously with 10,000 WT or Plrx(-) sporozoites, respectively. Parasitemia was followed by daily examination of Giemsa-stained blood smears. The occurrence of a single parasite marked the first day of patenty.

**Author Contributions**

Conceived and designed the experiments: KM KBuchholz SR RS KBecker. Performed the experiments: KBuchholz SR. Analyzed the data: KM KBuchholz SR RS KBecker. Wrote the paper: KM KBuchholz SR KBecker.

**References**

1. Becker K, Tilley L, Vennerstrom JL, Roberts D, Rogerson S, et al. (2004) Oxidative stress in malaria parasite-infected erythrocytes: host-parasite interactions. Int J Parasitol 34: 163–189.

2. Muller S (2004) Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. Mol Microbiol 53: 1291–1305.

3. Sobolewski P, Gramaglia I, Frangos JA, Intaglietta M, van der Heyde H (2005) Plasmodium berghei resists killing by reactive oxygen species. Infect Immun 73: 6704–6710.

4. Krauth-Siegel RL, Bauer H, Schirmer RH (2005) Dithiol proteins as guardians of the intracellular redox milieu in parasites: old and new drug targets in Plasmodium in Malaria.

[Oligonucleotide primers used for the quantitative real time PCR.](#)
trypanosomes and malaria-causing plasmodia. Angew Chem Int Ed Engl 44: 690–715.
5. Rahli S, Becker K (2006) Interference with redox-active enzymes as a basis for the design of antimalarial drugs. Mini Rev Med Chem 6: 163–176.
6. Rahli S, Schirmer RH, Becker K (2002) The thioredoxin system of Plasmodium falciparum and other parasites. Cell Mol Life Sci 59: 1024–1041.
7. Rahli S, Nickel C, Deponte M, Schirmer RH, Becker K (2003) Plasmodium falciparum thioredoxins and glutaredoxins as central players in redox metabolism. Redox Rep 8: 246–250.
8. Müller S (2003) Thioredoxin reductase and glutathione synthesis in Plasmodium falciparum. Redox Rep 8: 231–235.
9. Becker K, Koncarevic S, Hunt NH (2005) Oxidative stress and antioxidant defense in malarial parasites, in: Sherman I, ed (2005) Molecular Approaches to Malaria, Herndon, VA: American Society of Microbiology Press. pp 365–383.
10. Rietsch A, Beekwijk J (1998) The genetics of disulphide bond metabolism. Annu Rev Genet 32: 163–184.
11. Knauski Z, Gilberger TW, Walter RD, Cowman AF, Müller S (2002) Thioredoxin reductase is essential for the survival of Plasmodium falciparum erythrocytic stages. J Biol Chem 277: 25970–25975.
12. Becker K, Kanzok SM, Iozef R, Fischer M, Schirmer RH, et al. (2003) Plasmodiromedoxin, a novel redox-active protein unique for malarial parasites. Eur J Biochem 270: 1057–1064.
13. Kanzok SM, Fechner A, Bauer H, Ushchmidt JK, Müller HM, et al. (2001) Substitution of the thioredoxin system for glutathione reductase in Drosophila melanogaster. Science 291: 643–646.
14. Nickel C, Trujillo M, Rahli S, Deponte M, Radi R, et al. (2005) Plasmodium falciparum 2-Cys peroxiredoxin reacts with plasmoredoxin and peroxynitrite. Biol Chem 386: 1129–1136.
15. Gimsburg H, Famin O, Zhang J, Krugliak M (1998) Inhibition of glutathione-dependent degradation of FP by chloroquine and amodiaquine as a possible basis for their antimalarial mode of action. Biochem Pharmacol 56: 1305–1313.
16. Janse CJ, Waters AP, Kos J, Lugt CB (1994) Comparison of in vivo and in vitro antimalarial activity of artemisinin, dihydroartemisinin and sodium artesunate in the Plasmodium berghei-rat model. Int J Parasitol 24: 589–594.
17. Buchholz K, Schirmer RH, Eubel JK, Akoachere MB, Dandekar T, et al. (2008) Structural and biochemical characterization of a mitochondrial peroxiredoxin from Plasmodium falciparum. Mol Microbiol 61: 948–59.
18. Russel M, Holmgren A (1988) Construction and characterization of glutaredoxin-negative mutants of Escherichia coli. Proc Natl Acad Sci USA 85: 990–994.
19. Collinson EJ, Wheeler GL, Garrido EO, Avery AM, Avery SV, et al. (2002) The yeast glutaredoxins are active as glutathione peroxidases. J Biol Chem 277: 16712–16717.
20. Mueller EGD (1991) Thioredoxin deficiency in yeast prolongs S phase and shortens the G1 interval of the cell cycle. J Biol Chem 43: 1167–1174.
21. Thadby V, Mennard R (2002) Gene targeting in Plasmodium berghei. Methods Mol Med 72: 317–331.
22. Druclour T, Davies BW, Grant CM (2000) A single glutaredoxin or thioredoxin is essential for viability in the yeast Saccharomyces cerevisiae. Mol Microbiol 43: 1167–1174.
23. Plasmaredoxin in Malaria