ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 4, 2012
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DOI: 10.1089/ars.2011.4404

FORUM REVIEW ARTICLE

Peroxiredoxins in Parasites

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Abstract

Significance: Parasite survival and virulence relies on effective defenses against reactive oxygen and nitrogen species produced by the host immune system. Peroxiredoxins (Prxs) are ubiquitous enzymes now thought to be central to such defenses and, as such, have potential value as drug targets and vaccine antigens. ***Recent Advances:*** Plasmodial and kinetoplastid Prx systems are the most extensively studied, yet remain inadequately understood. For many other parasites our knowledge is even less well developed. Through parasite genome sequencing efforts, however, the key players are being discovered and characterized. Here we describe what is known about the biochemistry, regulation, and cell biology of Prxs in parasitic protozoa, helminths, and fungi. At least one Prx is found in each parasite with a sequenced genome, and a notable theme is the common patterns of expression, localization, and functionality among sequence-similar Prxs in related species. ***Critical Issues:*** The nomenclature of Prxs from parasites is in a state of disarray, causing confusion and making comparative inferences difficult. Here we introduce a systematic Prx naming convention that is consistent between organisms and informative about structural and evolutionary relationships. ***Future Directions:*** The new nomenclature should stimulate the crossfertilization of ideas among parasitologists and with the broader redox research community. The diverse parasite developmental stages and host environments present complex systems in which to explore the variety of roles played by Prxs, with a view toward parlaying what is learned into novel therapies and vaccines that are urgently needed. *Antioxid. Redox Signal*. 17, 608–633.

Introduction and Scope

Peroxiredoxins (Prxs; EC 1.11.1.15) are exquisitely efficient cysteine-dependent peroxidases functioning in antioxidant, regulatory, and signaling systems (90, 116, 206, 254). They are ubiquitous and frequently present at high abundance (268). Broad reviews of Prx structure, function, and physiology are available (90, 92, 250), but here we focus on Prxs of the single-celled protozoa, multicellular helminths, and intracellular fungi that parasitize humans and are major contributors to the global burden of disease (267). In these organisms, Prxs are important for defense against endogenous and host-derived reactive oxygen species (ROS) and peroxynitrite ($ONOO^-$) and are possibly involved in cellular signaling. We begin with an overview of structure–function relationships among Prxs and, based on the six Prx subfamilies, propose a systematic and informative nomenclature for parasite Prxs. In the survey section of this review, we list the Prxs of relevant parasites and review studies of their biochemical properties and cellular roles. Finally, we briefly discuss Prxs as potential drug and vaccine targets.

General Features of Prxs

Redox stress is a fact of life and organisms are exposed to oxidizing species from within and without, experiencing harmful oxidation of DNA, proteins, and lipids. Prxs, not recognized as widespread enzymes until 1994 (42), appear to be the first and dominant line of defense against hazardous ROS (263) and also are able to efficiently detoxify peroxynitrite/peroxynitrous acid ($ONOO^-/ONOOH$; $pK_a = 6.8$), a powerful oxidant and nitration agent produced by the reaction of superoxide ($O_2^{\bullet -}$) with nitric oxide ($NO^{\bullet}$), as previously reviewed (240); $NO^{\bullet}$, $ONOO^-/ONOOH$, and related molecules are often collectively referred to as reactive nitrogen species (RNS). In many eukaryotes Prxs also play a role in regulating signaling networks that use hydrogen peroxide (H_2O_2) as a second messenger (79, 89, 126, 213, 265).

Distinct conserved sequence patterns allow Prxs to be categorized into six subfamilies—Prx1/AhpC, Prx6, Prx5, PrxQ/BCP, Tpx, and AhpE (183). For simplicity, we will use Prx1 to refer to the Prx1/AhpC subfamily, and PrxQ to refer to the PrxQ/BCP subfamily. Conveniently, a Web-based searchable database, the PeroxiRedoxin classification indEX (PREX), has

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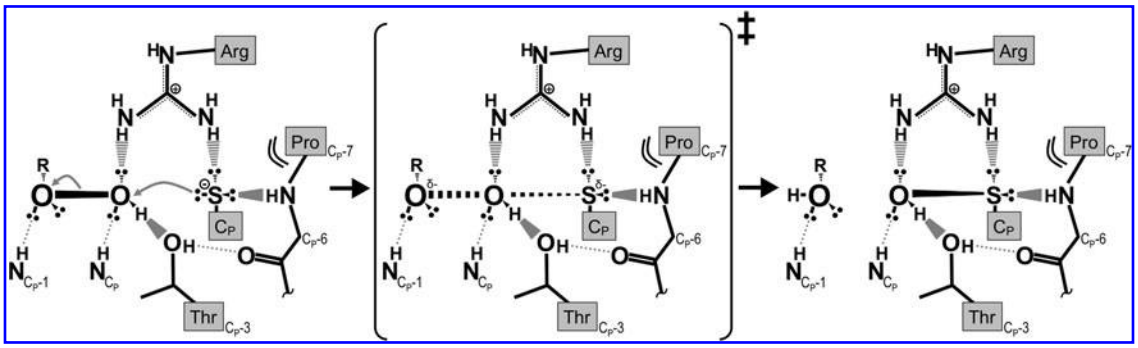

FIG. 1. A structurally detailed view of the Prx peroxidation reaction mechanism. Adapted from Ref. (92).

been developed that organizes Prxs into these families (235) (http://csb.wfu.edu/prex).

Catalytic properties of Prxs

Prxs catalyze the direct reduction of a variety of hydroperoxides (ROOH) to the corresponding alcohols (ROH; or H_2O in the reduction of H_2O_2) and water (184, 268). This occurs *via* a direct displacement S_N2 reaction, with a Prx active-site cysteine sulfur atom carrying out nucleophilic attack on the distal oxygen atom of the peroxide (77). The reaction proceeds through a planar transition state, releasing the alcohol and leaving the Prx cysteine oxidized to sulfenic acid (S_POH) (Fig. 1). To regenerate the reduced active-site cysteine for the next round of catalysis, the Prx participates in a series of reactions involving disulfide intermediates.

The complete catalytic cycle is diagrammed in Figure 2. In the first step of the reaction, within a fully folded (FF) Prx active site, the Prx peroxidatic cysteine (C_P) in its thiolate form (Prx^{FF}-S_P^-) reacts with ROOH to yield ROH and Prx^{FF}-S_POH (Step 1, peroxidation). The Prx-S_POH pK_a value has been measured as 6.1 (208) and 6.6 (103); however, its protonation state during catalysis is unclear. Next, the Prx undergoes a local unfolding (LU) of its active site (Prx^{LU}-S_POH) to expose the S_POH group for reaction with a resolving thiol (R′-S_RH) to form a disulfide bridge (Prx^{LU}-S_P-S_R-R′) and release water (Step 2, resolution). Prx^{LU}-S_P-S_R-R′ is then reduced by a cysteine thiol from a different protein (R″-SH), yielding the disulfide R″-S-S_R-R′ and restoring the reduced Prx^{LU}-S_P^- (Step 3, recycling). Finally, to complete the cycle, R″-S-S_R-R′ is reduced and the Prx active site refolds to the FF-conformation required for peroxide reduction. Certain Prxs present in eukaryotes (268) are sensitive to overoxidation of the peroxidatic S_POH, which occurs when a second molecule of peroxide reacts with S_POH to form cysteine-sulfinic acid (S_PO_2H; Step 4). The S_PO_2H of such sensitive Prxs can in many organisms be reactivated (Step 5) by ATP-dependent reduction by sulfiredoxin (Srx) or, perhaps, the less-characterized sestrin (46, 113). How these Prxs facilitate peroxide signaling is not yet well understood but may involve a floodgate-like mechanism (265) and/or the formation of larger aggregates with molecular chaperone or other activities (12, 89, 197). Prx1 activity has also been reported to be regulated by phosphorylation (264).

Prxs can contain one or two participating Cys residues. In addition to the C_P possessed by all Prxs, 2-Cys Prxs have the resolving thiol (R′-S_RH) present as a cysteine (C_R) either on the same chain (atypical 2-Cys Prxs) or on the other chain of a homodimer (typical 2-Cys Prxs). Figure 3 illustrates the rearrangements involved in a typical 2-Cys Prx catalytic cycle that allow the disulfide to form. R″ is often thioredoxin (Trx) (41), a Trx equivalent such as tryparedoxin (Txn) (187), plasmoredoxin (Plrx; 24), or a Trx-like domain of a Prx reductase as in the bacterial alkyl hydroperoxide reductase component F (AhpF) (205). For 1-Cys Prxs, molecules such as Trx (193) or GSH (157) provide the R′ thiol although the reducing equivalents can also be provided by ascorbate (171). The Trx or Trx equivalent is reduced by an NADPH-dependent reductase such as Trx reductase (TrxR). The NADPH is produced by the pentose phosphate pathway, connecting Prx activity to primary metabolism.

Early work on Prxs reported typical catalytic efficiency (k_{cat}/K_m) values of $10^5\ M^{-1}s^{-1}$. Such reported catalytic rates, much lower than those of catalase and glutathione peroxidase (Gpx), led to a perception that Prxs were only physiologically

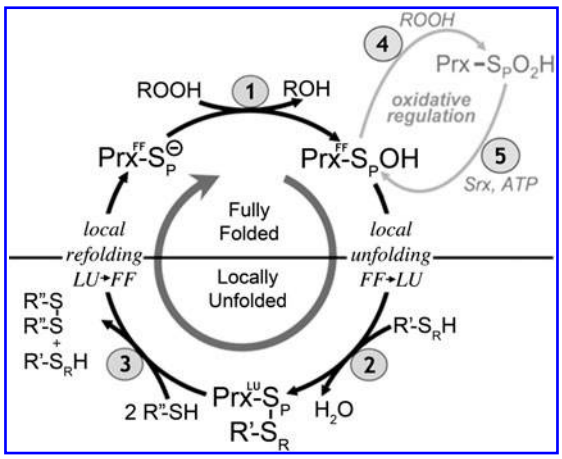

FIG. 2. The Prx catalytic cycle, showing both chemical steps and local unfolding/refolding steps. Shown are the three chemical steps (peroxidation [Step 1], resolution [Step 2], and recycling [Step 3]) of peroxide reduction, and the two chemical steps (overoxidation [Step 4] and ATP-dependent Srx-catalyzed recovery [Step 5]) seen for the oxidative regulation of sensitive, eukaryotic floodgate-type 2-Cys Prxs. The generic Prx is represented as a monomer with S_P designating the sulfur atom of C_P and S_R the resolving thiol. See the text for further descriptions. Adapted from Ref. (92).

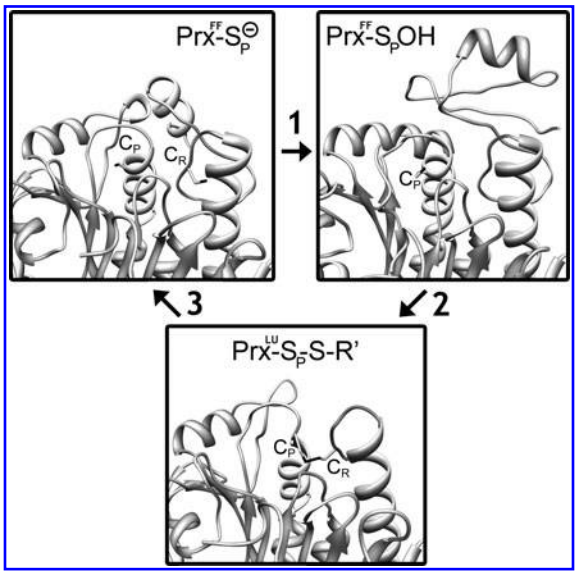

FIG. 3. Structures of three intermediates of the Prx catalytic cycle, using examples of dominant Prx1 and Prx6 family members. Steps in the cycle are numbered as in Fig. 2. Clockwise from upper left, the structures are *Pv*Prx1a (PDB Code 2I81), *Hs*Prx6 (1PRX), and *Pv*Prx1a (2H66).

relevant in organisms lacking the efficient heme- ($k_{cat}/K_m \sim 10^7\ M^{-1}s^{-1}$) or seleno-peroxidases ($k_{cat}/K_m \sim 10^8\ M^{-1}s^{-1}$) (77, 81). However, recent work has found that k_{cat}/K_m values of $\sim 10^7\ M^{-1}s^{-1}$ are typical for Prxs, with lower rates occurring when recycling of the Prx (Fig. 2, Step 3) by non-physiological redox partners is rate limiting (190). Given their high rate constants and high expression levels [as high as 0.5 m*M* (77)], Prxs are now considered the preferential reducing agent of ROOH, $ONOO^-$, and lipid peroxides in the cell (263), a view supported by a network model of cellular redox reactions (2). Prxs may also play a role in detoxifying peroxides of amino acids and proteins (196).

Remarkably, Prxs employ a simple cysteine sulfur atom as the nucleophile in the catalytic chemical reduction of ROOH yet achieve catalytic efficiencies nearly six orders of magnitude greater than expected for a thiolate (77). Recent structural analyses reveal that this catalytic power arises from an exquisitely structured active site that preferentially stabilizes the transition state; it does not simply activate the thiolate, but also orients and activates the peroxide substrate for attack (Fig. 1) (92).

Structural features of Prxs

The first Prx crystal structure (49) revealed the basic Prx fold (Fig. 4), which is related to that of Trx (192). Using a family-based naming of secondary structure elements (Fig. 4) (90), the C_P is always located in helix α2 on one face of the β-sheet, cradled between helices α3 and α5 (90). For 2-Cys Prxs, the C_R location tends to be characteristic of a given Prx subfamily, and as noted below can be near the C-terminus, or in α2, α3, or α5.

Prxs form two types of dimers. A-type dimers, thought to represent the ancestral mode of dimerization, interact in a tip-to-tip manner at strands β1 and β2, and at the loops preceding helices α2, α3, and α4 (Fig. 5A) (222). B-type dimers, seen for Prx1 and Prx6 subfamily members, form by an edge-to-edge β-sheet interaction (the B-interface) to make an extended, 10-strand β-sheet (Fig. 5B) (90). The B-type dimers commonly associate *via* the A-interface into ring-shaped decamers (or dodecamers or octamers; Fig. 5C) to stabilize the FF active site and allow maximal peroxidatic activity (23, 191). The LU form of the enzyme favors dimers, suggesting that decamer–dimer transitions occur during the catalytic cycle (266); regulation by phosphorylation may occur *via* decamer disruption (268).

The following sections briefly describe structure–function characteristics of each subfamily.

Prx1. The Prx1 subfamily includes all "typical" 2-Cys Prxs. This subfamily is the largest and most widely distributed, with members found in archaea, bacteria, and all

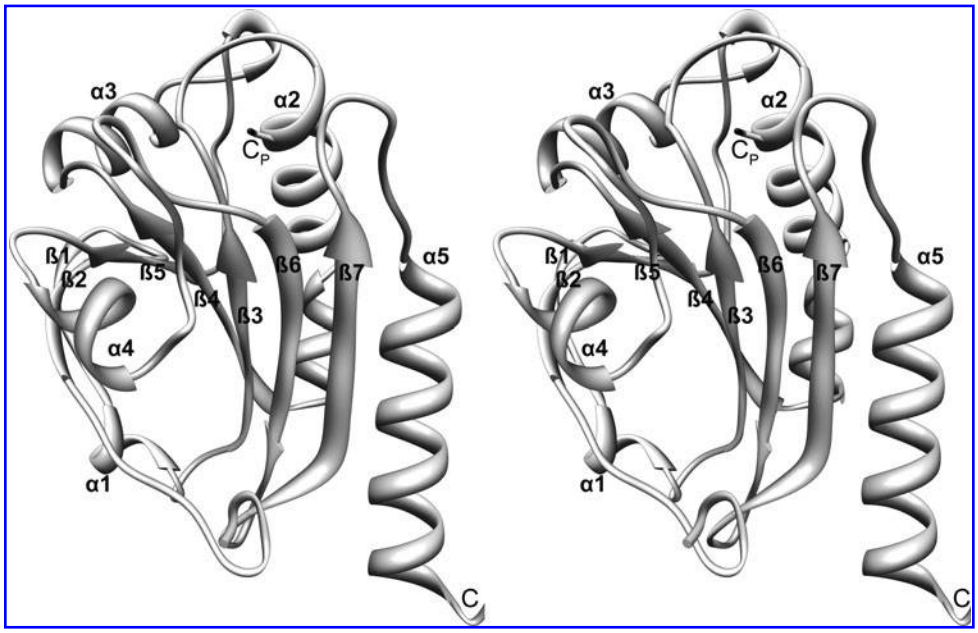

FIG. 4. The standard Prx fold. Shown is a stereo view of one chain of PDB entry 2I81 with labels giving the family numbering for secondary structural elements.

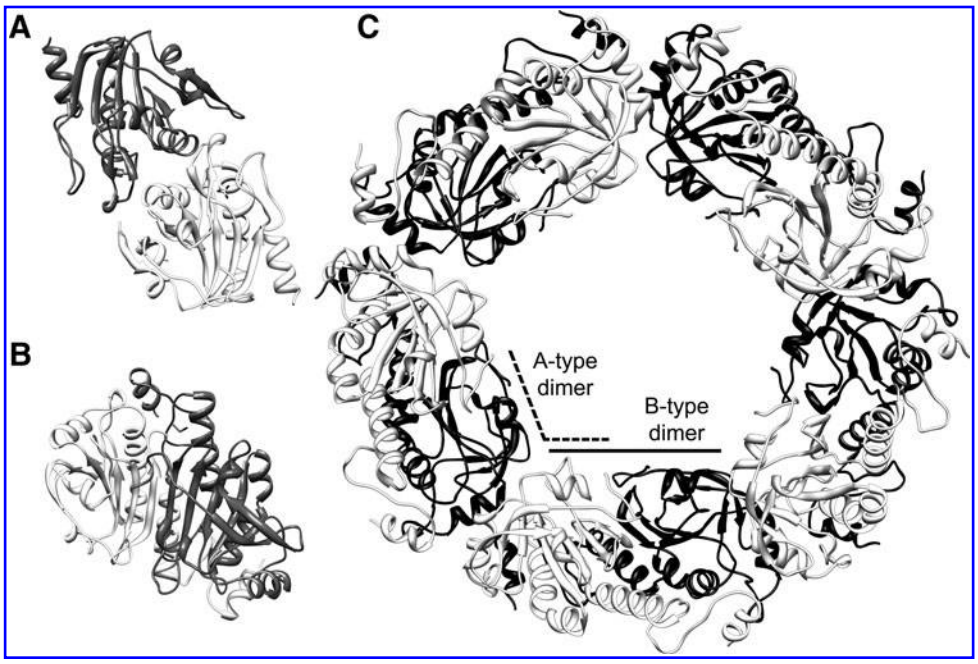

FIG. 5. Quaternary structure of Prxs. Shown are **(A)** an A-type dimer, **(B)** a B-type dimer, and **(C)** a decamer built using both A- and B-type interfaces; examples of A- and B-type dimers comprising the decamer are indicated by broken and solid lines, respectively. Structures shown are **(A)** *Pf*Prx5 (PDB entry 1XIY), **(B)** *Pf*Prx1m (2C0D), and **(C)** *Tcru*Prx1a (1UUL).

eukaryotic classes (183). Members include bacterial AhpC proteins; Txn peroxidases; yeast TSA1 and TSA2; and human Prxs I, II, III, and IV. These form doughnut-shaped decamers (Fig. 5C) and have C_R in a C-terminal extension of the partner chain of a B-type dimer (89). Thus, the disulfide form is a covalent dimer, and nonreducing SDS gels provide a simple method to test for Prxs in this form. Prx1s are typically the most abundant Prxs in an organism and, in eukaryotes, may be sensitive to overoxidation and involved in signaling. They generally prefer H_2O_2 over organic ROOH substrates (195).

Prx6. The Prx6 subfamily is named after human PrxVI (49). This subfamily is present in archaea, bacteria, and eukaryotes (183). Prx6 proteins resemble those of the Prx1 subfamily, containing a C-terminal extension, forming B-type dimers and, in some cases, oligomers (90). However, the vast majority of Prx6s are 1-Cys Prxs (61). The physiological reductant of most Prx6 members is unknown, and Trx notably fails to reduce human PrxVI (115).

Prx5. The Prx5 subfamily derives its name from human PrxV. Prx5s are present in bacteria, yeast, fungi, plants, and mammals (183), and have been localized to mitochondria, peroxisomes, and the cytoplasm (128). Members of the Prx5 subfamily form A-type dimers and include 1-Cys Prxs as well as 2-Cys Prxs that have C_R on helix α5 of the same chain (60, 90, 222). A unique feature of the Prx5 active site is a π-helical bulge (52) in helix α2 immediately following C_P (222). With the possible exception of the bacterial Prx5s fused to glutaredoxin (Grx) (124), the reductant for Prx5 subfamily members is Trx. Human PrxV prefers organic ROOH substrates and is an efficient reductant of $ONOO^-$ (250).

PrxQ. The PrxQ subfamily was found first in plant chloroplasts (132), and identified in *Escherichia coli* as the bacterioferritin comigratory protein (BCP), a name given before its function was known (109). This subfamily is predominantly bacterial, but also occurs in archaea and some eukaryotes, but not in mammals (183). This subfamily includes primarily monomeric Prxs (183) but some form A-type dimers (*e.g.*, Protein Data Bank codes 2CX4, 2YWN). The PrxQ subfamily includes both 2-Cys and 1-Cys Prxs (257). For the 2-Cys members, the common location for C_R is in helix α2, five residues after C_P; however, about 7% of the subfamily have C_R in helix α3 (147, 183).

Tpx. The Tpx subfamily is smaller and less phylogenetically diverse than the previous subfamilies. Known Tpx members are all bacterial (except for two trichomonad enzymes identified below) and are almost exclusively 2-Cys Prxs with C_R located in helix α3. Structural transitions involved in the local unfolding of the active site during catalysis have been examined in detail (93). Tpxs form A-type dimers that are unaffected by redox state and are essential for activity, as the substrate binding pocket includes residues from both subunits (93). Tpx proteins are commonly reduced by Trx. *E. coli* Tpx prefers hydrophobic, bulky cumene-OOH over H_2O_2 (K_m $\sim$9 μM *vs.* 1.7 mM) (20), and, based on sequence conservation, a similar preference is expected for all Tpxs (91). This suggests a role in detoxifying lipid hydroperoxides (40).

AhpE. The AhpE subfamily, named after *Mycobacterium tuberculosis* AhpE, contains the fewest members, all in aerobic gram-positive bacteria of the order Actinomycetales (183). It appears to include both 1-Cys and 2-Cys Prxs. *M. tuberculosis* AhpE has been structurally characterized (146), but its natural reducing partner remains unclear (103). As no parasites have a Prx from this subfamily, we will not describe it further.

Occurrence and Studies of Prxs in Parasites Affecting Human Health

In this review, parasites are organized into groups and subgroups as follows: protozoa—the single-celled apicomplexans, kinetoplastids, and other protists; helminths—the multicellular nematode, cestode, and trematode worms; and

parasitic fungi. Detailed life cycle information for each parasite discussed here can be found on the U.S. Centers for Disease Control & Prevention Web site (http://dpd.cdc.gov/DPDx). To identify all relevant Prxs, a primary search of our selected parasite genera (Appendix 1) used the PREX database (235); the EuPathDB bioinformatics databases (14–16, 85, 98) were consulted as a secondary source. One challenge in developing a list of Prxs is discerning whether highly similar Prx sequences from a single organism represent distinct gene products. Our simplistic solution to this problem was, for a given species, to list only the longest sequence within groups of Prxs with ≥95% amino acid sequence identity [as determined by Clustal Omega (231)]. For surveying the literature, the genera list (Appendix 1) was used to query National Center for Biotechnology Information (NCBI) PubMed (226) in combination with the terms "peroxiredoxin," "thiol peroxidase," "thioredoxin peroxidase," "tryparedoxin peroxidase," "thiol-specific antioxidant protein," and "peroxidoxin."

Of 97 unique parasite Prxs identified (Table 1), subfamily Prx1 is overwhelmingly the most common, with 80 members and at least one in every parasite considered. In some species, the multiple Prx1 isozymes may have similar functional properties but distinct localization or expression patterns such as is seen for human Prxs I-IV, all in the Prx1 subfamily (97, 105). Representatives of the Prx6, Prx5, PrxQ, Tpx, and AhpE subfamilies are much less frequently found, with 7, 4, 4, 2, and 0 occurrences, respectively. *Plasmodia*, the apicomplexan malaria parasites, are uniquely well endowed with a range of Prxs: two Prx1 proteins, a Prx6, a Prx5, and a PrxQ are present. Apart from *Plasmodia*, a PrxQ occurs only in the fungus *Cryptococcus neoformans*, a Prx5 is found only in the apicomplexan *Toxoplasma gondii*, and Prx6s were identified only among *C. neoformans*, *T. gondii*, and the nematode worms. The Tpx subfamily was represented only in the protozoan *Trichomonas vaginalis*.

As Table 1 was assembled, it became apparent that the nomenclature of Prxs from these parasites is highly inconsistent, confusing, and provides little or no information about the subfamily to which a given Prx belongs. For instance, 20 parasite Prx proteins named Tpx in the literature are not Tpx subfamily members, and names for members of the Prx1 subfamily include CPX, Prx3, Px2, Pxn2, Pxn3, Tpx-2, TrxPx1, TRYP6, TSA, and TxnPx, among others (Table 1). To resolve this confusion, we here propose a systematic, informative, and forward-looking nomenclature in which Prxs are named according to the subfamily to which they belong (*e.g.,* Prx6). Lettered suffixes (*e.g.,* Prx1a and Prx1b) are used in the Prx1 and Tpx subfamilies since multiple Prxs of these families can be found in a single organism; even if just one Prx1 is currently known in an organism, a suffix is assigned based on its sequence similarity to other Prxs (*e.g.,* Fig. 8). The suffix "m" (*i.e.,* Prx1m) is given to Prxs assigned as mitochondrially localized.

Table 1 lists these newly assigned names and their corresponding literature names, and notes experimental results that cannot be uniquely assigned to a specific Prx. To refer to a Prx from a certain organism, an italicized species-specific prefix is used (*e.g., Tcru*Prx1a for the first cytosolic Prx1 subfamily enzyme of *Trypanosoma cruzi*). We assume unless proven otherwise that the equivalent Prxs in organisms of the same genus will behave similarly, and so studies of such enzymes are grouped together. We also assume, in the absence of contrary evidence, that the members of a Prx subfamily will share the features of that subfamily, so studies confirming such properties are only mentioned briefly.

As we begin the survey, a few general observations can be made. First, despite the presence in many Prx1 subfamily members of motifs very similar to the "GGLG" and "YF" motifs associated with the overoxidation shunt (265), there is no biochemical evidence that any protozoan parasite Prx1 is susceptibile to overoxidation. Also, no protozoan parasite appears to have a gene encoding sulfiredoxin. A second point is that for many Prxs, the recycling step can occur *via* multiple pathways and so sorting out the main path is nontrivial and perhaps even of little importance. Overall, the most common path seems to be through Trx *via* an NADPH-TrxR-Trx-Prx cascade. As noted above, the apicomplexans and trypanosomatids feature Trx equivalents (*i.e.,* Plrx and Txn), which complement or replace the common system (Fig. 6). A third point is that among the Prxs listed in Table 1, there are only six for which crystal structures are available (Table 2). For the others, the amino acid sequence identity to crystallographically characterized Prxs is generally high (at least 40% for all Prxs except the PrxQs, which have greater than 34% identity to a known structure), so their three-dimensional structures can be inferred with reasonable accuracy.

Finally, we note that determining the physiological roles of Prxs can be difficult. Since all Prxs will turnover (although differentially) H_2O_2, $ONOO^-$, and organic ROOH, it must be realized that when any given Prx is artificially overexpressed in a tissue, it will by its very nature protect that tissue from oxidative assault. While it is tempting to say that this result proves that the Prx in question is involved in protection from oxidative stress, it does not. Such a result gives no insight into the true physiological role of the Prx, because adding an excess of any peroxidase will provide such protection. In contrast, the true physiological role of a given Prx depends on where and to what extent the enzyme is expressed under natural conditions. Similarly, when a *Prx* gene is knocked out or knocked down (*i.e.,* by RNA interference [RNAi]), the lack of a specific phenotype provides limited information about its physiological role, because with multiple antioxidant enzymes present, there is typically a high level of redundancy.

The following sections are grouped by parasite type and species, reviewing what is known about enzyme kinetic and substrate profile studies (available for ~50 Prxs), spatial and temporal protein localization experiments (immunoblot and immunolocalization, done for ~40 Prxs), proteomic and gene expression studies (reverse transcriptase–polymerase chain reaction [RT-PCR], performed for ~40 Prxs), and biological roles as dissected by gene overexpression, knockout (deletion), and knockdown (RNAi) studies (reported for ~30 Prxs). In many studies, enzymatic activity is evaluated indirectly by monitoring the prevention of peroxide-induced inactivation of glutathione synthase (GS) or the nicking of DNA (123, 148).

Prxs in apicomplexans

Apicomplexans are obligate intracellular parasites containing an apical complex that aids in entering host cells (28). Many also contain a chloroplast-derived apicoplast (216), which, for those that have it, is essential and is the site of

Table 1. Prxs Found in Parasites of Humans

Species[a]	*Name*	*Literature name(s)*	*Identifier (number of loci)*[b]	*References*
		PROTOZOA		
		Apicomplexans		
Cryptosporidium hominis	Prx1a		*Chro.40093*	
Cryptosporidium muris	Prx1a		*CMU_012880*	
	Prx1m		*CMU_034200*	
Cryptosporidium parvum	Prx1a	TrxPx	*cgd4_740*	(142)
Plasmodium falciparum	Prx1a	Prx-1, Px2, TPx-1, TPx-2, Trx-Px1	*PF14_0368*	(4, 118, 121, 129, 130, 137, 186, 210, 271)
	Prx1m	TPx-2	*PFL0725w*	(27, 121, 210, 271)
	Prx6	1-Cys-Prx, Prx-2, Tpx-1	*PF08_0131*	(94, 117, 120, 121, 130, 137, 181, 185, 271)
	Prx5	AOP	*MAL7P1.159*	(121, 185, 222)
	PrxQ	MCP1, nPrx	*PF10_0268*	(127, 214)
Plasmodium knowlesi	Prx1a		*PKH_126740*	
	Prx1m		*PKH_143220*	
	Prx6	1-Cys-Prx	*PKH_011610*	
	Prx5	AOP	*PKH_021360*	
	PrxQ	MCP1	*PKH_061160*	
Plasmodium vivax	Prx1a	TPx-1	*PVX_118545*	
	Prx1m	TPx-2	*PVX_123435*	
	Prx6	1-Cys-Prx	*PVX_093630*	
	Prx5		*PVX_081760*	
	PrxQ	MCP1	*PVX_111355*	
Toxoplasma gondii	Prx1a	Prx, Trx-Px1	*TGME49_017890*	(5, 141, 159)
	Prx1m	Prx3	*TGME49_030410*	(141)
	Prx6	Trx-Px2, Prx2, PrxII	*TGME49_109210*	(5, 61, 66, 95, 141)
	Prx5	AOP	*TGME49_086630*	
		Kinetoplastids		
Leishmania aethiopica	Prx1a	Pxn1	AAZ23599	(112)
	Prx1b	Pxn2	AAZ23602	(112)
Leishmania amazonensis	Prx1a	cTxnPX	AAX47428	(101, 149)
	Prx1m	mTxnPX	AAX47429	(101, 149)
Leishmania braziliensis	Prx1a	TxnPX	*LbrM.15.1080 (2)*	(55)
	Prx1m		*LbrM.23.0050*	
Leishmania chagasi	Prx1a	TxnPx, Prx1, Pxn1	AAG40074	(21, 22)
	Prx1b	Prx2, Pxn2	AAK69586	
	Prx1c	Prx3, Pxn3	AAK69587	
Leishmania donovani[c]	Prx1a	TxnPx[d]	AAK00633	(80, 106, 111), (125, 194, 270)[d]
	Prx1m	mPrx, TxnPx[d]	AAX73294	(96), (125, 194, 270)[d]
Leishmania guyanensis	Prx1a	PXN1, TXN1, TxnPx[d]	AAX18168	(1), (258)[d]
	Prx1b	PXN2, TXN2,TxnPx[d], TxnPx2	AAV31765	(1), (258)[d]
Leishmania infantum	Prx1a	cTxnPx, TRYP[d]	*LinJ.15.1100 (3)*	(37, 38, 149, 220), (51)[d]
	Prx1m	mTxnPx, TRYP[d]	*LinJ.23.0050*	(35–38, 54), (51)[d]
Leishmania major	Prx1a	TryP1, TRYP6, TXNPx	*LmjF15.1060 (7)*	(58, 72, 133, 144)
	Prx1m		*LmjF.23.0040*	
Leishmania mexicana	Prx1a		*LmxM.15.1040 (2)*	
	Prx1m		*LmxM.23.0040*	

(*continued*)

Table 1. (Continued)

Species[a]	*Name*	*Literature name(s)*	*Identifier (number of loci)*[b]	*References*
Leishmania tropica	Prx1a		AAZ23601	
	Prx1b		AAZ23600	
Trypanosoma brucei	Prx1a	c-TxnPx, CPX, TDPX, TryP, TRYP1	*Tb09.160.4250 (2)*	(32, 33, 39, 65, 135, 201, *247, 249, 260*)
	Prx1m	m-TcTxnPx, TRYP2	*Tb427.08.1990*	(39, 135, 175, 201, 247)
Trypanosoma cruzi	Prx1a	c-TcTxnPx, CPx	*Tc00.1047053504839.44 (2)*	(39, 78, 87, 135, 163, 188, 199–203, 249, 261, 275), (9, 17, 64, 151, 230)[d]
	Prx1m	m-TcTxnPx, MPx	*Tc00.1047053509499.14*	(39, 188, 198–201, 261), (9, 17, 64, 151, 230)[d]
		Other protozoa		
Blastocystis hominis	Prx1a		CBK22665	
	Prx1m		CBK25198	
Entamoeba dispar	Prx1a		*EDI_034430* (1–17)	(50, 153, 242)[d]
	Prx1b		*EDI_182130* (1–7)	(50, 153, 242)[d]
	Prx1c		*EDI_053480* (1–2)	(50, 153, 242)[d]
Entamoeba histolytica	Prx1a	Eh29, TSA	*EHI_122310* (1–8)	(30, 102, 110), (13, 48, 50, 57, 104, 153, 212, 221)
Entamoeba moshkovskii	Prx1a		BAD66879	(47, 48)[d]
Giardia lamblia	Prx1a		*GL50803_14521 (2)*	
	Prx1b		*GL50803_15383*	
Naegleria fowleri	Prx1a		n/a	(122)
Trichomonas vaginalis	Prx1a	Trx-Prx	*TVAG_114310*	(53, 143), (209, 276)[d]
	Prx1b		*TVAG_350540*	(209, 276)[d]
	Prx1c		*TVAG_038090 (2)*	(209, 276)[d]
	Prx1d		*TVAG_095250*	(209, 276)[d]
	Prx1e		*TVAG_075420*	(209, 276)[d]
	Prx1f		*TVAG_487570*	(209, 276)[d]
	Tpx-a		*TVAG_055200*	
	Tpx-b	Thiol peroxidase	*TVAG_165690 (1–2)*	(143)[d]
		HELMINTHS		
		Nematodes		
Brugia malayi	Prx1a	TPx-2, TPX	Q17172	(10, 86, 154, 155)
	Prx1b	TPx-1	P48822	(86)
	Prx6		AAF21098	
Loa loa	Prx1a	TPX	EFO27148	
	Prx1b	TPx1	EFO26191	
Onchocerca volvulus	Prx1a	PXN-2, Tpx-1, Tpx-2, TSA	AAC32810	(152, 227, 278)
	Prx6	Tpx-2	AAC27392	(44)
Wuchereria bancrofti	Prx1b	TPx-1	ACL82593	
		Cestodes		
Echninococcus granulosus	Prx1a	TPx	AAL84833	(100, 145, 158, 218, 234)
Echninococcus multilocularis	Prx1a	TRX	BAC11863	
Taenia crassiceps	Prx1a	2-CysPrx	ACM89282	(252)
Taenia solium	Prx1a	2-CysPrx	ACM89281	(170, 252)

(*continued*)

Table 1. (Continued)

Species[a]	*Name*	*Literature name(s)*	*Identifier (number of loci)*[b]	*References*
		Trematodes		
Clonorchis sinensis	Prx1b	Prx2	AEK86199	(19, 114, 274)
	Prx1m	Prx3	AEK86200	(19)
Fasciola gigantica	Prx1b	Prx-1, Prx2	ACU27401 (2)	(43, 211, 277)
Fasciola hepatica	Prx1a	FhePrx, Prx-1, Prx-2, Tpx	AAB71727	(67, 68, 108, 160, 161, 217, 229)
	Prx1b	Prx-3	ACI04165	
Opisthorchis viverrini	Prx1b		ACB13822	(239)
Schistosoma japonicum	Prx1a	TPx-1	CAX78585	(138, 139)
	Prx1b	TPx-2	AAW25625	(138, 139)
	Prx1m	Prx-3, TPx-3	AAW25436	(138, 139)
Schistosoma mansoni	Prx1a	Prx1, TPx1	AAG15507	(140, 176, 177, 224, 225)
	Prx1b	Prx2, TPx2	AAG15508	(68, 140, 176, 177, 224, 225, 256)
	Prx1c		XP_002577572	
	Prx1d		XP_002577887	
	Prx1m	Prx3, TPx3	AAG15506	(68, 140, 224, 225, 256)
		FUNGI		
Cryptococcus neoformans	Prx1	Tsa1[d]	AAP68994	(165, 167), (166)[d]
	Prx6	Tsa3[d]	XP_567781	(166)[d]
	PrxQ	Tsa4[d]	XP_569732	(166)[d]
Encephalitozoon cuniculi	Prx1	TDX	*ECU03_1190*	
Encephalitozoon intestinalis	Prx1	TSA1	*Ein03_1090*	

[a]Species names shown in bold type are those with complete genome sequences available (see, *e.g.*, http://eupathdb.org, http://www.sanger.ac.uk/resources/downloads/protozoa, and http://www.ncbi.nlm.nih.gov/genomes/leuks.cgi). Underlined portions of the name are used in the shorthand names of proteins from that organism.

[b]The EuPathDB gene ID, if available, is listed in italic type; otherwise, the NCBI accession number is provided. Where relevant, in parentheses is shown the number of loci (or range of possible loci) encoding gene products of ≥95% identity.

[c]The genome sequencing for this organism is in progress but is not publicly available.

[d]The references in parentheses describe one or more Prxs that do not clearly correspond to a systematic name.

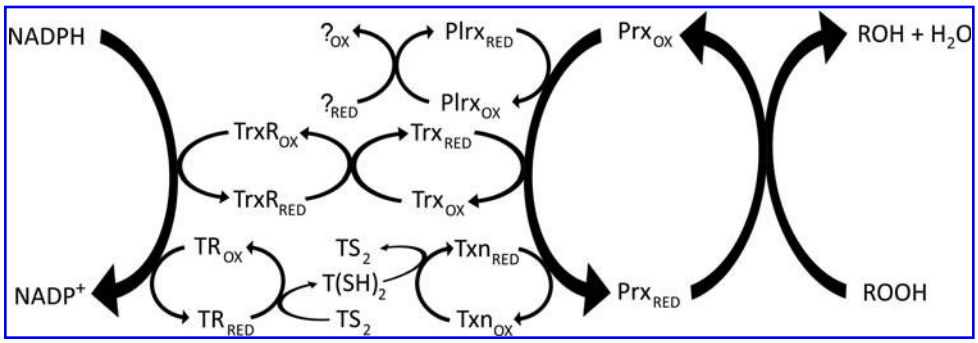

FIG. 6. Prx redox cascades showing special participants of *Plasmodia* (top pathway) and kinetoplastids (bottom pathway). TrxR, thioredoxin reductase; Plrx, plasmoredoxin; Trx, thioredoxin; TR, trypanothione reductase; TS_2, trypanothione; Txn, tryparedoxin.

isoprenoid, fatty acid, and heme biosynthesis (228). The apicomplexan parasites of humans are in the genera *Plasmodia*, *Toxoplasma*, and *Cryptosporidia*, and all have Prxs.

Plasmodia. Parasites of the genus *Plasmodia* are the cause of malaria. Their complex life cycle involves the blood-meal ingestion of male and female gametocytes by the *Anopheles* mosquito, wherein they multiply and form oocysts, which rupture to release infective sporozoites. In the human host, these sporozoites infect liver cells to form schizonts that rupture to release merozoites, which in turn infect red blood cells and develop into trophozoites. The trophozoites develop into schizonts that rupture to again release merozoites, which reinfect red blood cells to perpetuate the disease-causing blood stage or else differentiate into gametocytes.

While inside erythrocytes, *Plasmodia* are exposed to high levels of oxidative stress from host-defense ROS and the by-products of heme degradation. Plasmodial antioxidant enzymes are thought to be crucial for pathogenesis and are considered antimalarial therapeutic targets (62). Although *Plasmodia* possess a mitochondrial superoxide dismutase (SOD), they lack catalase and Gpx so their redox defense seems to rely heavily on Prxs (180). Their Prx recycling systems (Fig. 6) feature three Trxs (Trx1, Trx2, and Trx3) and also Plrx (24) and Grx (185), all found in characteristic cellular compartments (Fig. 7) (121). TrxR, the normal reductant for Trxs, has been shown to be nonessential (31); Plrx is known to reduce *Pf*Prx1a (186) and *Pf*Prx5 (185), but its reductant is unknown. Fascinatingly, *Plasmodia* are also reported to import host *Hs*PrxII (131) and other redox enzymes and equivalents (62).

Each plasmodial species considered here has a set of five Prxs: two from the Prx1 subfamily (Prx1a and Prx1m), one PrxQ, one Prx5, and one Prx6. The Prx1a and Prx1m enzymes cluster more closely with their cognates in other species than they do with each other (Fig. 8A), indicating that the Prx1a and Prx1m groups had already diverged in a common ancestor of the *Plasmodia. Plasmodia* spp. affecting nonhuman mammals have this same Prx complement, and are included in the discussion where relevant.

Localization studies: Studies using GFP fusion proteins (121) have localized *Plasmodium falciparum* Prx1a and Prx6 to the cytosol, Prx1m to the mitochondrion [see also Refs. (27, 62, 271)], and Prx5 to the apicoplast [see also Ref. (222)]. *Pf*PrxQ is exclusively localized to the nucleus (214), superceding previous work placing it in the cytosol and elsewhere (127). In the nucleus, it associates with chromatin (a novel observation for any Prx), and is enriched in coding regions (214).

Expression levels and other in vivo *studies: Pf*Prx1a is constitutively expressed throughout the life cycle, with higher levels in the erythrocyte stage (118, 119, 271). A *Pf*Prx1a knockout grows normally but is hypersensitive to exogenous ROS and RNS (129) and *Pf*Prx1a mRNA and protein levels increase under oxidative stress (4). Also, the Δ*prx1a* mutant in the mouse parasite *Plasmodium berghei* shows a 90% decrease in sporozoite formation, a 60% decrease in gametocyte yield, and attenuated infectivity in mice, although with no evidence of direct oxidative damage (272, 273).

*Pf*Prx1m is present in the highly metabolically active trophozoite and schizont stages, and *Pf*Prx6 is constitutively expressed, with transcript and protein levels elevated in the trophozoite and early schizont stages (271). Recombinant

Table 2. Available Prx Crystal Structures

PRX	*PDB code*	*Resolution (Å)*	*Redox state*	*Conformation*	*Oligomeric state*[a]	*Reference*
*Pv*Prx1a	2H66	2.5	S_{50}-$S_{170'}$	LU	$(\alpha_2)_5$	(255)[b]
*Pv*Prx1a	2I81	2.5	SH_{50}, $SH_{170'}$	FF	$(\alpha_2)_5$	n/a[c]
*Py*Prx1a	2H01	2.3	SH_{44}, $SH_{164'}$	LU	$(\alpha_2)_4$	n/a[c]
*Pf*Prx1m	2C0D	1.8	S_{67}-$SH_{187'}$	LU(alt)[d]	α_2(B)	(27)
*Tcru*Prx1a	1UUL	2.8	SH_{52}, $SH_{173'}$	FF	$(\alpha_2)_5$	(202)
*Pf*Prx5	1XIY	1.8	SO_3H_{59}	FF	α_2(A)	(222)
*Py*Prx6	1XCC	2.3	SH_{47}	FF	α_2(B)	(255)[b]

[a]An A-type or B-type dimer is noted in parentheses. For octamers and decamers, both interfaces are used.
[b]The publication summarizes a number of structures solved by the Structural Genomics Consortium (SGC; http://sgc.utoronto.ca) and shows only structure snapshots without specific discussion of *Pv*Prx1a or *Py*Prx6.
[c]The structure has been solved and deposited in the Protein Data Bank (http://pdb.org) by the SGC and is unpublished to date.
[d]The C_P loop has shifted, as is expected to occur after decamer dissociation.

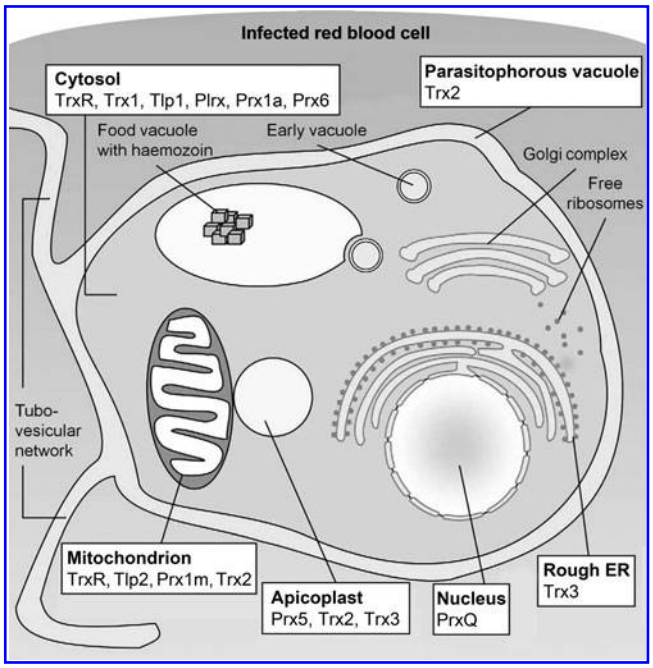

FIG. 7. Compartmentalization of Prxs and related redox cascade enzymes in *Plasmodia*. Adapted from Ref. (121). Plrx, plasmoredoxin; Tlp, thioredoxin-like protein.

*Pf*Prx6 coprecipitated with agarose-bound ferriprotoporphyrin IX (117) and so it was thought to protect against heme detoxification, although *Pf*Prx6 cellular distribution does not match that of heme (62). *Pf*Prx5 is expressed in all erythrocyte stages, with peak mRNA levels in trophozoites [microarray data PlasmoDB (16)], mirroring Grx expression (185).

Biochemical characterizations: All five *P. falciparum* Prxs have been biochemically characterized, along with certain homologs in other mammalian parasites, for example, *Plasmodium yoelii* Prx6 (119).

Prx1a. As expected for a Prx1, *Pf*Prx1a is a doughnut-shaped $(\alpha_2)_5$ decamer (4) and forms disulfide-linked dimers during its catalytic cycle (118). Mutagenesis confirms the locations of C_P and C_R (186), and proven substrates include H_2O_2 (4), $ONOO^-$ (186), *t*-butyl-OOH, and cumene-OOH (210). *Pf*Prx1a can be reduced by *E. coli* Trx (118), plasmodial Trx (4), and Plrx (186). Stopped-flow kinetics studies, with Trx as the reductant, gave $k_{cat}/K_m = 6.7 \times 10^6\ M^{-1}s^{-1}$ for H_2O_2 (4) and $k_{cat}/K_m = 1 \times 10^6\ M^{-1}s^{-1}$ for $ONOO^-$(186). Structural genomics projects have produced crystal structures for *Pv*Prx1a (two structures) and *Py*Prx1a (Table 2), but these have not been described in publications.

Prx1m. Recombinant *Pf*Prx1m is a functional peroxidase that is reduced by the mitochondrial *Pf*Trx2 about 10-fold more effectively than the cytosolic *Pf* Trx1 (27); neither Grx nor lipoamide could directly support *Pf* Prx1m activity. The crystal structure of *Pf*Prx1m has been solved in its LU disulfide form (Table 2), with the protein in the crystal forming a B-type dimer (27). The mitochondrial targeting sequence (residues 1–19) is disordered and thus seems unimportant in the folding or activity of the mature protein.

Prx6. Despite showing activity in a GS-oxidation protection assay (117, 185), recombinant Prx6 from *P. falciparum* exhibits low peroxidase activity with both H_2O_2 and *t*-butyl-OOH. Its functional reducing partners include Grx and a Trx, but not GSH, even though one of its eight Cys residues can be glutathionylated *in vitro* (185). The crystal structure of *Py*Prx6 has been determined by a structural genomics group (Table 2). Interestingly, *Py*Prx6 in the FF state with a reduced active site is present in the crystal as a B-type dimer, suggesting that not

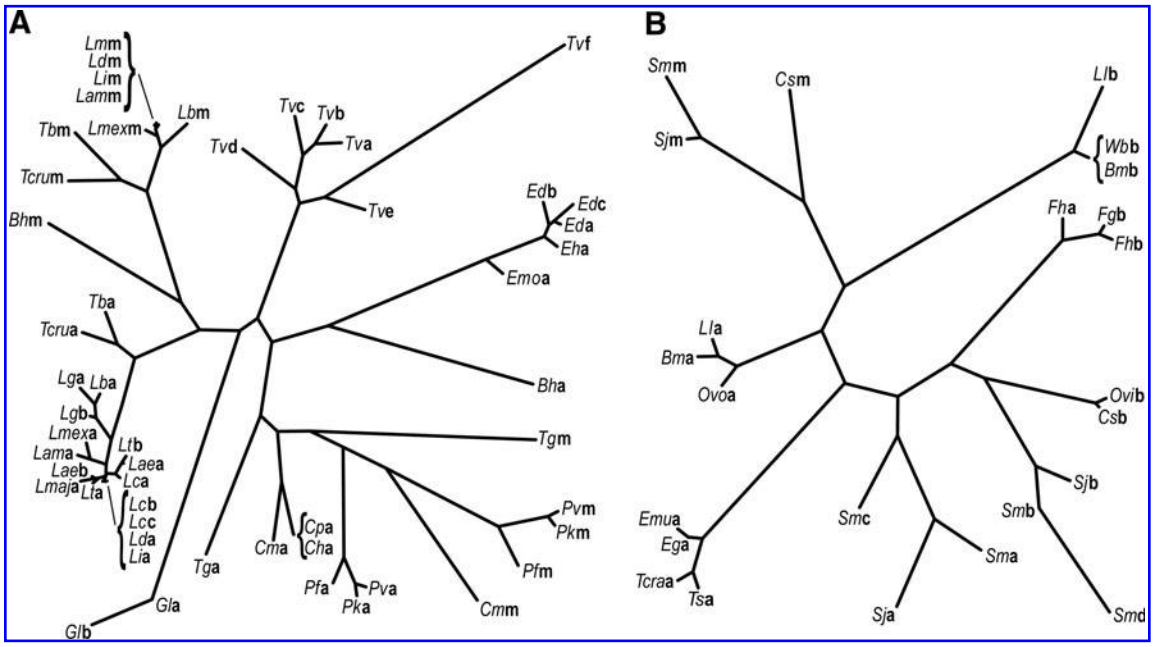

FIG. 8. Relatedness tree of Prx1 subfamily members. Shown are **(A)** a tree of the Prx1 subfamily members from protozoan parasites, and **(B)** a tree of the Prx1 subfamily members from helminthic parasites. For each Prx1 we show only its italicized species-specific prefix followed by its suffix in bold type. The tree was generated using Clustal Omega 1.0.2 multiple sequence alignment (231) followed by PHYML3.0 phylogeny calculation (88) and displayed using phylip 3.67: drawtree (76).

all Prx6 subfamily members form decamer-like ring complexes during their catalytic cycle.

Prx5. Recombinant *Pf*Prx5 functions as a 1-Cys Prx and has a preference for the organic ROOH substrates *t*-butyl-OOH and phosphatidylcholine-OOH, with H_2O_2 and cumene-OOH reduced less efficiently (185). Since the apicoplast is the site of fatty acid synthesis, this suggests that *Pf*Prx5 is important for the reduction of fatty acid peroxides. The recycling of *Pf*Prx5 is efficiently carried out by *Pf*Grx, and to a lesser extent *Pf*Trx1 and Plrx (185). The putative apicoplast proteins Trx2 and Trx3 (185) have not been tested. The existence of bacterial Grx-Prx5 fusion proteins (124) suggests that Grx is a plausible physiological redox partner of *Pf*Prx5. The *Pf*Prx5 crystal structure has been determined (Table 2) and is extensively described (222). This structure provided the first view of a C_P doubly overoxidized to C_P-SO_3^-, thought to be an artifact of long-term storage in an oxidizing environment.

PrxQ. *Pf*PrxQ has an N-terminal Prx domain and an uncharacterized, lysine-rich C-terminal domain (214). The recombinant Prx domain reduces H_2O_2 or cumene-OOH with $K_m \sim 10$ - 30 μM and a low specific activity of 6 μmol/min/mg protein. Its preferred reductant appears to be *Pf*Grx1 over *Pf*Trx1 (which does not show saturation). Its nuclear location and genome-wide chromatin association (214) suggests that it could play a sentinel role against oxidative DNA damage.

Toxoplasma. *T. gondii*, the causative agent of toxoplasmosis, infects about one-third of the world's population, causing serious illness in immune-challenged or pregnant individuals. The life cycle involves a bradyzoite stage with sexual reproduction in cats and an asexual, tachyzoite stage in cats, rodents, birds, or humans. The *T. gondii* genome contains four Prxs: Prx1a, Prx1m, Prx5, and Prx6. Except for $PrxQ_1$ these are a one-to-one match to those found in *Plasmodia*. Unlike *Plasmodia*, *T. gondii* has a functional cytosolic catalase (66).

Localization studies: Immunofluorescence microscopy has localized *Tg*Prx1a and *Tg*Prx6 to the cytosol and *Tg*Prx1m to the mitochondria (141). An independent study confirms this and identifies cytosolic Trx as a Prx reductant (5). Finally, a Prx (presumably *Tg*Prx1m) and an SOD isozyme undergo dual targeting to the apicoplast and mitochondria (204).

Expression levels and other in vivo *studies: T. gondii* was shown to express Prx1a and Prx1m constitutively and Prx6 in the tachyzoite and bradyzoite stages (141). A study both deleting and overexpressing *Tg*Prx6 and catalase showed that both similarly protected against oxidative stress (141).

Biochemical characterizations: Biochemical characterizations exist for *Tg*Prx1a and *Tg*Prx6.

Prx1a. Recombinant *Tg*Prx1a turns over H_2O_2 and *t*-butyl-OOH with a reported K_m in the nanomolar range and $k_{cat}/K_m \sim 10^6$–10^7 $M^{-1}s^{-1}$ (5). As no nanomolar substrate concentrations were tested, these K_m values could be artificially low if the recycling reaction is rate limiting. Also, treatment of macrophages *in vitro* with recombinant *Tg*Prx1a increases their replication rates and influences their developmental fate (159). *Tg*Prx1a is also reported to interact with a histone lysine methyltransferase to influence chromatin structure and regulate the expression of antioxidant defenses (223).

Prx6. Unusual for this subfamily (183), *Tg*Prx6 appears to be a 2-Cys Prx with Cys209 serving as C_R (61). The enzyme appears active by GS protection assay, but direct peroxide reduction using Trx, Grx, or GSH could not be shown. The protein mostly forms dimers, but also associates in tetramers and hexamers (61). In a drug candidate screen, *Tg*Prx6 was covalently modified at C_P by the drug conoidin, but the inhibition of *Tg*Prx6 was not involved in conoidin bioactivity (95).

Cryptosporidia. Three *Cryptosporidia* species cause the water-borne disease cryptosporidiosis, characterized by gastroenteritis and diarrhea. The genomes of *Cryptosporidium parvum* and *Cryptosporidium hominis* encode only a Prx1a and that of *Cryptosporidium muris* a Prx1a and a Prx1m. Although the localization of *Cm*Prx1m has not been investigated, its sequence contains a weak mitochondrial localization signal and clusters nearer to the plasmodial Prx1m enzymes than the other cryptosporidial Prx1s (Fig. 8A). The simpler Prx set in *Cryptosporidia* compared with *Plasmodia* and *Toxoplasma* makes sense in light of their further reductive evolution including loss of the apicoplast. Also, the absence of Prx1m in *C. parvum* and *C. hominis* is noteworthy in light of their simpler mitochondrion-like organelles (mitosomes) that lack DNA and most ATP synthase subunits and that do not carry out oxidative phosphorylation (169).

The only characterized cryptosporidial Prx is Prx1a from *C. parvum*. Proteomic analysis after high doses of gamma radiation revealed nearly 10-fold elevated *Cp*Prx1a mRNA levels, peaking approximately 48 h after exposure (142). As radiation exposure can produce toxic levels of H_2O_2 (18), this induction may be an oxidative stress response.

Prxs in kinetoplastids

The kinetoplastid parasites (order Trypanosomatida) are named for the kinetoplast, a characteristic granular substructure associated with the basal body of the flagellum and containing the DNA of the lone, extended mitochondrion. They include *Leishmania* species responsible for disfiguring (cutaneous and mucocutaneous) and fatal (visceral) leishmaniases, as well as *Trypanosoma brucei* and *T. cruzi*, respectively the causes of African and American trypanosomiasis (sleeping sickness and Chagas disease).

As intracellular pathogens, the kinetoplastids must defend against ROS and $ONOO^-$, with the latter demonstrated to be necessary and sufficient to control *Leishmania donovani* infections in a mouse model (59, 182). Recently, using *T. cruzi*, direct support for $ONOO^-$ as an intraphagosomal cytotoxin in host defense has been obtained (9). This implies that enzymes like Prxs capable of reducing $ONOO^-$ are crucial for virulence.

The kinetoplastid redox system is comprised of subfamily Prx1 peroxiredoxins (commonly called Txn peroxidases in the literature), their reducing partner Txn, the small-molecule dithiol trypanothione [TS_2, oxidized; $T(SH)_2$, reduced], and trypanothione reductase (TR), as depicted in Figure 6 (65, 135). Each species with a sequenced genome has one cytosolic Prx1 (*i.e.* Prx1a) and one Prx1m, and we suppose that the other species also have Prx1m enzymes. In these kinetoplastids, from two to seven highly similar, tandem cytosolic Prx1 genes are present. Although few of these are recognized as distinct by our 95% criteria (*e.g.,* only two of seven loci in *Leishmania major*), it is certain that at least some actually are distinct (*e.g., Leishmania aethiopica* and *Leishmania chagasi* Prx1a-type genes are known to be differentially expressed).

Leishmania. The *Leishmania* life cycle involves the development of the infectious promastigote in the gut of the phlebotomine sandfly and development of the disease-causing amastigote in human macrophages.

Localization studies: The *L. chagasi* enzymes *Lc*Prx1a and *Lc*Prx1b (22), and *L. amazonensis*, *Lam*Prx1a were localized to the cytosol (101, 149). *Ld*Prx1m has been shown in promastigotes to be found in the kinetoplast region of the mitochondrion and throughout the entire mitochondrion in amastigotes (96). An interesting twist occurs for *Leishmania braziliensis*, for which a Prx was identified as secreted (55). The exact Prx was not specified, but as it was homologous to *Lg*Prx1b, we denote it *Lb*Prx1a. Because the *Lb*Prx1a sequence has no apparent signal peptide, and also because about 98% of the *L. donovani* secretome lacks classical N-terminal secretion signals (232), it was suggested that *Leishmania* may employ nonclassical secretion pathways.

Expression levels and other in vivo *studies:* In *L. aethiopica*, Prx1a expression is restricted to amastigotes and stationary phase promastigotes, whereas Prx1b is expressed throughout the life cycle (112). Similarly, in *L. chagasi*, Prx1a is expressed in amastigotes, with Prx1b and Prx1c predominantly expressed in promastigotes, the latter at a much lower level (21). Prx1m expression levels in *L. donovani* were shown to increase strongly in promastigotes in the late logarithmic phase and in amastigotes (96). Also, *Lam*Prx1a and *Lam*Prx1m were upregulated by exposure to arsenite (101, 149). In *L. major*, Prx1a is expressed in promastigotes, and accounts for 1%–4% of total cellular protein (133).

Studies in *Leishmania guyanensis* show that oxidative challenges are present throughout normal growth, as *Lg*Prx1a and *Lg*Prx1b populate the disulfide form (*i.e.,* covalent dimers) during all developmental stages. Provocatively, it was observed that more of the disulfide form was seen in an aggressively metastatic *L. guyanensis* strain than in a nonmetastatic strain (1). In another isolate, an unspecified cytosolic *Lg*Prx1 also was identified as a virulence factor (258).

Prx overexpression, as expected, protects parasites from oxidative challenge. Cell culture studies include *Leishmania amazonensis* cells overexpressing *Lam*Prx1a or *Lam*Prx1m (101), and *L. chagasi* cells overexpressing *Lc*Prx1a (21), and *in vivo* results are available for *Leishmania infantum* overexpression of either *Li*Prx1a or *Li*Prx1m (38). In *L. donovani*, overexpression of *Ld*Prx1a in the promastigote results in protection against a combination of H_2O_2 and $NO^{\bullet}$ and increased virulence (106) and overexpression of *Ld*Prx1m decreases H_2O_2-induced apoptosis (96). In *L. infantum*, *Li*Prx1a overexpression preferentially protects against H_2O_2 challenge and *Li*Prx1m overexpression protects only against *t*-butyl-OOH (38).

Biochemical characterizations: Prx1a and Prx1b. The cytoplasmic Prxs from *L. chagasi* and *L. donovani* have been biochemically characterized. Recombinant *Lc*Prx1a has been shown to reduce H_2O_2, alkyl ROOH, $ONOO^-$, and possibly even hydroxyl radicals or nitric oxide (21), whereas *Lc*Prx1b was seen to only reduce H_2O_2 (22). Biochemical characterization of *Ld*Prx1a, done with reducing partners Txn1 and Txn2 from model trypanosome *Crithidia fasciculata*, revealed its ping-pong reaction kinetics and very high activity with *t*-butyl-OOH ($k_{cat}/K_m = 2.4\times10^7\ M^{-1}s^{-1}$); specific activity using *t*-butyl-OOH was similar with H_2O_2 and cumene-OOH, but was relatively low with linoleic acid-OOH and phosphatidylcholine-OOH (80). Interestingly, the specific activity of *Ld*Prx1a with *t*-butyl-OOH using the *Cf*Txn1 and *Cf*Txn2 is on the same order of magnitude as that of the native *C. fasciculata* Prx in this system.

Prx1m. Mitochondrial Prxs from *L. donovani* and *L. infantum* have been characterized. *Ld*Prx1m was shown to reduce peroxides and protect DNA from oxidative damage (96). Recombinant *Li*Prx1m had efficient peroxidase activity in a redox system comprised of NADPH, *T. cruzi* TR, $T(SH)_2$, and *C. fasciculata* Txn1 and Txn2 (35). *Cf*Txn2 bound *Li*Prx1m well ($K_m = 31.9\ \mu M$), and the catalytic efficiency (k_{cat}/K_m) for *t*-butyl-OOH was $4.1\times10^6\ M^{-1}\ s^{-1}$. Curiously, *Li*Prx1m was reported to be inhibited in a time- and concentration-dependent manner by linoleic acid-OOH and phosphatidylcholine-OOH (35).

Trypanosoma. The trypanosomes have complex life cycles involving the tsetse fly (for *T. brucei*) or the triatomine bug (for *T. cruzi*) in which the epimastigote form develops and then differentiates into the infective metacyclic trypomastigote (MT). In the human host, *T. brucei* MTs develop into bloodstream trypomastigotes and disseminate to a variety of tissues (including blood, lymph, and spinal fluid). In contrast, *T. cruzi* MTs develop into amastigotes, and differentiate into trypomastigotes at the insect wound site, and then enter the bloodstream. Interestingly, *T. brucei* and *T. cruzi* lack Grx and Trx (135, 136), and the complete Prx cascade has been shown in *T. brucei* to be essential for parasite survival [reviewed in Ref. (8)] and is considered an important target for drug design. Both *T. brucei* and *T. cruzi* have two Prxs: Prx1a and Prx1m.

Localization studies: As expected, *T. cruzi* and *T. brucei* Prx1a and Prx1m are localized to the cytosol and mitochondrion, respectively (247, 261).

Expression levels and other in vivo *studies: Tb*Prx1a and *Tb*Prx1m are both expressed in the bloodstream and procyclic stages of the parasite (247). Similarly, *Tcru*Prx1a was seen to be expressed throughout the life cycle (203). In support of the role of Prxs in maintaining infectivity, overexpression of *Tcru*Prx1a both protect against exogenous $ONOO^-$ and yield higher growth rates under $ONOO^-$ challenge (199). These protective effects are lost if the C_P residue is mutated. *T. cruzi* parasites overexpressing both *Tcru*Prx1a and *Tcru*Prx1m exhibit increased virulence (199) and levels of *Tcru*Prx1a and *Tcru*Prx1m directly correlate with degree of parasitemia in mouse models (200).

Biochemical characterizations: Prx1a. Recombinant *Tb*Prx1a, in the presence of the recombinant native Prx redox cascade components (NADPH, $T(SH)_2$, and *T. brucei* Txn and TR), efficiently reduced H_2O_2 and could reduce $ONOO^-$ with a $k_{cat}/K_m = 9\times10^5\ M^{-1}s^{-1}$ (249). Similarly, the reduction of $ONOO^-$ by *Tcru*Prx1a was reported to be $7.2\times10^5\ M^{-1}s^{-1}$ (249) and independently around $1\times10^6\ M^{-1}s^{-1}$ (201). In the latter study, H_2O_2 reduction by *Tcru*Prx1a was very efficient ($k_{cat}/K_m = 3\times10^7\ M^{-1}s^{-1}$; 201); *t*-butyl-OOH reduction in a different study was much less so ($k_{cat}/K_m = 3.2\times10^4\ M^{-1}s^{-1}$) (87).

The crystal structure of *Tcru*Prx1a has been determined in its FF thiol form, providing the first structure of an active Prx known to have efficient $ONOO^-$ reductase activity (202). It adopted the expected toroidal pentamer of dimers. Recently, molecular dynamics simulations have explored active-site pK_a values and oligomerization interactions (275).

Prx1m. A recent, comparative kinetics study showed that the remarkably efficient reduction of $ONOO^-$ by *Tcru*Prx1m (k_{cat}/K_m of $1.8\times10^7\ M^{-1}s^{-1}$) is about 10-fold greater than that by *Tcru*Prx1a and more than double its own k_{cat}/K_m for the reduction of H_2O_2 (201).

Prxs in other selected protozoan parasites of humans

Here we cover localization, *in vivo* studies, and biochemical characterization of Prxs in metamonads (*T. vaginalis* and *Giardia lamblia*), *Naegleria fowleri*, and species of the genus *Entamoeba*.

T. vaginalis. *T. vaginalis* causes the sexually transmitted infection trichomoniasis, considered the most common parasitic infection in industrialized countries. It is a metamonad, which are flagellate anaerobic/microaerobic protozoa with a life cycle including swimming trophozoite and cystic stages. *T. vaginalis* prefers oxygen concentrations below 0.25 μM and is unable to tolerate oxygen concentrations above 60 μM, or about 5% O_2 (189). As an anaerobe, it lacks mitochondria and instead has a specialized organelle called the hydrogenosome. Hydrogenosomes, particularly well studied in *T. vaginalis*, produce ATP and hydrogen gas using an oxygen-sensitive pyruvate:ferredoxin oxidoreductase and a hydrogenase (179).

To maintain low intracellular oxygen concentrations, *T. vaginalis* employs cytosolic and hydrogenosomal oxidases and SOD (70). *T. vaginalis* lacks detectable Gpx or heme-dependent peroxidase activity (70), but has multiple Prxs: at least six Prx1 subfamily members and two Tpxs. There may be even more, as one of the Prx1 gene sequences filtered by our >95% identity cutoff appears to represent a distinct gene. Of note, this is the only eukaryote with a Tpx subfamily Prx (183). Proteomics identified *Tv*Tpx-a and a Trx as components of the *T. vaginalis* hydrogenosome (209), and later, two TrxR enzymes bearing novel internal hydrogenosome targeting sequences were also discovered (162). Thus, a complete Prx system exists in the hydrogenosome.

In expression studies of *Tv*Prx1a, a Western blot detected two protein bands of 22 and 20 kDa. The former is the expected size for *Tv*Prx1a; the latter may be a crossreacting Prx. High oxygen or the absence of ascorbate led to elevated Prx1a mRNA and increases in both Western bands. Adding cysteine slightly suppressed Prx1a mRNA levels, abolishing the 22 kDa protein band, but increasing the 20 kDa band (53).

In a proteomic study of antiprotozoal 5-nitroimidazole drugs, *Tv*Prx1a and *Tv*Tpx-b were two of only seven proteins identified as covalently modified by metronidazole and tinidazole (143). A twofold reduction in peroxidase activity was observed in extracts of metronidazole-treated cells, but a similar loss in TrxR activity was seen, so the Prxs are not necessarily implicated. Also, metronidazole-resistant cells had elevated levels of Prx1a, Prx1b, Prx1c, and a protein 96.4% identical to Prx1c (EuPathDB gene ID TVAG_455310), which could be a distinct gene product (143). An siRNA knockdown of an unspecified Prx and TrxR led to no clear detrimental effects although the cell cycle was somewhat extended [according to an abstract (276)].

Only one *T. vaginalis* Prx, *Tv*Prx1a, has been studied *in vitro*. Recombinantly expressed *Tv*Prx1a, *Tv*TrxR, and *Tv*Trx, together, were found to effectively reduce H_2O_2, cumene-OOH, and *t*-butyl-OOH (53).

G. lamblia. *G. lamblia* [synonymous with *G. intestinalis* (174)], also a metamonad, is the cause of giardiasis, "beaver fever." By our criteria, it has two distinct Prx1 subfamily members. However, we found no associated published studies. The *Gl*Prx1b sequence is strongly predicted by SignalP-HMM (26, 71) to encode a secreted protein. As the *G. lamblia* Prxs are not closely related to those of *T. vaginalis* (Fig. 8A), they need not behave similarly.

N. fowleri. *N. fowleri* is a free-living amoeba that causes primary amoebic meningoencephalitis, a rare but acute and frequently fatal central nervous system disease. The genome has not yet been sequenced, but an 18-kDa Prx (matching a Prx1 from *Aedes aegypti* by its N-terminal sequence) was identified as an excretory–secretory pathogenic factor in *N. fowleri* infection (122). While it was not cloned and so may not be a Prx1, the sequenced genome of the nonpathogenic *Naegleria gruberi* (84) contains a Prx1 and a Prx5 (NCBI accession numbers XP_002678814 and XP_002680895, respectively). Though neither is characterized, the TargetP server (71) identifies a mitochondrial transit peptide in NgPrx5.

Entamoebae. *Entamoeba histolytica* is an anaerobic protozoan responsible for amoebic dysentery and amoebic liver abscess. Two morphologically indistinguishable, nonpathogenic relatives, *Entamoeba dispar* (63) and *Entamoeba moshkovskii* (7), commonly co-occur with *E. histolytica* and are also discussed here. *E. histolytica* does not possess mitochondria and lacks GSH as well as the enzymes central to GSH metabolism (73). The *E. histolytica* and *E. dispar* genomes are sequenced but not yet fully assembled and, using our 95% identity criteria, have one and three Prx1 subfamily members, respectively, all very similar in sequence (Fig. 8a). With 8 and 20 possible loci in the two species, we suspect that more of these will represent unique *Prx* genes, especially since seven were cloned from a single strain of *Entamoeba nuttalli*, a rhesus monkey parasite (243, 244).

*Eh*Prx1a was initially discovered as a 29-kDa antigen Eh29. It is by far the primary source of free thiol on the protein surface (82), and formed the basis of a vaccine conferring protection in an animal model (236). The protein was shown to be a Prx (30) dependent on Trx and TrxR (207) and could also be reducible by the *E. histolytica* trypanothione–dependent TR system (245). *Eh*Prx1a is 50 times more abundant than the *Ed*Prx1 and is mostly present on the outer surface of cells (poised to counteract host redox defenses), and this may in part explain the invasiveness of *E. histolytica versus E. dispar* and *E. moshkovskii* (50). The only studied *Ed*Prx1 seems restricted to the cytoplasm (50) and *Emo*Prx1a to the cytoplasm and nucleus (48); *Eh*Prx1a may also be present in the cytoplasm (30, 242). *Eh*Prx1a was found to be upregulated about twofold in trophozoites under oxygen stress conditions (3) or exposure to 50 m*M* Trichostatin A, a histone deacetylase inhibitor (104). Increased expression of *Eh*Prx1a (along with SOD) is also associated with metronidazole resistance (246, 259).

The recombinant *Ed*Prx1 (N-terminally fused to *E. coli* Trx) yielded a k_{cat}/K_m of $\sim10^5\ M^{-1}s^{-1}$ for H_2O_2. Its turnover of various organic ROOHs was 10%–40% less efficient (50). At saturating H_2O_2, the rate of a corresponding *Eh*Prx1a was about half that of the *Ed*Prx1 (50). Independently, *Eh*Prx1a was found by DNA nicking assay to be slightly less active than *Emo*Prx1a (48). The Prxs from *E. histolytica* and *E. dispar*

have a distinctive ~40 residue cysteine-, lysine-, and glutamate-rich sequence at the N-terminus, reportedly essential to enzyme activity, although some apparently truncated loci lack this sequence (30).

Prxs in helminths

The helminths are multicellular, parasitic worms, and those examined here are roundworms (Phylum Nematoda) and two classes of flatworms (Phylum Platyhelminthes), namely, tapeworms (Class Cestoda) and flukes (Class Trematoda). In total, 25 Prxs—all in subfamily Prx1 or Prx6—were identified in 17 species. No crystal structures of helminth Prxs have been solved.

One important question is whether, as multicellular eukaryotes, helminths have floodgate-type Prxs (89) that participate in cell signaling *via* an overoxidation shunt. While overoxidation-sensitive Prxs have been documented in *Schistosoma mansoni* (225), there are no recognizable sestrin genes in helminths, including *Caenorhabditis elegans* (248). Also, although *C. elegans* possesses a sestrin homolog and a Prx sensitive to overoxidation, its subsequent reduction is very slow. Furthermore, under ordinary conditions, accumulation of the overoxidized Prx could be detected neither in wild-type *C. elegans* nor in a sestrin deletion strain (248).

Nematoda. Nematodes cause widespread, disabling diseases known as filiariases. Of the five species surveyed, the filarial parasites with genomes sequenced have two or three Prxs each. Of these, *Brugia malayi* Prx1b and *Onchocerca volvulus* Prx1a have been expressed *in vitro*, and found to have peroxidase activity by DNA protection assay (86, 152). These two Prxs are transcribed and translated in larvae and adult worms. In the adult, *Bm*Prx1b was localized to the hypodermis and lateral chord and was not secreted by or at the surface of the larval or adult worms (86). In contrast, *Ovo*Prx1a is found in the larval and adult hypodermis and cuticle and appears to be secreted (152, 278). The closely related homolog *Bm*Prx1a (86) (Fig. 8B) could be likewise surface-localized or secreted, as it is antigenic in mice (10) and in humans (154, 155). The 1-Cys *Bm*Prx6 has been cloned (44) and patented as a vaccine antigen (U.S. Patent 6,352,836); its ortholog from the dog heartworm *Dirofilaria immitis* is secreted and has been found in developmental stages and localization patterns similar to the filiarial Prx1a enzymes, above (45).

Although no Prx has been identified in *Ascaris lumbricoides* (which lacks a sequenced genome), a closely related Prx1 (accession number Q9NL98) from the swine parasite *Ascaris suum* has been shown to be active *in vitro* by DNA nicking assay and to be expressed in adult female worms (251). Two uncharacterized Prxs (accession numbers YP_198397.1 and YP_197960.1) are found in the genus *Wolbachia*, the *B. malayi* bacterial endosymbiont (83).

Cestoda. Cestodes cause cysticercosis when larvae penetrate the intestinal wall and disseminate into muscle and central nervous system tissues. Although no genome of the four cestodes surveyed has been sequenced, each has one identified Prx. These are all Prx1 subfamily members and cluster by sequence (Fig. 8B). *Echinococcus granulosus* Prx1a is expressed in all tissues of the protoscolex and brood capsules and has DNA protection assay activity (100, 145). Its expression in the juvenile stage can be inferred from its isolation from a protoscolex cDNA library (NCBI accession number AAL84833). In cystic echinococcosis patients, *Eg*Prx1a elicits a strong humoral immune response, but since anti-*Eg*Prx1a antibodies are not protective against infection, their role, if any, is unclear (234).

Echninococcus multilocularis Prx1a is expressed at least in the larval stage, having been cloned from a metacestode cDNA library (NCBI accession number BAC11863). *Taenia solium* and *Taenia crassiceps* have high H_2O_2 tolerance, and constitutively express Prx1a (252). Antibodies against *Ts*Prx1a were used to show its presence throughout development, as well as the presence of homologs in *T. crassiceps* cysticerci and *Taenia saginata* adults (170). Recombinant *Ts*Prx1a was found to have Trx-dependent peroxidase activity with H_2O_2 and cumene-OOH (170).

Trematoda. Trematodes include the blood and liver flukes, which cause schistosomiasis and fascioliasis, respectively. The six species included in our survey are two blood flukes from the genus *Schistosoma* and four liver flukes, two from the genus *Fasciola* and two from the family Opisthorchiidae. All of these have Prxs only from subfamily Prx1. Trematodes do not possess catalase and their Gpx enzymes are generally restricted to the vitelline glands and intrauterine eggs (168) and exhibit little activity against H_2O_2 (156).

Schistosoma: The schistosomal species *S. mansoni* and *Schistosoma japonicum* are among the causative agents of schistosomiasis (bilharzia), a persistent infection occurring primarily in poor communities in the tropics, with over 200 million people infected and 700 million at risk. Three schistosomal Prx1 enzymes have been studied, referred to here as Prx1a, Prx1b, and Prx1m (the latter with a mitochondrial targeting sequence). The orthologous enzyme pairs from the two species cluster together in the relatedness tree (Fig. 8B), suggesting their similar function. The PREX database lists two additional Prx1 enzymes in *S. mansoni*, with *Sm*Prx1c resembling *Sm*Prx1a, and *Sm*Prx1d resembling *Sm*Prx1b (Fig. 8B).

Transcription of the three studied *S. mansoni* Prxs increases during development (224), peaking in the redox-stress tolerant adult forms (224). *Sm*Prx1a was expressed at a higher level than the others. In *S. japonicum*, all Prxs are constitutively expressed throughout development (138). *Sj*Prx1a has been localized to the surface of the miracidium and in the space between the miracidium and eggshell. In the adult fluke, it is found in the tegument. *Sj*Prx1a is secreted in culture and in host tissues surrounding eggs (138). Likewise, *Sm*Prx1a is present in male and female adult worms (140), and is also found in and secreted by eggs (262). Eggs also secrete a reducing partner of *Sm*Prx1a, *Sm*Trx1 (6). *Sj*Prx1b is found in eggs only inside the miracidia; in the adult, it is found in subtegumental tissues, the parenchyma, the vitelline gland, and the gut epithelium (138).

Knockdown of *Sm*Prx1a expression in culture results in sevenfold decreased survival of parasites and increased protein and lipid oxidation (224). Also, whereas knocking down *Sm*Prx1a and *Sm*Prx1b individually in *S. mansoni* sporocysts exhibited no phenotype, a combined *Sm*Prx1a/*Sm*Prx1b knockdown resulted in significantly smaller larvae (176) and reduced survival upon exposure to H_2O_2 or when cultured together with susceptible snail hemocytes (177). Similarly, with *Sj*Prx1a knocked down, larval growth is retarded by organic ROOH treatment, whereas a knockdown of *Sj*Prx1b

had less pronounced effects (139). Study of recombinant *Sm*Prx1a, *Sm*Prx1b, and *Sm*Prx1c showed that each exhibits a catalytic efficiency (k_{cat}/K_m) of $\sim 10^4$–10^5 $M^{-1}s^{-1}$ (225). *Sm*Prx1a is robust to oxidative inactivation by H_2O_2, whereas *Sm*Prx1b and *Sm*Prx1m are sensitive to H_2O_2 and have a preference for organic ROOH substrates (225), so the latter enzymes may play a role in redox signaling, if this occurs in *Schistosoma*. Also, *Sm*Prx1b and *Sm*Prx1m could be reduced by both Trx and, unusually, GSH (225).

Fasciola: The *Fasciola* species *F. gigantica* and *F. hepatica* lack catalase and have little or no Gpx (43, 160). Three Prx1 genes from the genus have been identified (43). One *F. hepatica* Prx is secreted and so we denote it Prx1a (229). Although it lacks a secretion signal peptide (71), *Fh*Prx1a is strongly predicted by the Secretome Server (25) to follow a nonclassical eukaryotic secretion pathway and *Fh*Prx1b is not. Although not biochemically characterized, *Fh*Prx1b (which is 99% identical in sequence to *Fg*Prx1b) has a weakly predicted mitochondrial transit peptide of 24 residues at its N-terminus, and *Fh*Prx1a does not (71). *Fh*Prx1a has demonstrated efficacy as a vaccine antigen in an animal model (161), whereas an *Fg*Prx1b-based vaccine has failed in a separate animal trial (211).

*Fh*Prx1a protein is abundant in the parasite and has been cloned, recombinantly expressed, and characterized: it was shown to be active by DNA protection assay; to reduce H_2O_2, cumene-OOH, and *t*-butyl-OOH with catalytic efficiencies (k_{cat}/K_m) $\sim 5\times 10^5$ $M^{-1}s^{-1}$; and to be inactivated *via* overoxidation at 0.5 m*M* H_2O_2 (217, 229). *Fg*Prx1b gene expression is constitutive throughout development, and localized to the gut epithelium, tegument, and reproductive system (43). In very early stage juveniles, the gut epithelium exhibits increased transcript levels (43).

Opisthorchiidae: Despite the lack of sequenced genomes, several Prxs have been identified in the liver flukes *Clonorchis sinensis* and *Opisthorchis viverrini*. In these organisms, thus far only a Prx1b and a Prx1m have been found. Both Prx genes are expressed during the entire life cycle, with *Cs*Prx1b transcript levels observed (by semiquantitative RT-PCR) to increase steadily from the juvenile to the adult stages and the *Cs*Prx1m gene expressed at a constant, much lower level (19). Although both proteins can be detected throughout the worm tissues, *Cs*Prx1b is much more abundant and is also secreted (19), as confirmed by a secretion proteomics study (114). The *Ovi*Prx1b gene is expressed in all parasite tissues and the protein may also be secreted, as it was found in host secondary bile ducts too small for infection (239). Thus, *Ovi*Prx1b and *Cs*Prx1b appear to combine the properties of the Prx1a and Prx1b of schistosomes. Recombinantly expressed *Cs*Prx1b and *Cs*Prx1m showed activity in a DNA protection assay (239) and yielded k_{cat}/K_m values on the order of 10^{-3}–10^{-4} $M^{-1}s^{-1}$ for H_2O_2, cumene-OOH, and 13-hydroperoxy-octadecadienoic acid (19). *E. coli* and yeast reducing partners were used, however, so these probably underestimate their true physiological rates.

Prxs in fungi

The intracellular pathogenic fungi with identified Prxs are *C. neoformans* and the *Encephalitozoon* species, *E. cuniculi* and *E. intestinalis*. The sequenced genomes show that *C. neoformans* has three Prxs (from subfamilies Prx1, Prx6, and PrxQ), and each *Encephalitozoon* species has a single Prx from subfamily Prx1. In *C. neoformans*, *Cn*Prx1a was shown to be inducible by H_2O_2 (164, 165) and the gene encoding each Prx has been individually deleted: the deletion of the Prx1a gene exhibits reduced growth rate, increased sensitivity to ROS and RNS, and decreased virulence in a mouse model; deleting the genes encoding *Prx6* and *PrxQ* yields no related phenotype (164). *E. cuniculi* lacks catalase and has no detectable peroxisome, and is expected to rely on Prx1a along with a GSH-dependent peroxidase and SOD for coping with ROS (75). One proteomics study found *Ec*Prx1a to be expressed in the late sporogonial stages (29).

Prxs as Potential Drug, Vaccine, or Diagnostic Targets

Certain Prx systems seem to be essential for life, but current drugs tend to target enzymes upstream of the Prxs (8, 107). For example, arsenic-based drugs for African trypanosomiasis and antimony-based therapeutics used against leishmaniasis are thought to target $T(SH)_2$ and TR (56, 74, 178, 269). Similarly, in *L. infantum* a cytosolic Txn has been shown to be essential (215).

Although few selective Prx inhibitors are known, a structure–activity relationship study with derivatives of conoidin A, a covalent inhibitor of *T. gondii* Prx6, led to analogs with IC_{50} values ranging from about 10 to 100 μM (150). Although conoidin is toxic to *T. gondii* cells, *Tg*Prx6 is not its molecular target (95). The inhibition of mammalian PrxI and PrxII by conoidin makes it less attractive as an antiparasitic lead compound, yet since few Prx inhibitors are known, Conoidin A and its derivatives may be of value as probes in Prx chemical genetics studies (238).

One validated Prx drug target is *Tb*Prx1a, shown to be essential by RNAi (260). Inspired by the potential of some heteroaromatic quinols as cancer drugs, a series of substituted quinols were tested against *T. brucei* bloodstream trypomastigotes (134). Three of the derivatives exhibited half-maximal effective concentration (EC_{50}) values below 100 n*M*, although all exhibited poor specificity for *T. brucei* cells (only two- to threefold compared with MRC5 human fibroblasts). The most promising compound (PMX464) was shown to be trypanocidal at five times the EC_{50} value. Other molecular targets of PMX464 were a Gpx-like protein (with an inhibition profile nearly identical to *Tb*Prx1a) and $T(SH)_2$ (with 14-fold greater affinity than GSH and cysteine).

A likely barrier to the development of clinical Prx inhibitors is the strong conservation of the FF active site among all Prxs (90), which makes specificity an issue. Some authors suggest exploiting species-specific features such as the angle of approach of the C_R in the resolving step of the reaction [human erythrocyte TPx-B compared with *Tcru*Prx1a (202)] or specific characteristics of the dimerization interface(s).

Prxs have been implicated in several instances of drug resistance. Antimony resistance resulted from overexpression of *Ld*Prx1a in *L. donovani* promastigotes and amastigotes (106). *Tcru*Prx1a and *Tcru*Prx1m expression was found to be increased in benznidazole-resistant *T. cruzi* strains (11, 188). Likewise, metronidazole-resistant *E. histolytica* exhibited an upregulated Prx1 (259).

Prxs have shown promise as vaccine antigens. *Lmaj*Prx1a is part of a heterologous prime-boost vaccine that promotes long-term protection against *L. major* infection in mice (237), a vaccine protective against infection in mouse and monkey models (34), and a trivalent vaccine successful in phase I

clinical trials (51). *Ld*Prx1a has been patented as a vaccine antigen candidate (U.S. Patent 7795406).

Prx-based helminth vaccine development has also been pursued. The *Bm*Prx1a gene has been used in a DNA cocktail vaccine efficacious in mice (10) and a peptide derived from a *Bm*Prx1a epitope is strongly immunogenic in mouse and human cell lines, and strongly and selectively immunoreacts to sera from immune and infected individuals (154, 155). An *Fh*Prx1a-derived vaccine protected goats from liver damage after *Fasciola* infection (161), but the *Fg*Prx1b-derived vaccine failed to demonstrate efficacy in buffaloes (211). Also, a multiple antigen peptide combining *S. mansoni* aldolase with *Sm*Prx1a elicited a specific immune response in mice (69). On a related note, Prxs may have diagnostic value in testing for *Leishmania* species (219, 233), *E. granulosus* (158), *F. gigantica* (43, 277), and *Taenia* species (253).

Treatment, prevention, and diagnostic tools are very limited for many parasitic diseases, especially those that disproportionately affect people in resource-poor communities (99). Despite the urgent need for improved interventions, financial support for relevant product development is generally weak (172, 173), limiting the progression of candidate drugs, vaccines, and diagnostics through clinical testing. Fundamental research in parasite biology and biochemistry is therefore essential to ensure that the therapeutic, vaccination, and diagnostic approaches pursued in the clinic are the ones most likely to succeed. Thus, it is encouraging to survey the extensive work on parasite Prxs and to note numerous efforts underway to understand and exploit their properties for the benefit of hundreds of millions of patients and people at high risk of parasitic infection.

Acknowledgments

The authors wish to thank Kim Nelson and Laura Soito for in-depth queries of the PREX database and discussion of results. We would like to acknowledge the support of National Institutes of Health Grant R01 GM050389 to L.B.P. and P.A.K.

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Date of first submission to ARS Central, November 14, 2011; date of acceptance, November 18, 2011.

Abbreviations Used

AhpF = alkyl hydroperoxide reductase component F
BCP = bacterioferritin comigratory protein
C_P = peroxidatic cysteine
C_R = resolving cysteine
EC_{50} = half-maximal effective concentration
FF = fully folded
Gpx = glutathione peroxidase
Grx = glutaredoxin
GS = glutathione synthase
LU = locally unfolded
MT = metacyclic trypomastigote
NCBI = National Center for Biotechnology Information
PDB = Protein Data Bank
Plrx = plasmoredoxin
PREX = the PeroxiRedoxin classification indEX
Prx = peroxiredoxin
Prx1 = a Prx subfamily or subfamily member
Prx5 = a Prx subfamily or subfamily member
Prx6 = a Prx subfamily or subfamily member
PrxQ = a Prx subfamily or subfamily member
RNAi = RNA interference
RNS = reactive nitrogen species
ROS = reactive oxygen species
RT-PCR = reverse transcriptase–polymerase chain reaction
SOD = superoxide dismutase
S_P = sulfur atom of the peroxidatic Cys
S_POH = sulfenic acid form of the peroxidatic Cys
S_PO_2H = sulfinic acid form of the peroxidatic Cys
S_R = sulfur atom of the resolving thiol
Srx = sulfiredoxin
Tpx = a Prx subfamily named thiol peroxidase, or member of the same
Trx = thioredoxin
TrxR = thioredoxin reductase
TR = trypanothione reductase
TS_2 = trypanothione (oxidized)
$T(SH)_2$ = trypanothione (reduced)
Txn = tryparedoxin

(*Appendix follows* →)

Appendix 1. List of Parasite Genera and Important Pathogenic Species Included in Prx Searches

Genus	*Important species*[a]
Acanthamoeba	*astronyxis, castellanii, divionensis, hatchetti, lenticulata, lugdunensis, polyphaga, rhysodes, ulbertsoni*
Ancylostoma	*duodenale*
Angiostrongylus	*cantonensis, costaricensis*
Anisakis	*simplex*
Ascaris	*lumbricoides*
Austrobilharzia	*variglandis*
Balamuthia	*mandrillaris*
Balantidium	*coli*
Baylisascaris	*procyonis*
Blastocystis	***hominis***
Brugia	***malayi***, *timori*
Capillaria	*hepatica, philippinensis*
Clonorchis	***sinensis***, *viverrini*
Cryptococcus	***neoformans***
Cryptosporidium	***parvum***, ***hominis***, ***muris***
Cyclospora	*cayetanensis*
Cystoisospora	*belli* (formerly *Isospora belli*)
Dicrocoelium	*dendriticum*
Dientamoeba	*fragilis*
Dioctophyme	*renale*
Diphyllobothrium	*latum*
Dipylidium	*caninum*
Dracunculus	*medinensis*
Encephalitozoon	***cuniculi***, ***intestinalis***
Echinococcus	***granulosus***, ***multilocularis***, *oligarthrus, vogeli*
Echinostoma	*echinatum*
Entamoeba	***dispar***, ***histolytica***, ***moshkovskii***
Enterobius	*vermicularis, gregorii*
Fasciola	***hepatica***, ***gigantica***
Fasciolopsis	*buski*
Giardia	***lamblia***
Gnathostoma	*hispidum, spinigerum*
Heterophyes	*heterophyes*
Hymenolepis	*diminuta, nana*
Leishmania	***aethiopica***, ***amazonensis***, ***braziliensis***, ***chagasi***, ***donovani***, ***major***, ***mexicana***, ***guyanensis***, ***infantum***, *panamensis, peruviana,* ***tropica***, *venezuelensis*
Loa	***loa***
Mansonella	*streptocerca*
Metagonimus	*yokogawai*
Naegleria	***fowleri***
Necator	*americanus*
Onchocerca	***volvulus***
Opisthorchis	***viverrini***, *felineus*
Paragonimus	*africanus, caliensis, kellicotti, skrjabini, uterobilateralis, westermani*
Plasmodium	***falciparum***, ***knowlesi***, *malariae, ovale,* ***vivax***
Pneumocystis	*carnii* (also known as *P. jirovecii*)
Pseudoterranova	*decipiens*
Rhinosporidium	*seeberi*
Schistosoma	*haematobium,* ***japonicum***, ***mansoni***, *mekongi*
Spirometra	*erinacei, mansoni, mansonoides, ranarum*
Strongyloides	*stercoralis*
Taenia	***crassiceps***, ***solium***, *saginata*
Toxocara	*canis, cati*
Toxoplasma	***gondii***
Trichinella	*britovi, nativa, nelsoni, spiralis*
Trichobilharzia	*regenti*
Trichomonas	***vaginalis***
Trichuris	*trichiura, vulpis*
Trypanosoma	***brucei***, ***cruzi***
Wuchereria	***bancrofti***

[a]Species indicated in bold type are those with at least one Prx protein or gene that is mentioned in the review. For all others, we found no Prx gene or protein mentioned in the literature or present in sequence databases.

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