A Genome-wide Chromatin-associated Nuclear Peroxiredoxin from the Malaria Parasite *Plasmodium falciparum*\(^*\)**

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Malaria parasites are subjected to high levels of oxidative stress during their development inside erythrocytes and the ability of the parasite to defend itself against this assault is critical to its survival. Therefore, *Plasmodium* possesses an effective antioxidant defense system that could potentially be used as a target for the development of inhibitor-based therapy. We have identified an unusual peroxiredoxin protein that localizes to the nucleus of *Plasmodium falciparum* and have renamed it PfnPrx (PF10_0268, earlier called MCP1). Our work reveals that PfnPrx has a broad specificity of substrate being able to utilize thioredoxin and glutaredoxin as reductants and having the ability to reduce simple and complex peroxides. Intriguingly, chromatin immunoprecipitation followed by deep sequencing reveals that the enzyme associates with chromatin in a genome-wide manner with a slight enrichment in coding regions. Our results represent the first description of a dedicated chromatin-associated peroxiredoxin and potentially represent an ingenious way by which the parasite can survive the highly oxidative environment within its human host.

Malaria is one of the most common infectious diseases in the world with ~500 million cases each year and one to three million deaths (1). The disease has an enormous burden on human health and causes decreased productivity and economic growth and increased poverty (2). The lack of an effective vaccine, the health and causes decreased productivity and economic growth and increased poverty (2). The lack of an effective vaccine, the

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it was shown that the phosphorylation of peroxiredoxins affects their subcellular distribution and enzymatic activity (13).

Usually organisms contain more than one peroxiredoxin, which are found in different subcellular locations such as cytosol, mitochondria, peroxisomes, and nucleus (8). This is also the case for Plasmodium. To date, five malarial peroxiredoxins have been characterized: cytosolic PfTrx-Px1 and mitochondrial PfTrx-Px2 (14–16), cytosolic PfTrx-Px3 (17, 18), PfAOP, potentially localizing to the apicoplast (19), and finally a glutathione-dependent-like peroxidase with a preference for thioredoxin as reducing substrate (7). P. falciparum also possesses several proteins belonging to the thioredoxin superfamily, which provide reducing equivalents to the peroxiredoxins (reviewed in Ref. 6).

In addition to the five characterized peroxiredoxins, the malaria parasite possesses an additional protein with a conserved AhpC-TSA domain called MCP1 (merozoite capping protein-1, PF10_0268) (20, 21). MCP1 was described as a cytosolic protein localizing to the invasion tight junction, a zone of tight apposition between the merozoite and the red blood cell membrane formed during the invasion process (20). However, it is not known whether the AhpC-TSA domain of MCP1 is functional (20, 21). Additionally, MCP1 has two other defined domains with a negatively charged middle region enriched in glutamate and a C-terminal positively charged section enriched in lysine. It was speculated from the solubility properties of MCP1 and the C-terminal lysine-rich domain that this region may be required for binding to the cytoskeleton within the invading merozoite (21).

In this work, we show that MCP1 is a peroxiredoxin with unusual biochemical characteristics. As this enzyme exclusively localizes to the nucleus (in contrast to previous reports) we renamed it PfnPrx (P. falciparum nuclear peroxiredoxin). In addition, we demonstrate that PfnPrx is associated with the chromatin of the parasite in a genome-wide manner, suggesting a potentially essential role in the protection of nuclear DNA against oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Parasite Cultures**—P. falciparum asexual stage parasites were maintained in human erythrocytes (blood group O +) at a hematocrit of 4% with 0.5% (w/v) AlbumaxTM (Invitrogen) (22). P. falciparum 3D7 parasites were originally obtained from David Walliker at Edinburgh University. Cultures were synchronized as described previously (23).

**Antisera**—Rabbit and mouse antibodies were generated against PfnPrx1-100, containing the conserved oxidoreductase domain, from a GST fusion protein expressed from a plasmid construct using the following primers: PfnPrxF (5′-CGCGGATCCATGCTCATAATTAGCAGAAAATAC-3′) and PfnPrxR (5′-CCGCTCGAGTAAGGAAGTTCTAGCTTTTC-3′). PCR products were digested with BamH1/Xho1 (underlined letters in primers), purified and cloned into the plasmid pGEX4T-1 (Amersham Biosciences). For immunoblots, saponin-lysed parasite pellets from highly synchronous 3D7 parasites were separated in sample buffer on 4–12% (w/v) SDS-NuPAGE gels (Invitrogen) under reducing conditions and transferred to nitrocellulose membranes (Schleicher & Schuell). Affinity purified PfnPrx rabbit anti-serum and anti-PfnPrx mouse monoclonal were diluted in 0.1% (v/v) Tween 20-phosphate-buffered saline with 1% (w/v) skim milk. Appropriate secondary antibodies were used, and immunoblots were developed by ECL (Amersham Biosciences). For the time course of expression analysis, proteins extracted from an equal number of cells were used for each time point.

**Fluorescence Imaging**—Fluorescence images of parasites were captured using a Carl Zeiss Axioskop microscope with a PCO Sensicam and Axiovision software (version 2). For immuno-fluorescence assays of free and/or invading merozoites, highly synchronous schizont stage 3D7 parasites in the process of rupture/reinvasion were smeared and fixed in 100% methanol at −20 °C. After blocking in 3% (w/v) bovine serum albumin (Sigma), the cells were incubated for 1 h with the appropriate antisera: rabbit anti-PRON4 (1/200) (24, 25), mouse monoclonal anti-PfnPrx (1/200). Bound antibodies were then visualized with Alexa Fluor 488/594 anti-rabbit IgG or anti-mouse IgG (Molecular Probes) diluted 1:1000. Parasites were mounted in Vectashield (Vector Laboratories) containing DAPI (Roche Molecular Biochemicals).

**Immunoelectron Microscopy**—Parasites for electron microscopy immunolabeling were fixed and prepared as described previously (26). The primary antibody used was the rabbit anti-PfnPrx. Samples were washed and incubated with secondary antibodies conjugated to 15-nm colloidal gold (BB International). Samples were then post-stained with 2% aqueous uranyl acetate then 5% triple lead and observed at 120 kV on a Philips CM120 BioTWIN Transmission Electron Microscope.

**Vector Construction, Transfection, and Southern Blotting**—To create the plasmid used for the integration of GFP at the 3′ end of the nPrx gene by single crossover, a PCR fragment containing exon 3 of nPrx without a stop codon was cloned into the BglII-AvrII sites of the pARL1-GFP vector (27, 28). Parasites were transfected, and integrants were selected as described previously (27). Integration was monitored by Southern blots according to standard procedures.

**Protein Purification and Enzymatic Assays**—A PCR fragment containing nucleotides 1 to 492 of PfnPrx was amplified from P. falciparum cDNA and cloned in the BamH1-Xho1 site of the pET-45b expression vector (Novagen). The latter directs expression of the protein with an N-terminal His10 tag to facilitate purification of the recombinant protein. Generation of the nPrxC56S mutant was performed by site-directed mutagenesis with the QuickChange II XL kit from Agilent Technologies. Induction and purification of His10-nPrx1-164 were performed according to standard procedures. P. falciparum TrxR and PfTrx1 were generated as described (29, 30). P. falciparum glutaredoxin 1 (PfGrx1, PF0271c) was amplified from 3D7 genomic DNA and cloned in the BamH1-Xho1 site of the pET-45b expression vector (Novagen). Induction of the protein was performed at 27 °C overnight.

The overall antioxidant activity of PfnPrx was estimated by analyzing its ability to protect Escherichia coli glutamine synthetase (Sigma) from inactivation by a thiol-metal catalyzed oxidation system (DTT/Fe3+/O2) (31). The P. falciparum nPrx kinetic parameters were determined (30) using a spectrophotometric assay to determine the initial rates of the peroxidase reaction.
reaction as described previously for *Toxoplasma gondii* Trx-Px2 (30, 32). For the assays using the thioredoxin system, the reaction mix contained 100 mM HEPES, pH 7.6, 1 mM EDTA, 0.25 units of *P. falciparum* thioredoxin reductase, 2 μM PfnPrx, 200 μM NADPH, varying concentrations of *P. falciparum* cytosolic thioredoxin PfTrx1 (20–100 μM) at a constant concentration of 20 μM hydrogen peroxide.

For assays using the GSH system with *P. falciparum* Grx1, the reaction mix contained 100 mM HEPES, pH 7.6, 1 mM EDTA, 0.5 units glutathione reductase, 1 mM GSH, 2 μM PfnPrx, 200 μM NADPH, varying concentrations of *P. falciparum* cytosolic glutaredoxin PfGrx1 (1–30 μM) at a constant concentration of 20 μM hydrogen peroxide or varying concentrations of hydrogen peroxide (1–50 μM) and cumene hydroperoxide (1–100 μM) at a constant concentration of 10 μM PfGrx1. No NADPH oxidation was observed without addition of PfGrx1 indicating that PfnPrx cannot be reduced by GSH itself. The decrease in absorbance at 340 nm due to NADPH oxidation was determined over 30 s with time points taken every 10 ms. Assays were performed at 25 °C. The linear rates were determined and fitted to the Michaelis-Menten equation using Graphit (version 5.0; Erithracus).

**RESULTS**

**Identification of PfnPrx (Earlier MCP1) as a Nuclear Peroxiredoxin**—To investigate the role of PfnPrx (MCP1) in the merozoite invasion process, we generated an antibody against

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**A.** Domain organization of PfnPrx PF10_0268. The alignment shows the conserved AhpC-TSA family domain with the peroxidatic cysteine. Comparison was realized with Praline software. The Genbank™ accession numbers of the aligned sequences are as follows: PfnPrx, AAC46600; *Saccharomyces cerevisiae* DOT5, P40553; PfTPx1, XP_001348542; PfTPx2, XP_001350554; Pf1-Cys_Prx, AAG14353; and PfAOP, AY306209. The asterisks represent conserved residues, and the arrow highlights the peroxidatic cysteine.

**B.** Time course of PfnPrx expression during the blood stage using an affinity-purified rabbit anti-serum raised against the first 100 residues of PfnPrx.

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**FIGURE 1.** PfnPrx is expressed throughout the erythrocytic stage. **A,** domain organization of PfnPrx PF10_0268. The alignment shows the conserved AhpC-TSA family domain with the peroxidatic cysteine. Comparison was realized with Praline software. The Genbank™ accession numbers of the aligned sequences are as follows: PfnPrx, AAC46600; *Saccharomyces cerevisiae* DOT5, P40553; PfTPx1, XP_001348542; PfTPx2, XP_001350554; Pf1-Cys_Prx, AAG14353; and PfAOP, AY306209. The asterisks represent conserved residues, and the arrow highlights the peroxidatic cysteine. **B,** time course of PfnPrx expression during the blood stage using an affinity-purified rabbit anti-serum raised against the first 100 residues of PfnPrx.

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**Chromatin Immunoprecipitation and Deep Sequencing—Chromatin immunoprecipitation of nPrx-GFP from formaldehyde cross-linked and sonicated chromatin was performed as described (33) using an anti-GFP antibody (AbCam 290). Immunoprecipitated as well as input chromatin was decross-linked at 45 °C in the presence of 0.5 mM NaCl and tested by quantitative PCR (for primer list, see supplemental Table 1) or used for Illumina sequencing library preparation according to the linear amplification protocol (34). Sequencing libraries were loaded on the Illumina Genome Analyzer IIx and sequenced for 36 cycles from one side of the fragments. The quality filtered 35-bp sequence reads were mapped against the *P. falciparum* genome assembly (PlasmoDB version 6.1) using the standard Illumina pipeline. Coverage plots were generated using uniquely mapped sequence reads by counting the number of overlapping tags in 100-bp windows and visualized in SignalMap (NimbleGen). The ratio track was obtained by dividing the ChIP-seq tag counts with the input tag counts and displayed on a log2 scale. For generation of average gene profile the coding body of all “normal size” genes (1–10 Kb) were divided into 20 equal size windows and five 150-bp windows immediately up- or downstream represented flanking sequences. The ratios of the tag counts in the ChIP versus input data set has been computed in each individual windows and averaged in the corresponding window of all genes. For scatter plot analysis, the ratios of ChIP and input tag counts have been calculated in the coding body of each gene have been plotted against the RNAseq tag density (tag/1000 bp transcript) of an independent 3D7 schizont population (34).
the conserved oxidoreductase domain (Fig. 1A) (20). Western blots on schizont stage protein extracts showed a single specific band of ~55 kDa, which was slightly higher than the predicted 45 kDa (Fig. 1B, 48 h lane) and is in agreement with previous results (20). All of the other *P. falciparum* peroxiredoxins having sizes between 22 and 28 kDa, we can be confident that our antibody is not cross-reacting with any of them. We next performed a time course analysis for expression of PfnPrx (MCP1) using parasite protein extracts taken at 8-h intervals throughout the erythrocytic asexual cycle. The PfnPrx (MCP1) protein was present as a single 55 kDa band in all samples analyzed with the highest expression between 32 to 48 h (Fig. 1B) which corresponds to the schizont stage where the parasite undergoes nuclear division and also when proteins involved in merozoite invasion are expressed. This pattern is also in agreement with the RNA expression analyses published previously (35).

To determine the subcellular localization of the PfnPrx (MCP1) protein, we performed immunofluorescence assays on parasites from different stages of the erythrocytic cycle. The anti-nPrx (MCP1) monoclonal antibody overlapped almost completely with the DAPI-stained parasite DNA in rings, trophozoites, and schizonts, suggesting that PfnPrx was located in the nucleus (Fig. 2A), a subcellular localization different to that described previously (20). Analysis of the protein sequence with the PredictProtein software (36) also revealed a potential nuclear localization signal in the C terminus of PfnPrx (KKPAKKVVKKK). To analyze the subcellular localization of PfnPrx (MCP1) in more detail, we performed immunofluorescence experiments on merozoites during erythrocyte invasion.
in which we observed that PfnPrx also localized to the nucleus of the parasite. No anti-PfnPrx (MCP1) signal was found at the tight junction, visualized by staining with an antibody against the RON4 protein, a well characterized marker of this transient structure (25). To determine whether this localization was due to our antibody recognizing a protein other than PfnPrx (MCP1), we engineered a parasite line in which the green fluorescent protein (GFP) gene was integrated by single crossover at the 3′/H11032 end of the PfnPrx (MCP1) gene leading to the production of a fluorescent PfnPrx (MCP1) (Fig. 3, A and B). Western blot analysis showed that PfnPrx (MCP1) was detected as an 80-kDa protein with both the PfnPrx (MCP1) mouse monoclo-
nal antibody or an anti-GFP monoclonal antibody confirming expression of the chimeric protein and providing additional evidence for the specificity of our mouse monoclonal anti-nPrx antibody (Fig. 3C). To determine the localization of the PfnPrx-GFP fusion, the 3D7 PfnPrx-GFP line was analyzed by epifluorescence microscopy. As shown in Fig. 3D, the PfnPrx-GFP fluorescence overlaps with the DAPI-stained parasite DNA in rings, trophozoites, and schizonts confirming that nPrx (MCP1) is found in the nucleus of the parasite throughout the blood stage cycle. To determine more precisely the localization of PfnPrx in the nucleus, we performed immunoelectron microscopy on *P. falciparum* schizont stage sections. PfnPrx localizes predominantly to the more electron dense regions at the nuclear periphery, and this is believed to represent heterochromatin (Fig. 2C) (37). Consequently, because of the nuclear localization and functional data presented below, we renamed this protein PfnPrx (*P. falciparum* nuclear peroxiredoxin).

**FIGURE 4.** PfnPrx is a peroxiredoxin with unusual characteristics. A, purification of the N-terminal domain of PfnPrx. B, glutamine synthetase (GS) protection assays revealing that PfnPrx is an antioxidant protein and that this activity was dependent on its conserved peroxidatic cysteine. C–E, peroxidase assays showing that glutaredoxin (C) is a potent reductant of PfnPrx and that PfnPrx efficiently reduces hydrogen peroxide (D) and cumene hydroperoxide (E). 2 μM PfnPrx was used for all assays, 20 μM hydrogen peroxide was used for the variable Pf glutaredoxin 1 assay, and 10 μM PfGrx1 was used for the variable peroxide assays. Results are representative of at least three independent experiments.
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protein (amino acids 1–164) was recombinantly expressed and purified (Fig. 4A), and its potential peroxidase activity was assessed using various enzymatic assay systems. Initially, we determined whether the protein protects glutamine synthetase from inactivation by a thiol-metal catalyzed oxidation system (DTT/Fe³⁺/O₂) (31). 4.5 μg of the recombinant peroxiredoxin-like domain of PfPrxA rescued glutamine synthetase activity by 50% (Fig. 4B), confirming that the AhpC-TSA domain was catalytically active, therefore suggesting that PfPrxA has antioxidant capacity. The specificity of this catalytic activity was further investigated by exchanging the predicted active site cysteine residue at position 56 by serine, which totally abolished the glutamine synthetase protection activity of the recombinant protein (Figs. 1A and 4B). The fact that the recombinant AhpC-TSA domain of PfPrxA showed the ability to protect glutamine synthetase activity is presumably attributable to its capacity to reduce hydrogen peroxide. This suggests that this part of the protein acts as a peroxidase that might potentially protects nuclear components such as DNA from oxidative insults. To identify its natural reducing substrate, the catalytic activity of PfPrxA was analyzed in the presence of P. falciparum thioredoxin 1, glutathione, and glutaredoxin 1. PfPrxA had the ability to reduce hydrogen peroxide, and this activity increased with increasing concentrations of PfTrx1 without reaching saturation at concentrations as high as 100 μM (supplemental Fig. 1), suggesting that it is most likely not the favored reductant of the enzyme.

In addition to the thioredoxin system, P. falciparum possesses a functional glutathione system, and we studied the possibility that it could be involved in the reduction of PfPrxA. No PfPrxA peroxidase activity on hydrogen peroxide was detected when using GSH as a reductant, whereas addition of glutaredoxin 1 (PfGrx1, 15) to the assay mix supported the reduction of PfPrxA with saturation kinetics (Fig. 4C) and a reasonable reaction rate. At a concentration of 20 μM H₂O₂, the apparent Kₘ for PfGrx1 was determined to be ~4 μM, showing that it was an excellent reductant for PfPrxA. When looking at the affinity of PfPrxA for H₂O₂, an apparent Kₘ of 14 μM was calculated when 10 μM of PfGrx1 was used (Fig. 4D), demonstrating that H₂O₂ was a good substrate for PfPrxA and corroborating the results obtained with the glutamine synthetase protection assay. In addition, PfPrxA was able to reduce cumene hydroperoxide with an apparent Kₘ of ~30 μM (Fig. 4E). The specific activity of the recombinant protein was in the range of 6 μmol/min/mg protein, which is low compared with the activity of other Plasmodium peroxiredoxins (16, 30). It cannot be excluded that regulatory or structural features important to define substrate specificity are missing from the recombinant PfPrxA given that only the AhpC-TSA domain of PfPrxA rather than the full-length protein was analyzed.

As PfPrxA is localized to the nucleus, it would be expected that its favored physiological reductant should also localize to this organelle or at least have the ability to translocate to it from the cytoplasm upon oxidative stress. To investigate the cellular localization of PfGrx1, we generated parasites expressing a PfGrx1-GFP fusion. PfGrx1-GFP mostly localizes to the cytoplasm of the parasite though some of protein overlaps with the DAPI-stained nuclear DNA, suggesting that it has the ability to translocate between the two compartments (supplemental Fig. 2). However, we cannot at this stage exclude that the nuclear staining is the result of a mislocalization of the fusion protein due to its overexpression from an episome.

Having demonstrated that PfPrxA had the ability to detoxify peroxides in vitro, we hypothesized that it could potentially be involved in protecting the nucleus of the parasite from oxidative stress. However, it has not been possible to demonstrate a consistent increase in the level of PfPrxA transcript or protein when submitting the parasites to exogenous oxidative stress in vitro using a glucose/glucose oxidase system (data not shown).

PfPrxA Is Potentially Essential in Erythrocytic Stage—To gain further insights into the role of PfPrxA in the blood stage cycle of the malaria parasite, we attempted to generate a knock-out line in both P. falciparum and Plasmodium berghei, a rodent malaria parasite often used as a model for human malaria due to the ease with which knock-out strains can be generated. Despite several attempts, we were unable to generate a nPrx knock-out line in either of the Plasmodium species tested. This was in contrast with the ability to easily tag the PfPrxA with GFP, suggesting that the protein performs an essential/important function in the erythrocytic stage of the malaria parasite (data not shown).

ChiP-seq Analysis Reveals Genome-wide Binding of PfPrxA to Chromatin—The co-localization of PfPrxA with the DAPI-stained DNA suggested that it was potentially associating with chromatin. To investigate this possibility, we performed a genome-wide ChiP-seq analysis on schizont stage 3D7nPrx-GFP parasites using an anti-GFP antibody. Intriguingly, visual inspection of the data showed nearly even distribution of PfPrxA across the P. falciparum genome (Fig. 5A). An obvious exception was the strong depletion of PfPrxA from the centromeric regions (Fig. 5B). In addition, PfPrxA was found to be slightly enriched in the coding body of genes (Fig. 5B), which was also apparent on the average gene profile (Fig. 5C). Enrichment of PfPrxA in coding regions and its depletion from centromeres were confirmed by ChIP-quantitative PCR and were found to be consistent across all stages of intraerythrocytic development (Fig. 5D). Importantly, recoveries with the GFP antibody for chromatin isolated from nontagged 3D7 parasites were very low for all sites tested, proving the specificity of our assay (data not shown). Finally, we determined whether enrichment of PfPrxA showed a correlation with transcriptional activity. As demonstrated by scatter plot analysis (Fig. 5E), PfPrxA occupancy in coding regions showed limited variation across genes and no or minimal correlation with steady state mRNA levels. Notably, outlier genes with high PfPrxA occupancy tended to be tRNA or rRNA genes. In conclusion, the ChiPseq data demonstrates the intimate association of PfPrxA with the malaria genome.

To determine whether the association of PfPrxA with the chromatin was mediated through interactions with other proteins, we performed immunoprecipitation on the 3D7nPrx-GFP line using an anti-GFP antibody. Mass spectrometry analysis revealed, in addition to nPrx-GFP, a high number of different proteins such as core histones, heat shock proteins, proteasome components, and a few transcription factors, but none with very high peptide coverage (Fig. 6). This high variety
FIGURE 5. PfnPrx shows genome wide chromatin association. A and B, broad distribution of PfnPrx (A), its enrichment in coding and depletion at centromeric region (B) demonstrated by coverage plots and ratio track of PfnPrx-GFP ChIP-seq data obtained from schizont stage parasites. C, average gene profile of PfnPrx occupancy. D, quantitative PCR data confirming enrichment of PfnPrx in coding region and its depletion at centromeres at three different stages of intraerythrocytic development. E, scatter plot analysis of correlation between PfnPrx enrichment in coding body of genes and steady state mRNA levels (RNA-seq data of schizont stage parasites from Bartfai et al. (33)).
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Peroxiredoxins are a family of anti-oxidants that protect the cell from metabolically produced reactive oxygen species. The *P. falciparum* PfnPrx prefers glutaredoxin over thioredoxin as a reducing substrate and shows saturation kinetics. The protein reduces hydrogen peroxide and cumene hydroperoxide equally well, suggesting that it does not discriminate between anorganic or organic hydroperoxides. Its specificity for glutaredoxin as a reducing substrate is unusual especially because most other known peroxiredoxins or peroxiredoxin-like proteins react with thioredoxin or glutathione. Peroxiredoxins with this substrate specificity have so far only been described in plants, although some of these can also be reduced by thioredoxins (9, 39, 40, and reviewed in Ref. 41). Intriguingly, PfnPrx was associated strongly with the genome of the parasite, suggesting that the protein has a role in either protecting nuclear components such as DNA from oxidative damage or being involved in maintaining chromatin structure, DNA repair, or mechanisms that affect transcriptional activities. In fact, it has been shown that redox active thiol-containing proteins and peptides are critical for these processes because they can respond to oxidation or reduction processes that allows for the regulation of their activities (42). There are a number of reports that show interaction of human peroxiredoxin 1 with the nucleus. This binding capacity and the downstream effects on transcriptional regulation can be either dependent or independent of the peroxidase activity of the protein, and it has been suggested that apart from acting as an antioxidant the protein can act as a chaperone to stabilize other DNA-binding factors (43–45).

The broad distribution of PfnPrx across the *P. falciparum* genome suggests that it plays a general role across the chromosomes and it could possibly be associated with nucleosomes. Studies on humans, *Drosophila*, and worms have shown that nucleosome occupancy increases in coding regions and that this is independent of transcriptional status (46–48). In addition, our previous studies on core histone H3 demonstrated a slight enrichment in the coding body of genes similar to that observed for PfnPrx. Such a similar enrichment profile is consistent with a potential association of PfnPrx with nucleosomes (49). Due to its very highly positively charged C terminus, PfnPrx could potentially interact directly with the phosphate backbone of the DNA, without the need to link through an interacting partner. It is indeed well documented that the lysine-rich C-terminal tails of linker histone H1s bind directly to the linker DNA entering and exiting a nucleosome leading to the formation of higher order folding states of chromatin (50, 51). Although linker histones of multicellular eukaryotes display a tripartite structure made up of a conserved globular domain flanked by two less structured N- and C-terminal domains, their counterparts in several protists and bacteria only contain the lysine-rich C terminus (reviewed in Ref. 52). It is worth mentioning that although H1-like basic proteins have been found in dinoflagellates (53) and ciliates (54), none have been identified for any of the apicomplexans, also members of the alveolates family. It is tempting to speculate that the lysine-rich C terminus of PfnPrx could potentially play a similar role in chromatin structure; however, our attempts to recombinantly express the full-length PfnPrx or its C-terminal tail to investigate a potential association with nucleosomes in vitro have unfortunately been unsuccessful so far. In any case, if PfnPrx is involved in the protection of the genome against oxidative stress, the pairing of a peroxiredoxin domain with a highly basic domain suggests an ingenious means of making sure that the enzyme would always be in a position to quickly get rid of any oxidative insult as soon as it is produced, therefore limiting the chances of DNA damage.

Moreover, we were unable to disrupt the PfnPrx gene either in *P. falciparum* or *P. berghei*, suggesting that it plays an essential role for nuclear integrity or function possibly through protecting nuclear DNA and chromatin-binding, thiol-containing proteins from oxidative damage or because it is intimately involved in the regulation of gene expression. In conclusion, we have identified a dedicated nuclear peroxiredoxin with broad substrate specificity that associates with chromatin throughout the genome potentially demonstrating a specific adaptation of the malaria parasite to its hostile environment.

**FIGURE 6.** PfnPrx-GFP associates with a broad range of proteins. The immunoprecipitated proteins were grouped in categories and plotted according the total number of peptides identified. HSPs, heat shock proteins; RNA/DNA, proteins involved in RNA/DNA metabolism and/or binding. The list of proteins with accession numbers is available in the supplemental Table 1.

No GFP peptides were recovered because a *P. falciparum*-specific database was used for peptide identification.
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REFERENCES

1. Snow, R. W., Guerra, C. A., Noor, A. M., Myint, H. Y., and Hay, S. I. (2005) Nature 434, 214–217
2. Sachs, J., and Malaney, P. (2002) Nature 415, 680–685
3. White, N. J. (2008) Science 320, 330–334
4. Becker, K., Tilley, L., Vennerstrom, J. L., Roberts, D., Rogerson, S., and Ginsburg, H. (2004) Int. J. Parasitol. 34, 163–189
5. Hunt, N. H., and Stocker, R. (1990) Blood Cells 16, 499–526
6. Müller, S. (2004) Mol. Microbiol. 53, 1291–1305
7. Sztajer, H., Gamain, B., Aumann, K. D., Slomianny, C., Becker, K., Brige-Dorflöhr, R., and Brohöf, L. (2001) J. Biol. Chem. 276, 7397–7403
8. Wood, Z. A., Schröder, E., Robin Harris, J., and Poole, L. B. (2003) Trends Biochem. Sci. 28, 32–40
9. Rouhier, N., Gelhaye, E., and Jacquot, J. P. (2002) J. Biol. Chem. 277, 13609–13614
10. Wood, Z. A., Poole, L. B., Hantgan, R. R., and Karplus, P. A. (2002) Biochemistry 41, 5493–5504
11. Butlerfield, I. H., Merino, A., Golub, S. H., and Shau, H. (1999) Antioxid. Redox Signal 1, 385–402
12. Cox, A. G., Winterbourn, C. C., and Hampton, M. B. (2010) Methods Enzymol. 474, 51–66
13. Zykova, T. A., Zhu, F., Vakorina, T. I., Zhang, J., Higgins, L., Urusova, D. V., Krnajski, Z., Gilberger, T. W., Walter, R. D., and Müller, S. (2001) Mol. Biochem. Parasitol. 112, 219–228
14. Boucher, I. W., McMillan, P. J., Gabrielsen, M., Akerman, S. E., Brannigan, J. A., Schnick, C., Brzozowski, A. M., Wilkinson, A. J., and Müller, S. (2006) Mol. Microbiol. 60, 948–959
15. Kawazu, S., Tsuji, N., Hatabu, T., Kawai, S., Matsumoto, Y., and Kano, S. (2000) Mol. Biochem. Parasitol. 109, 165–169
16. Krajzsk, Z., Walter, R. D., and Müller, S. (2001) Mol. Biochem. Parasitol. 113, 303–308
17. Sarma, G. N., Nickel, C., Rahls, S., Fischer, M., Becker, K., and Karplus, P. A. (2005) J. Biol. Chem. 280, 1021–1034
18. Klotz, F. W., Hadley, T. J., Aikawa, M., Leech, J., Howard, R. J., and Miller, L. H. (1989) Mol. Biochem. Parasitol. 36, 177–185
19. Hudson-Taylor, D. E., Dolan, S. A., Klotz, F. W., Fujioka, H., Aikawa, M., Koonin, E. V., and Miller, L. H. (1995) Mol. Microbiol. 15, 418–471
20. Trager, W., and Jensen, J. B. (1976) Science 193, 673–675
21. Lambros, C., and Vanderberg, J. P. (1979) J. Parasitol. 65, 418–420
22. Richard, D., Kats, L. M., Langer, C., Black, C. G., Mitriz, K., Boddy, J. A., Cowman, A. F., and Coppol, R. L. (2009) PLoS Pathog. 5, e1000328
23. Richard, D., MacRaid, C. A., Riglar, D. T., Chan, J. A., Foley, M., Baum, I., Ralph, S. A., Norton, R. S., and Cowman, A. F. (2010) J. Biol. Chem. 285, 14815–14822
24. Rug, M., Wrickham, M. E., Foley, M., Cowman, A. F., and Tilley, L. (2004) Infect. Immun. 72, 6095–6105
25. Gilberger, T. W., Thompson, J. K., Reed, M. B., Good, R. T., and Cowman, A. F. (2003) J. Cell Biol. 162, 317–327
26. Przyborski, J. M., Miller, S. K., Pfähler, J. M., Henrich, P. R., Rohrbach, P., Crabb, B. S., and Lanzer, M. (2005) EMBO J. 24, 2306–2317
27. Gilberger, T. W., Walter, R. D., and Müller, S. (1997) J. Biol. Chem. 272, 29584–29589
28. Akerman, S. E., and Müller, S. (2003) Mol. Biochem. Parasitol. 130, 75–81
29. Kim, K., Kim, I. H., Lee, K. Y., Rhee, S. G., and Stadtman, E. R. (1988) J. Biol. Chem. 263, 4704–4711
30. Akerman, S. E., and Müller, S. (2005) J. Biol. Chem. 280, 564–570
31. Rouhier, N., Koh, C. S., Gelhaye, E., Corbier, C., Favier, F., Didierjean, C., and Jacquot, J. P. (2008) Biochim. Biophys. Acta 1780, 1249–1260
32. Go, Y. M., and Jones, D. P. (2010) Antioxid. Redox Signal 13, 489–509
33. Wang, X., He, S., Sun, J. M., Delcuve, G. P., and Davie, J. R. (2010) Mol. Biol. Cell 21, 2987–2995
34. Neumann, C. A., and Fang, Q. (2007) Curr. Opin. Pharmacol. 7, 375–380
35. Park, S. Y., Xu, X., Ip, C., Mohler, J. L., Bogner, P. N., and Park, Y. M. (2007) Cancer Res. 67, 9294–9303
36. Tilgner, H., Nikolau, C., Alhammer, S., Sammeth, M., Beato, M., Valcarcel, J., and Guigo, R. (2009) Nat. Struct. Mol. Biol. 16, 996–1001
37. Schwartz, S., Meshorer, E., and Ast, G. (2009) Nat. Struct. Mol. Biol. 16, 990–995
38. Andersson, R., Enroth, S., Rada-Iglesias, A., Wadelius, C., and Komorowski, J. (2009) Genome Res. 19, 1732–1741
39. Salcedo-Amaya, A. M., van Driel, M. A., Akalo, B. T., Trelle, M. B., van den Elzen, A. M., Cohen, A. M., Janssen-Megens, E. M., van de Veij-Bolmer, M., Selzer, R. R., Iniguez, A. L., Green, D. R., Sauерwein, R. W., Jensen, O. N., and Stunnenberg, H. G. (2009) Proc. Natl. Acad. Sci. U.S.A. 106, 9565–9560
40. Syed, S. H., Goutte-Gattat, D., Becker, N., Meyer, S., Shukla, M. S., Hayes, J. J., Everaers, R., Angelov, D., Bednar, J., and Dimitrov, S. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 9695–9690
41. Hendzel, M. J., Lever, M. A., Crawford, E., and Th’ng, J. P. (2004) J. Biol. Chem. 279, 20028–20034
42. Kasinsky, H. E., Lewis, J. D., Dacks, J. B., and Ausiello, J. (2001) FASEB J. 15, 34–42
43. Vernet, G., Sala-Rovira, M., Maeder, M., Jacques, F., and Herzog, M. (1990) Biochim. Biophys. Acta 1048, 281–289
44. Hayashi, T., Hayashi, H., and Iwai, K. (1987) J. Biochem. 102, 369–376
