

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Thiol-Dependent Peroxidases in *Mycobacterium tuberculosis* Antioxidant Defense

Martín Hugo^{1,2}, Rafael Radi^{1,2} and Madia Trujillo^{1,2}

¹Departamento de Bioquímica

²Center for Free Radical and Biomedical Research,
Facultad de Medicina, Universidad de la República,
Uruguay

1. Introduction

Mycobacterium tuberculosis (*M. tuberculosis*) is the causative agent of tuberculosis disease. According to the Global Tuberculosis Control 2010 report of the World Health Organization http://www.who.int/tb/publications/global_report/en/, approximately one-third of the world's population is latently infected with *M. tuberculosis* and about two million people die of this disease every year. The emergence of multi- and extensively- drug resistant strains to the currently available drugs makes the development of new therapeutic strategies a priority. However, the mechanisms underlying pathogenesis, virulence and persistence of infections caused by *M. tuberculosis* are not completely understood (Nathan, 2009; Lawn & Zumla, 2011).

M. tuberculosis is one of the most successful human pathogens. It has evolved diverse strategies to ensure growth and survival inside the hostile environment of macrophages, its primary host cells (Ehrt & Schnappinger, 2009; Meena & Rajni, 2010). The molecular mechanisms of *M. tuberculosis* pathogenesis are under active investigation, since they could provide the basis for a rationalized drug design. These include inhibition of phagosome maturation into phagolysosomes (Armstrong & Hart, 1971; MacMicking *et al.*, 2003), inhibition of the acidification of *Mycobacterium*-harboring phagosomes (Sturgill-Koszycki *et al.*, 1994), DNA repair and protein repair or degradation (Boshoff *et al.*, 2003; Gandotra *et al.*, 2007; Lee *et al.*, 2009), as well as decomposition of cytotoxic reactive nitrogen and oxygen species formed upon phagocytosis (Nathan & Shiloh, 2000; Shiloh & Nathan, 2000; Bedard & Krause, 2007). These should be considered as complementary survival mechanisms. Herein, we will focus in the antioxidant systems of *M. tuberculosis*, and particularly, in thiol-dependent peroxidases.

2. Formation of reactive oxygen and nitrogen species by activated macrophages

Upon phagocytosis, NADPH oxidase (NADPHox) assembles into an enzymatically active complex that transfers electrons from NADPH to molecular oxygen producing superoxide

oxidizing and nitrating moiety (Ferrer-Sueta & Radi, 2009; Alvarez *et al.*, 2011). In the absence of direct targets, peroxyntous acid ($pK_a = 6.5-6.8$, (Goldstein & Czapski, 1995; Pryor & Squadrito, 1995; Kissner *et al.*, 1997)) homolyses into nitrogen dioxide ($\bullet NO_2$) and $\bullet OH$ in 30% yields ($k = 0.9 \text{ s}^{-1}$ at pH 7.4 and $37^\circ C$) (Goldstein & Czapski, 1995; Gerasimov & Lymar, 1999). However, the importance of this reaction *in vivo* is probably limited, since in cells, most peroxyntous is expected to be involved in direct reactions. For instance, peroxyntous can react with carbon dioxide (CO_2) present in mM concentrations in biological systems ($k = 4.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 and $37^\circ C$), leading to the formation of up to 35% carbonate ($CO_3^{\bullet-}$) and $\bullet NO_2$ radicals, which are also oxidizing species (Lymar & Hurst, 1995; Denicola *et al.*, 1996; Bonini *et al.*, 1999; Augusto *et al.*, 2002). $\bullet OH$ and $\bullet NO_2$ can participate in lipid peroxidation reactions, resulting in fatty acid hydroperoxide formation (Barber & Thomas, 1978; Prutz *et al.*, 1985). These can also be synthesized by enzymatic mechanisms through lipoxygenase (LOX)-catalyzed reactions (Sevanian *et al.*, 1983). Fatty acid hydroperoxides can be released from membranes by the action of phospholipase A_2 (PLA_2) (Bonney *et al.*, 1985). Free arachidonic acid is toxic for *M. tuberculosis* acting in a synergistic way with reactive nitrogen species (Akaki *et al.*, 2000). Although the mechanism of synergism has not been resolved, the fact that free fatty acid-dependent toxicity to *Helicobacter pylori* increases in peroxidase-deficient strains indicates that fatty acid hydroperoxides could participate in cytotoxicity (Wang *et al.*, 2006). In summary, inside the phagosomes of activated macrophages, and among other reactive species, different peroxides can be formed, including H_2O_2 , peroxyntous and fatty acid hydroperoxides (Figure 1). All of these species have been reported to be cytotoxic against microorganisms including bacteria (Clifford & Repine, 1982; Denicola *et al.*, 1993; Hurst & Lymar, 1997; Evans *et al.*, 1998; Wang *et al.*, 2006). The enzymatic mechanisms that allow reactive nitrogen and oxygen species detoxification, in general, and peroxide reduction, in particular, thus enabling the bacterium to infect and persist inside the phagosome of activated macrophages, is a field of active investigation.

3. Singular aspects of the antioxidant defense systems of *M. tuberculosis*

The antioxidant defenses of *Mycobacterium tuberculosis* are unusual in many aspects. As most other Actinobacteria, it lacks glutathione, and contains millimolar concentration of 1-D-myo-inosityl-2-deoxy-2-(N-acetyl-L-cysteinyl)amino-D-glucopyranoside, or mycothiol (MSH), as main low molecular weight thiol (Newton & Fahey, 2002). MSH is maintained in the reduced form by mycothione reductase using NADPH as electron donor (Patel & Blanchard, 2001). It participates in drug detoxification pathways by forming adducts with alkylating agents and antibiotics that are subsequently cleaved by MSH S-conjugate amidase to generate a mercapturic acid (excreted outside the cell) and glucosamine inositol (used to regenerate MSH) (Newton *et al.*, 2000). MSH can function as a resource for metabolic precursors and for energy production (Bzymek *et al.*, 2007). Mycothiol-deficient *M. smegmatis* strains are more sensitive to $\bullet NO$ - and H_2O_2 -mediated toxicity than wild type strains (Rawat *et al.*, 2002; Miller *et al.*, 2007). However, there is currently no evidence for MSH acting as a reducing substrate for any peroxidase. Mycobacteria, among other organisms, also synthesize ergothioneine, which is a thiourea derivative of histidine containing a sulfur atom in the imidazole ring. Its synthesis is increased in *M. smegmatis* mutants in MSH synthesis suggesting a compensation mechanism (Ta *et al.*, 2011), although the actual function of this unusual thiol remains to

be investigated (Seebeck, 2010). Related to enzymatic mechanisms of reactive oxygen species detoxification, *M. tuberculosis* expresses a Fe-dependent superoxide dismutase, SODA (Rv3846), which is released to the extracellular medium and is considered to be important for pathogenesis (Edwards *et al.*, 2001); it also express a Cu-dependent SODC (Rv0432) that is not essential for intracellular growth within macrophages and seems to play a minor role in pathogenicity (Dussurget *et al.*, 2001). *M. tuberculosis* contains different thioredoxin-related enzymes which are maintained at reduced state by thioredoxin reductase and NADPH (Jaeger *et al.*, 2004). In spite of the absence of glutathione, *M. tuberculosis* genome codifies for different glutaredoxin-like proteins whose functional role awaits further investigation (Cole *et al.*, 1998). The bacterium expresses a heme-dependent peroxidase (catalase peroxidase, KatG) and several thiol-dependent peroxidases of the peroxiredoxin (Prx) type (see below). Moreover, *M. tuberculosis* lacks a functional OxyR, that in *E. coli* controls the transcription of a regulon of ~ 20 antioxidant genes (Zahrt & Deretic, 2002). The regulation of oxidative stress responses in *M. tuberculosis* is at least partially dependent on the alternative sigma factor H/antisigma factor H, a zinc-thiolate redox sensor (Raman *et al.*, 2001).

4. Catalase peroxidase, the heme-dependent peroxidase of *M. tuberculosis*

M. tuberculosis constitutively expresses a catalase peroxidase (EC 1.11.1.6) (MtKatG, Rv1908)(Diaz & Wayne, 1974). The enzyme has attracted considerable attention due to its role in the activation of the first line antituberculosis prodrug isonicotinic acid hydrazide (isoniazid, INH) and the fact that loss-of-function mutations are a major mechanism of resistance to INH (Zhang *et al.*, 1992). *In vitro* generated MtKatG negative strains were non pathogenic. Virulent catalase-negative clinical isolates overexpressed the thiol-dependent peroxidase alkyl hydroperoxidase reductase C (AhpC), indicating the need of another peroxidase to assure protection of the pathogen against oxidizing species (Sherman *et al.*, 1996). More recently, a mechanism of INH resistance in *M. tuberculosis* through down-regulation of KatG was proposed based on the observation that mutations in the *furA*²-*katG* intergenic region conferred INH resistance (Ando *et al.*, 2011). The protein has been identified in the cytosol, membrane fraction and culture filtrates of *M. tuberculosis* (Gu *et al.*, 2003; Mawuenyega *et al.*, 2005; Malen *et al.*, 2007). It displays a broad peroxidase activity, as well as a high catalase activity ($k_{\text{cat}}/K_M = 2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$)(Johnsson *et al.*, 1997), catalyzing the dismutation of H_2O_2 into dioxygen and water. It also reduces peroxynitrite ($k = 1.4 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 and 25 °C (Wengenack *et al.*, 1999)). The catalytic mechanism of H_2O_2 reduction by KatG involves the initial two-electron oxidation of the enzyme to compound I ((Fe IV=O)•). KatG contains a unique post-translational modification in the form of a three amino acid adduct (Met255-Tyr229-Trp107) with a specific role in the catalase reaction since mutation of any of the three residues virtually eliminates catalase but not peroxidase activity (Jakopitsch *et al.*, 2004; Ghiladi *et al.*, 2005). It has been proposed that catalase activity in KatG is associated with a radical formation in the Met-Tyr-Trp adduct, whereas during the peroxidase activity a tyrosyl radical is formed (Zhao *et al.*, 2010). In the case of peroxynitrite reduction, oxidation of resting state KatG to compound II (Fe IV=O) plus •NO₂ has been proposed (Wengenack *et al.*, 1999).

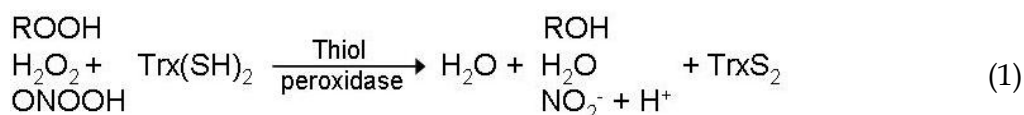
² FurA is a negative regulator of KatG expression in *Mycobacterium smegmatis* (Zahrt *et al.*, 2001)

In addition to *MtKatG*, the genome of *M. tuberculosis* codifies for a putative lignin peroxidase (Rv1900c) and other putative non-heme non-thiol -dependent peroxidases whose functional characterization is lacking (Cole *et al.*, 1998)(<http://www.webtb.org/>).

5. Thiol-dependent peroxidases of *M. tuberculosis*

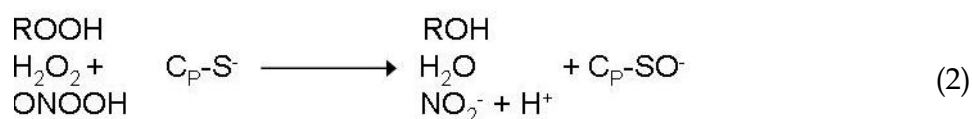
5.1 Thiol-dependent peroxidases

Peroxidases with catalytic activities dependent on critical cysteine residues are called thiol-dependent peroxidases. These enzymes catalyze the reduction of H_2O_2 , organic hydroperoxides and/or peroxyntrous acid (ONOOH) to water, organic alcohols and nitrite, respectively, at the expense of a reducing substrate, usually thioredoxin (Trx) or a Trx-related protein, via a double-displacement or ping-pong kinetic mechanism (Flohe *et al.*, 2003; Wood *et al.*, 2003; Trujillo *et al.*, 2007).



where ROOH is organic peroxide; ONOOH is peroxyntrous acid; NO_2^- is nitrite; ROH is organic alcohol; $\text{Trx}(\text{SH})_2$ is reduced thioredoxin and TrxS_2 is oxidized thioredoxin.

The oxidizing part of the catalytic cycle involves a $\text{S}_\text{N}2$ reaction occurring through a nucleophilic attack of the deprotonated thiol at the so called peroxidatic cysteine residue (C_P) on one of the peroxide oxygens. In the transition state, the negative charge is distributed among the two oxygen and the sulfur atoms, and the reaction is completed by the break of the peroxide bond forming an alcoxide as leaving group, which may protonate depending on its basicity. Thus, the thiolate in C_P suffers a two-electron oxidation to sulfenic acid (E-SOH).



The rest of the catalytic cycle differs depending on the kind of thiol-dependent peroxidase. In most cases, it consists on the formation of a disulfide bridge through the reaction between the sulfenic acid intermediate in C_P and another cysteine residue, which is called the resolving cysteine residue (C_R), which is then reduced by thioredoxin (Trx) (or another thiol-disulfide oxidoreductase protein) that is maintained at reduced state by thioredoxin reductase and NADPH (Poole, 2007). For all thiol-dependent peroxidases tested so far, the acidity constants of the peroxidatic thiols are quite high ($\text{pK}_\text{a} \sim 5 - 6.3$, (Bryk *et al.*, 2000; Ogusucu *et al.*, 2007; Trujillo *et al.*, 2007; Nelson *et al.*, 2008; Hugo *et al.*, 2009)). Thus, under physiological conditions they are expected to be mostly under thiolate form, the reactive species. However, the rate constants of reactions of C_P in thiol-dependent peroxidases with peroxide substrates are several orders of magnitude faster than the corresponding reactions of low molecular weight or most protein thiolates, indicating the existence of protein factors involved in specific peroxide reduction by these enzymes that are only starting to be unraveled (Trujillo *et al.*, 2007; Flohe *et al.*, 2010; Hall *et al.*, 2010; Ferrer-Sueta *et al.*, 2011).

Other intriguing aspect related to thiol-dependent peroxidase catalytic mechanism is the molecular mechanisms of the oxidizing substrate specificity: although in most cases thiol-dependent peroxidases can catalyze the reduction of a broad range of peroxides, preferential substrates vary, and do not reflect the expected trend that correlates thiolate reactivity with leaving group pK_a ³ (Trujillo *et al.*, 2007) that was reported for the reactivities of other thiolate with peroxides (Trindade *et al.*, 2006; Trujillo *et al.*, 2007).

Thiol-dependent peroxidases can be classified into two main groups⁴ based on sequence homology: glutathione peroxidases (Gpxs) and peroxiredoxins (Prxs). Since there are not genes for enzymes of the GPx type in *M. tuberculosis* genome, but there are several members of the Prx family, we will focus in the latter group of enzymes through the rest of this chapter.

5.2 Peroxiredoxins (EC 1.11.1.15)

Prxs are a family of thioredoxin-scaffold enzymes with thiol-dependent peroxidase activity (Chae *et al.*, 1994). They are ubiquitous, present in all living kingdoms and in different cellular compartments. They are also abundant, with concentrations usually in the μM range (Hofmann *et al.*, 2002). Due to their peroxidase activity, these enzymes play a role in antioxidant defenses. Moreover, at the light of the signaling role ascribed to H_2O_2 and other peroxides, Prxs are also regarded as key players in redox signaling processes and regulation of transcription factors (Rhee *et al.*, 2005; Hall *et al.*, 2009; Brigelius-Flohe & Flohe, 2011; Rhee & Woo, 2011). Peroxiredoxins have been functionally classified into 1-Cys Prxs and 2-cysteine Prxs according to the number of cysteine residues that participate in catalysis (Poole, 2007). The first part of the catalytic cycle is common for all kinds of Prxs and consists on the reduction of the peroxide substrate with concomitant oxidation of the C_P to a sulfenic acid derivative. In the case of 1-Cys Prxs, this sulfenic acid is reduced by different reducing pathways that depend on the particular 1-Cys Prx and that for most of them are still unclear. In 2-Cys Prxs, the sulfenic acid in C_P reacts with another Cys residue also required for catalysis, C_R that can be either in the same or in a different protein subunit (atypical or typical 2-Cys Prxs, respectively), forming a disulfide bridge that is reduced by Trx or a Trx-related protein. More recently, a Prx classification base on sequence homology has been proposed in the peroxiredoxin classification index (PREX) database (<http://csb.wfu.edu/prex/index.php>) (Nelson *et al.*, 2011; Soito *et al.*, 2011). Subfamilies thus identified are denoted by the name of one or more canonical member, as indicated below:

- **Alkyl hydroperoxide reductase C (AhpC) - Peroxiredoxin 1 (Prx1).** This subfamily is both the largest and the most widely distributed, with members found in archaea, bacteria, and all classes of eukaryotes. These proteins are functionally classified as typical 2-Cys Prxs.

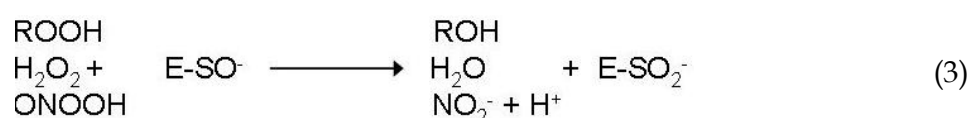
³ pK_a value of the alkoxide formed upon peroxide reduction

⁴ Other thiol-dependent peroxidases non-structurally related to Gpxs and Prxs exist. For example, in many bacteria, a thiol-dependent organic hydroperoxide reductase (Ohr) is involved in organic hydroperoxide detoxification. However, the *ohr* gene is absent in *M. tuberculosis* genome

- **Bacterioferritin comigratory protein (Bcp)- Peroxiredoxin Q (PrxQ)** (for *Escherichia coli* Bcp and plant PrxQ, respectively). Present mostly in bacteria, also in yeast and plants but not in mammals. They can function either as atypical 2-Cys Prxs or as 1-Cys Prxs.
- **Thiol peroxidase (Tpx)** (for *E. coli* Tpx). Tpx subfamily members are all bacterial and are almost exclusively classified as atypical 2-Cys Prxs.
- **Peroxiredoxin 5 (for *Homo sapiens* Prx5)**. Members of this subfamily are found from bacteria to mammals with members present in plants, fungi, and yeast. They are functionally classified as either 1-Cys Prxs or atypical 2-Cys Prxs.
- **Peroxiredoxin 6 (for *H. sapiens* Prx6)**. Members of this subfamily are found in bacteria, plants, yeast and mammals. In general, they function as 1-Cys Prxs.
- **Alkyl hydroperoxide reductase E (AhpE)** (for *M. tuberculosis* AhpE). Found in aerobic gram-positive bacteria of the order Actinomycetes and some archaea. AhpE from *Mycobacterium tuberculosis* has been functionally classified as a 1-Cys Prx, but information regarding the catalytic mechanisms of other members of this group is lacking.

Further information regarding this sequence-based classification of Prxs can be found in the PREX database and references therein.

Peroxiredoxins are now known to be, at least in some cases, very efficient peroxidases (Trujillo *et al.*, 2007; Parsonage *et al.*, 2008; Manta *et al.*, 2009). The local sequence motif at the active site, ProXXXThrXXCys, is very conserved among different Prx subfamilies, although Thr is replaced by Ser in few known Prx sequences and a peroxidatic selenocysteine (Sec) instead of Cys has been reported in a Prx from *Eubacterium acidamidophilum* (Sohling *et al.*, 2001; Hofmann *et al.*, 2002; Poole, 2007; Nelson *et al.*, 2011). Prxs also contain a highly conserved Arg. These conserved residues along with several backbone interactions determine a low pK_a value of C_P and contribute to the catalytic mechanism of Prxs, in which transition state stabilization has been proposed to be involved (Hall *et al.*, 2010), although the precise mechanism of catalysis is still to be unraveled. Prx concentrations in different cells and tissues are frequently regulated, and usually increase under conditions of oxidative stress. Moreover, their catalytic activities are also regulated by different mechanisms, including protein phosphorylation (Chang *et al.*, 2002; Woo *et al.*, 2010) and inactivation due to overoxidation of the C_P , which involves the two- electron oxidation of the sulfenic form of the enzyme to sulfinic acid (Yang *et al.*, 2002). Recent data from our group indicated that the mechanism of C_P overoxidation is similar to that of oxidation, with the deprotonated sulfenate (or its tautomeric sulfoxide form) and the protonated peroxide as the reacting species (Hugo *et al.*, 2009; Reyes *et al.*, 2011).



In 2-Cys Prxs, the susceptibility to overoxidation depends on the structural GGLG and YF motifs present mostly in eukaryotic 2-Cys Prxs (Yang *et al.*, 2002) but also in some prokaryotic organisms including cyanobacteria (Pascual *et al.*, 2010). These structural motifs make disulfide formation with C_R to occur at a slower rate and thus, 2-Cys Prxs that possess them are more prone to oxidative inactivation (Wood *et al.*, 2003). Cysteine sulfinic acid,

previously considered as an irreversible post-transductional modification, is now known to be reversed by enzymatic mechanisms in different 2-Cys Prxs (Chang *et al.*, 2004; Iglesias-Baena *et al.*, 2011) and has been suggested to be involved in signaling processes (Iglesias-Baena *et al.*, 2010). Moreover, overoxidized forms of some members of the Prx family gained function as molecular chaperones (Moon *et al.*, 2005; Lim *et al.*, 2008).

5.3 Peroxiredoxins from *M. tuberculosis*

The genome of *M. tuberculosis* codifies for different thiol-dependent peroxidases of the Prx type, namely AhpC, TPx, AhpE, and two putative Bcps proteins (Cole *et al.*, 1998), which have been detected in the cytosolic, membrane and culture medium fractions (Figure 2). We will describe below the main functional characteristics of *M. tuberculosis* Prxs as well as reported evidences of their participation in peroxide detoxification in cellular or animal models of tuberculosis disease.

5.3.1 Alkyl hydroperoxide reductase C (*MtAhpC*, Rv2428)

AhpCs are thiol-dependent peroxidase member of the AhpC-Prx1 subfamily of Prxs. *MtAhpC* is functionally classified as a typical 2-Cys Prx, although site directed mutagenesis experiments revealed that it has three instead of two Cys residues involved in catalysis: C_P (Cys 61), the putative C_R (Cys 174) and a third Cys (Cys 176) whose role in catalysis is not completely clear but could provide an alternative route of disulfide bond formation (Guimaraes *et al.*, 2005). Whereas Cys 61 plays a central role in catalysis, the enzyme remains partially functional in the absence of Cys 174 and 176 and possibly adopts a 1-Cys-like mechanism (Chauhan & Mande, 2002; Koshkin *et al.*, 2004). *MtAhpC* has been detected both in the bacterial cytosol (Covert *et al.*, 2001) and as a membrane associated protein (Gu *et al.*, 2003). AhpC forms part of bacterial alkyl hydroperoxide reductase (Ahp) system (Storz *et al.*, 1987). In enterobacteria, this system commonly consists of two components, AhpC and a flavin-containing disulfide reductase (AhpF) that reduces AhpC at NADH expense, and both enzymes are jointly up-regulated under oxidative stress conditions targeting the oxyR regulon (Tartaglia *et al.*, 1989). However, AhpF is lacking in all mycobacteria. In this context, two reducing systems for *M. tuberculosis* AhpC (*MtAhpC*) have been proposed. Firstly, alkyl hydroperoxide reductase D (AhpD), that contains a CXXC motif, can reduce *MtAhpC*. The *ahpD* gene is found immediately downstream of *ahpC*, in the position occupied by *ahpF* in *S. typhimurium* genome, and both proteins are controlled by the same promoter (Hillas *et al.*, 2000). Oxidized AhpD is regenerated by dihydrolipoamide acyltransferase (DlaT); in turn, dihydrolipoamide dehydrogenase (Lpd) mediates the reduction of DlaT at NADH expense and completes the catalytic cycle (Bryk *et al.*, 2002). *dlaT* (Rv2215) encodes the E2 component of the pyruvate deshydrogenase complex, and *lpdC* (Rv0462), the only functional Lpd in *M. tuberculosis* (Argyrou & Blanchard, 2001), most probably codifies the E3 components of the pyruvate deshydrogenase complex (Tian *et al.*, 2005). Secondly, thioredoxin C (TrxC), but not thioredoxin B (TrxB) or A (TrxA), was also able to act as AhpC reducing substrates (Jaeger *et al.*, 2004), and the catalytic cycle is completed by thioredoxin reductase (*MtTR*) and NADPH. Catalytic efficiency of TrxC-mediated AhpC reduction was ~ 100 fold lower than that measured using AhpD as reducing substrate (2.5×10^4 versus 2.7×10^6 M⁻¹s⁻¹, respectively)(Jaeger *et al.*, 2004). However, the preferential reducing substrate would be determined not only by catalytic efficiencies but also by the steady-state concentrations of

reducing substrates at reduced state. *MtTrxC* is consistently seen as a major spot in bacterial proteomes while the spot corresponding to *MtAhpD* is of much lower intensity (Jungblut *et al.*, 1999; Mollenkopf *et al.*, 1999), indicating a lower concentration of *MtAhpD* compared to *MtTrxC* in these cells. Moreover, *MtTR* is also an abundant protein in *Mycobacteria* (Jungblut *et al.*, 1999; Mollenkopf *et al.*, 1999), and it is expected to keep *TrxC* at reduced state as long as NADPH is not limiting (Jaeger *et al.*, 2004). These data suggest that, despite the lower catalytic efficiency of *MtTrxC* compared to *MtAhpD* in *MtAhpC* reduction, both enzymatic pathways could be contributing to *MtAhpC*-mediated peroxide detoxification *in vivo*. Concerning the oxidizing substrate specificity, *AhpC* are broad-spectrum peroxidases that catalyze the reduction of H_2O_2 , organic hydroperoxides and peroxynitrite. The catalytic efficiency of *t*-BuOOH reduction (an artificial hydroperoxide used as a mimic of natural organic hydroperoxides) by *MtAhpC* was reported as $\sim 10^4 \text{ M}^{-1} \text{ s}^{-1}$ (Jaeger *et al.*, 2004). The enzyme could also reduce another artificial organic hydroperoxide, cumene hydroperoxide at similar rates. H_2O_2 and linoleic acid hydroperoxides, but not phosphatidylcholine hydroperoxide, were also reduced by *MtAhpC*. This enzyme, together with other bacterial *AhpC* enzymes, where the first Prxs for which a peroxynitrite reductase activity was demonstrated ($k = 1.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ at pH 6.85 and RT (Bryk *et al.*, 2000)). H_2O_2 was a preferential substrate of *MtAhpC*, although precise activity measurements were difficult to estimate due to the basal activity of the TR/Trx system (Jaeger *et al.*, 2004). In the case of another *AhpC* protein (from *Salmonella typhimurium*) the catalytic efficiency for H_2O_2 reduction was reported as $3.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (Parsonage *et al.*, 2008). Thus, oxidizing substrate selectivity of bacterial *AhpC* seems to follow the same trend as for other members of the *AhpC*-Prx1 subfamily, where reduction of peroxynitrite is somewhat slower than that of H_2O_2 at near-physiological pHs, and occur with similar pH independent rate constants⁵ (Manta *et al.*, 2009). There is no data regarding the pK_a of C_P and redox potential of *AhpC* from *M. tuberculosis*. The pK_a values of C_P in *S. typhimurium* *AhpC* was first determined as < 5 (Bryk *et al.*, 2000) and more recently reported as 5.8 (Nelson *et al.*, 2008), indicating that C_P would be mostly deprotonated at physiological pH. The midpoint reduction potential of the enzyme was reported as $-178 \pm 0.4 \text{ mV}$, somehow lower than that reported for mammalian Prx 3 ($E^\circ = -290 \text{ mV}$) (Cox *et al.*, 2010) and plant 2-Cys Prxs and PrxQ ($E^\circ = -288$ to -325 mV) (Dietz *et al.*, 2006). Data regarding redox potential of *MtAhpD* is lacking. In turn, *M. tuberculosis* Trx redox potentials have not been investigated so far, but redox potential of other bacterial Trxs has been reported to be low (-270 mV for *E. coli* Trx (Krause *et al.*, 1991). Since the standard midpoint reduction potential for H_2O_2 reduction to water and for ONOOH reduction to nitrite and water are 1.77 and 1.6 V, respectively (Latimer, 1938; Koppenol & Kissner, 1998), the thermodynamic driving forces would highly favor the flux of electrons from Trxs to these peroxides through *AhpC*. In addition to its peroxidase

⁵ According to the mechanism of reaction in which the thiolate form of the C_P reacts with the protonated peroxide, and considering that all the reported pK_a values of Prxs including *AhpC* are < 6.3 (68), (70), (71), and that the pK_a for the first H_2O_2 deprotonation is far above physiological pH, the pH-independent rate constant for H_2O_2 -mediated Prx oxidation is practically the same as the rate constant determined at physiological pH. However, the pK_a value of peroxynitrous acid is around 6.8 (reported values of ONOOH $\text{pK}_a = 6.5$ -6.8 (23), (24), (25)) and therefore, only 50 % or 20 % of peroxynitrite would be as be protonated at pH 6.8 or 7.4, respectively. Thus, the pH-independent rate constant of Prx oxidation by peroxynitrite would be 2 or 5 times higher than the value determined at pH 6.8 or 7.4.

activity, some bacterial AhpCs have other functions: *Helicobacter pylori* AhpC can form high molecular weight aggregates with chaperone activity under oxidative stress conditions (Huang *et al.*, 2010). Moreover, AhpC from some Gram negative microorganisms show a deglutathionylating activity that depends on C_R rather than on C_P (Yamamoto *et al.*, 2008).

Size exclusion chromatography indicated that wild-type *MtAhpC* performs as a heterogeneous mixture of oligomers under non-reducing conditions, whereas under reduced state the enzyme is a homogeneous oligomer formed by 10- or 12-subunits. The C176S mutant form of AhpC is dimeric under oxidized state, and forms oligomers of 10-12 subunits upon reduction. The crystallographic structure of C176S *MtAhpC* trapped as an intermediate of its catalytic cycle (where condensation had already occurred but still the enzyme was under its oligomeric form) was consistent with the formation of a ring shaped oligomer of 12 subunits, a hexamer of dimers (Guimaraes *et al.*, 2005). The relationship between *MtAhpC* oligomerisation and activity has not been addressed. In the case of *Salmonella typhimurium* AhpC, decameric under reduced state, the analysis of mutated forms of the enzyme at the decamer-building interface indicated that the oligomerization is quite important, but not essential to activity (Parsonage *et al.*, 2005).

The role of *MtAhpC* in the detoxification of peroxides *in vivo* was first suggested by the fact that pathogenic, INH-resistant strains lacking KatG over-expressed *MtAhpC*, which would represent a compensatory mechanism allowing the bacteria to get rid of cytotoxic peroxides (Sherman *et al.*, 1996). Overexpression of *MtAhpC* in those strains was associated to mutations in the gene promoter (Wilson & Collins, 1996). Thus, *MtAhpC* was proposed as a potential drug target. However, data obtained using *M. tuberculosis* strains lacking *MtAhpC* are not straightforward. AhpC expression in virulent strains of *M. tuberculosis* grown *in vitro* was repressed and increased under conditions of static growth, probably reflecting adaptation of the bacterium during its infection cycle (Springer *et al.*, 2001). AhpC expression was also induced by hypoxia (Sherman *et al.*, 2001). *S. typhimurim* lacking *ahpC* became hypersusceptible to reactive nitrogen species and *MtAhpC* complemented the defect. The enzyme also protected human cells from toxicity caused by reactive nitrogen species (Chen *et al.*, 1998). Whereas inactivation of *MtAhpC* caused no effect on bacterial growth during acute infection in mice and had no effect on *in vitro* sensitivity to H₂O₂, it caused an increase susceptibility to organic hydroperoxide and peroxynitrite-mediated toxicity (Springer *et al.*, 2001; Master *et al.*, 2002). Inactivation of *MtAhpC* caused a decrease in the survival of *M. tuberculosis* in non-stimulated macrophages but not in macrophages stimulated with interferon- γ (Master *et al.*, 2002). Strains lacking *DlaT* showed retarded growth, were highly susceptible to killing by acidified nitrite *in vitro*, showed decreased intracellular survival during macrophage infection and were less virulent in a mouse model of tuberculosis (Shi & Ehrt, 2006). Overall, these data indicate the importance of both *MtAhpC* and *MtDlaT*, its reductant through *MtAhpD*, for *M. tuberculosis* to overcome oxidative stress encountered inside its primary host cells and to establish a successful infection.

5.3.2 Thiol peroxidase (*MtTPx*, Rv1932)

The second Prx from *M. tuberculosis* to be identified belonged to the TPx subfamily (Jaeger *et al.*, 2004), enzymes widely distributed among Gram-positive and Gram-negative bacteria. In the case of *E. coli* Tpx, the enzyme is localized in the periplasmic space. In *M. tuberculosis*,

TPx was firstly characterized as an extracellular antigen that induces a strong proliferative response in animals (Weldingh *et al.*, 1998). *MtTPx* was repeatedly found in culture filtrates; it has also been found associated to membranes and in cytosolic fractions (Rosenkrands *et al.*, 2000; Covert *et al.*, 2001; Malen *et al.*, 2007; Malen *et al.*, 2010).

TPxs are atypical 2-Cys Prxs. They typically contain three cysteine residues where Cys60 is C_P, C93 is C_R and Cys80⁶ is catalytically irrelevant. However, site directed mutagenesis studies revealed that *MtTPx* lacking Cys 93 remained active for a limited period of time before getting inactivated by C_P overoxidation to sulfinic acid, and therefore the role of Cys93 is likely the formation of an intramolecular disulfide with the sulfenic acid in C_P and to avoid C_P overoxidation under conditions of restricted availability of reducing substrates (Trujillo *et al.*, 2006). *MtTPx* reacts very rapidly with peroxynitrite ($k = 1.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 and 25 °C)⁷. Reduction of *t*-BuOOH was slower ($k \sim 1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.4 and 25 °C). Reduction of H₂O₂ was faster than that of *t*-BuOOH, although the exact number was difficult to estimate. The enzyme was hardly active towards linolenic acid hydroperoxide and could not reduce phosphatidylcholine hydroperoxide. Concerning the reductive part of the catalytic cycle, both *MtTrxB* and *MtTrxC* reduced *MtTPx* with similar catalytic efficiencies (4.6 and $5.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, respectively). Since according to proteomic data currently available *MtTrxC* would be much more abundant than *MtTrxB*, the former would play a major role as *MtTPx* reducing substrate. Mycothiol plus mycothione reductase/NADPH were not able to reduce *MtTPx* (Jaeger *et al.*, 2004).

The crystallography structure of *MtTPx* (Rho *et al.*, 2006) and on the inactive mutant C60S *MtTPx* (Stehr *et al.*, 2006), as for other bacterial TPxs, indicated that Cys60 in *MtTPx* forms part of a typical catalytic triad with Thr57 and Arg130. The enzyme is dimeric both in the crystal structure and in solution (Rho *et al.*, 2006; Stehr *et al.*, 2006). In C60S *MtTPx*, a cocrystallized acetate molecule interacted with Ser60, Arg130 and Thr57 (Stehr *et al.*, 2006). Similarly, the wild type enzyme also showed anions near the active site. Co-crystallization with anions is frequently observed in Prxs; it has been proposed the existence of an anion-binding site in the neighborhood of reactive thiols in proteins, that could participate in transition state stabilization and thus, in the acceleration of peroxides reduction in general (Hall *et al.*, 2010; Ferrer-Sueta *et al.*, 2011).

M. tuberculosis strains lacking functional *MtTPx* had a lower peroxidase activity than their wild type counterparts, indicating that the enzyme importantly contributes to the total peroxidase activity in *M. tuberculosis*. Moreover, *MtTPx* mutants were more sensitive to H₂O₂ and •NO-mediated toxicity, but the effect was recovered when they were complemented with the *tpx* gene. Strains lacking *MtTPx* failed to grow and survive in macrophages, particularly after activation by interferon- γ . Growth was significantly restored in the macrophages from iNOS knockout mice. This is consistent with the ability of the enzyme to rapidly reduce peroxynitrite *in vitro*. Moreover, strains lacking *MtTPx*

⁶ Cysteine numbers correspond to the sequence in TPx from *M. tuberculosis*.

⁷ The pK_a value of C_P in *MtTPx* or other bacterial TPx has not been reported previously. Considering a pK_a value of <6.3, as for all other Prxs investigated so far, more than 90 % of C_P would be as thiolate and 20% of peroxynitrite as ONOOH at pH 7.4. Thus, the pH-independent rate constant of C_P oxidation by peroxynitrite would be 5 times higher than the value determined at pH 7.4, $7.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. It would be even higher if the pK_a of C_P of *MtTPx* was > 6.3.

failed to initiate an acute infection and to maintain a persistent infection, and were less virulent than wild type strains (Hu & Coates, 2009). In the *M. bovis* strain BCG, TPx is induced in response to exposure to diamide, an agent that causes thiol oxidation (Dosanjh *et al.*, 2005).

5.3.3 Alkyl hydroperoxide reductase E (*MtAhpE*, Rv2238c)

The genome of *M. tuberculosis* also codifies for a one-cysteine Prx, alkyl hydroperoxide reductase E, which is highly conserved among many Mycobacteria (Cole *et al.*, 1998; Passardi *et al.*, 2007). *MtAhpE* belongs to a novel family of Prxs, comprising bacterial and archaean AhpE and AhpE-like enzymes (Passardi *et al.*, 2007; Soito *et al.*, 2011). This protein has been identified in the membrane fraction of *M. tuberculosis* H37Rv using a proteomics approach (Gu *et al.*, 2003). The expression of *MtAhpE* increases during the dormant phase of tuberculosis disease (Murphy & Brown, 2007). Although *MtAhpE* shows greater sequence similarity with mammalian typical two-Cys Prxs than with one-Cys Prxs (Passardi *et al.*, 2007; Soito *et al.*, 2011), it has only one Cys residue and functions by a one-Cys mechanism. Accordingly, in the oxidized form of the enzyme C_P is as sulfenic acid, as revealed by crystallographic studies and by mass spectrometry analysis (Li *et al.*, 2005; Hugo *et al.*, 2009). We have reported the peroxidase activity of *MtAhpE*, being the first member of the AhpE family to be functionally characterized (Hugo *et al.*, 2009). The physiological reducing substrate(s) for *MtAhpE* (as well as AhpE-like Prxs) is/are still unknown, but its catalytic activity was demonstrated using the artificial substrates dithiotreitol (DTT) and thionitrobenzoic acid (TNB). Neither N-acetylcysteine nor glutathione could reduce oxidized *MtAhpE* but led to mixed disulfides formation. Concerning oxidizing substrate specificity, *MtAhpE* reduces peroxynitrite three orders of magnitude faster than H₂O₂ (1.9×10^7 versus 8.2×10^4 M⁻¹ s⁻¹ at pH 7.4 and 25 °C, respectively⁸). These rate constants were measured directly by taking advantage of the decrease in Trp-dependent fluorescence intensity that the enzyme exhibits upon oxidation. Moreover, the kinetics of peroxide-mediated inactivation by overoxidation of C_P to sulfinic acid was measured following the increase in the enzyme's intrinsic fluorescence intensity ($k = 40$ M⁻¹s⁻¹ for H₂O₂-mediated overoxidation)(Hugo *et al.*, 2009). This value was very similar to that previously calculated for mammalian Prx 1 oxidative inactivation by H₂O₂ (57 M⁻¹ s⁻¹) (Wood *et al.*, 2003; Stone, 2004). The pK_a of the thiol (in reduced *MtAhpE*) and of the sulfenic acid (in oxidized *MtAhpE*) were reported to be 5.2 and 6.6, respectively. Thus, taking into account the intrabacterial pH of wild-type *M. tuberculosis* (6.8–7.5 (Vandal *et al.*, 2008)), >95 % of the reduced and >50 % of the oxidized form of C_P in *MtAhpE* would be deprotonated, and therefore, at their reactive forms with peroxides (Hugo *et al.*, 2009). More recently, we have performed a comprehensive study on *MtAhpE* oxidizing substrate specificity as well as on its oxidative inactivation (Reyes *et al.*, 2011). For most peroxides tested, oxidation as well as oxidative inactivation rates

⁸ Considering a mechanism of reaction where thiolate and sulfenate as well as protonated peroxides are the reactive species, the reported pK_a values of the thiol and sulfenic acid in reduced and oxidized *MtAhpE* (Hugo *et al.*, 2009) and the pK_a of the H₂O₂ and peroxynitrite above indicated, pH independent rates constants can be calculated as very similar (for H₂O₂) and ~ 5 fold higher (for peroxynitrite) that the corresponding values measured at pH 7.4.

correlated with leaving group pK_a , indicating that both reactions occur by similar mechanisms, i.e. reaction of the thiolate or sulfenate anion at C_P with the protonated peroxide. In contrast, the hydroperoxide at position 15 of arachidonic acid (15-HpETE) and linolenic acid-derived hydroperoxides reacted surprisingly fast, with rate constants of $\sim 10^8$ and $\sim 10^5 \text{ M}^{-1} \text{ s}^{-1}$ for *MtAhpE* oxidation and overoxidation, respectively. The molecular basis for the fast reactivity of *MtAhpE* with fatty acid hydroperoxides is intriguing. The quaternary structure of *MtAhpE* in solution is tightly regulated by the oxidation state of the C_P , the enzyme being a dimer under reduced state and slowly forming high molecular weight aggregates upon oxidation (Hugo *et al.*, 2009). Analysis of the reported crystallographic structure of the protein under reduced state (Li *et al.*, 2005) showed a hydrophobic groove present in the dimeric enzyme, and formed by residues from both subunits, which is proposed as an anchoring site for fatty acid hydroperoxide binding (Reyes *et al.*, 2011). These data set *MtAhpE* (and probably other AhpE-like Prxs) as potential Prxs specialized for fatty acid hydroperoxide detoxification. However, the roles of *MtAhpE* in reduction of these or other peroxides *in vivo*, as well as in macrophage infection or bacterial virulence, remain to be investigated.

Prx	reductant	pK_a of C_P	$k_2 \text{ H}_2\text{O}_2$ ($\text{M}^{-1} \text{ s}^{-1}$)	$k_2 \text{ ONOOH}$ ($\text{M}^{-1} \text{ s}^{-1}$)	$k_2 \text{ } t\text{-BuOOH}$ ($\text{M}^{-1} \text{ s}^{-1}$)	$k_2 \text{ LOOH}$ ($\text{M}^{-1} \text{ s}^{-1}$)
AhpC	AhpD, TrxC	^a 5.8 ($C_P\text{-SH}$)	^a 3.7×10^7	^b 1.3×10^6	$1\text{-}2.3 \times 10^4$	^c 6.9×10^3
TPx	TrxB, TrxC	ND	ND	1.5×10^7	$0.9\text{-}3.4 \times 10^5$	0
AhpE	ND	5.2 ($C_P\text{-SH}$) 6.6 ($C_P\text{-SOH}$)	8.2×10^4	1.9×10^7	8×10^3	^d 1.8×10^8 ^e 2.7×10^8

^aFor *StAhpC*; ^bAt pH 6.85 and RT; ^cCalculated from (Jaeger *et al.*, 2004; Parsonage *et al.*, 2008), for linoleic acid hydroperoxide; ^dFor 15-HpETE; ^eFor α -linolenic acid hydroperoxide; ND is non determined. In the case of H_2O_2 reduction by *MtAhpC* and *MfTPx*, reactions were faster than with *t*-BuOOH, but precise rate constants were difficult to estimate (Jaeger *et al.*, 2004).

Table 1. Functional data on Prxs from *M. tuberculosis*: acidity constants, reducing substrates and kinetics of peroxide reduction.

5.3.4 Bacterioferritin comigratory proteins (Bcp, Rv2521; BcpB, Rv1608c)

The genome of *M. tuberculosis* also codifies for two putative Prxs of the Bcp type (Cole & Barrell, 1998). Evidence for the first Bcp (Rv2125) expression at a protein level exists, both in the membrane fraction (Gu *et al.*, 2003) and in the cytosol of H37Rv strains (Mawuenyega *et al.*, 2005). The protein has been shown to be target of modification by the small protein Pup, a post-translational modification that targets proteins for degradation by the *M. tuberculosis* proteosome (Pearce *et al.*, 2006; Festa *et al.*, 2010). To note, pupylation and proteosome function are essential for the virulence of this bacterium, for reasons still unknown (Darwin *et al.*, 2003; Gandotra *et al.*, 2007). In the case of BcpB (Rv1608c), it was identified associated to the membrane fraction of *M. tuberculosis* H37Rv (Gu *et al.*, 2003). The genes for both putative Bcps are considered as non-essential according to mutagenesis analysis in H37Rv strain (Sasseti *et al.*, 2003). Structural and functional data regarding both putative Bcps from *M. tuberculosis* and their role in infection processes await further investigation.

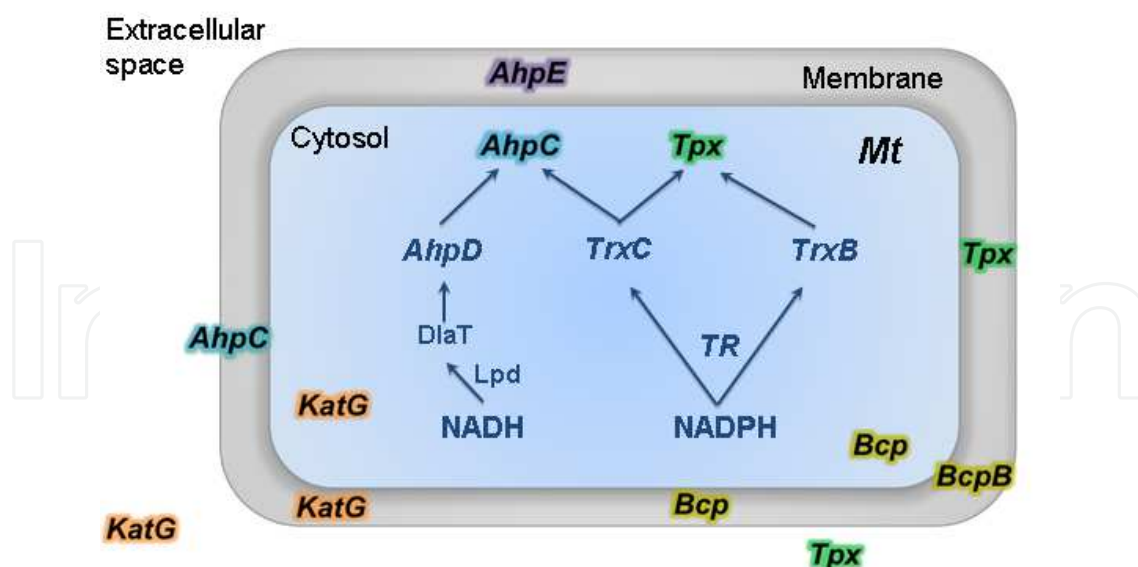


Fig. 2. Cellular localization and reducing substrates of peroxidases from *M. tuberculosis*. The five Prxs and the heme peroxidase KatG have distinct, although overlapping cellular distributions. *MtKatG* (orange) has been found in the cytosol, membrane and extracellular space. *MtAhpC* (blue) is a cytosolic enzyme also that was also found associated to the bacterial membrane. *MtTPx* (green) was detected in culture media repeatedly. It has also been found in membrane fractions and in the cytosol. *MtAhpE* (violet), and the putative BcpB and Bcp (yellow) were detected in cell membrane fractions, and the latter also in the cytosol. Reducing systems for *MtAhpC* and *MtTPx* (in grey) are shown without considering their cellular localization. *MtAhpE* and *MtBcps* reducing substrates are still unknown.

6. Conclusions

M. tuberculosis is an extremely successful pathogen, despite of being exposed to cytotoxic peroxides formed inside the phagosome of activated macrophages, its primary host cells. The bacterium expresses a heme-dependent peroxidase, KatG, and various thiol-dependent peroxidases of the Prx type. From the data reviewed herein, it becomes clear that Prxs from *M. tuberculosis* differ in cellular location, and have diverse oxidizing and reducing substrate specificities, that may explain in part the presence of different subfamilies of Prxs in the bacterium. Available data indicate that at least two of them (*MtAhpC* and *MtTPx*) play a role in pathogenesis. The third one, *MtAhpE*, has an outstanding reactivity with fatty-acid derived hydroperoxides, but since natural reducing substrate(s) has not been identified so far, its peroxidase catalytic activity *in vivo* remains to be confirmed. Similarly, further investigation is required to characterize the two putative Bcp proteins from *M. tuberculosis*.

7. Acknowledgments

This work was supported by grants from the Howard Hughes Medical Institute (HHMI), NIH and CSIC, Universidad de la República, Uruguay, to RR. MT was partially supported by PEDECIBA Biología, Uruguay. MH was partially supported by a fellowship from ANII, Uruguay. RR is an International Research Scholar of the HHMI. We thank Lucía Piacenza

(Facultad de Medicina, Universidad de la República, Uruguay) for her kindly help with the artworks.

8. References

- Akaki, T., Tomioka, H., Shimizu, T., Dekio, S. & Sato, K. (2000). Comparative roles of free fatty acids with reactive nitrogen intermediates and reactive oxygen intermediates in expression of the anti-microbial activity of macrophages against *Mycobacterium tuberculosis*. *Clin Exp Immunol* 121(2): 302-310.
- Alvarez, M. N., Peluffo, G., Piacenza, L. & Radi, R. (2011). Intraphagosomal peroxynitrite as a macrophage-derived cytotoxin against internalized *Trypanosoma cruzi*: consequences for oxidative killing and role of microbial peroxiredoxins in infectivity. *J Biol Chem* 286(8): 6627-6640.
- Ando, H., Kitao, T., Miyoshi-Akiyama, T., Kato, S., Mori, T. & Kirikae, T. (2011). Downregulation of katG expression is associated with isoniazid resistance in *Mycobacterium tuberculosis*. *Mol Microbiol* 79(6): 1615-1628.
- Argyrou, A. & Blanchard, J. S. (2001). *Mycobacterium tuberculosis* lipoamide dehydrogenase is encoded by Rv0462 and not by the lpdA or lpdB genes. *Biochemistry* 40(38): 11353-11363.
- Armstrong, J. A. & Hart, P. D. (1971). Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *J Exp Med* 134(3 Pt 1): 713-740.
- Augusto, O., Bonini, M. G., Amanso, A. M., Linares, E., Santos, C. C. & De Menezes, S. L. (2002). Nitrogen dioxide and carbonate radical anion: two emerging radicals in biology. *Free Radic Biol Med* 32(9): 841-859.
- Babior, B. M. (1984). Oxidants from phagocytes: agents of defense and destruction. *Blood* 64(5): 959-966.
- Barber, D. J. W. & Thomas, J. K. (1978). Reaction of radicals with lecithin bilayers. *Radiat Res* 74: 51-65.
- Bedard, K. & Krause, K. H. (2007). The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 87(1): 245-313.
- Blelski, B. H. J. & Allen, A. O. (1977). Mechanism of the Disproportionation of Superoxide Radicals. *J Phyl Chem* 81: 1048-1050.
- Bonini, M. G., Radi, R., Ferrer-Sueta, G., Ferreira, A. M. & Augusto, O. (1999). Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide. *J Biol Chem* 274(16): 10802-10806.
- Bonney, R., J., Opas, E. E. & Humes, J. L. (1985). Lipoxygenase pathways of macrophages. *Fed Proc* 44: 2933-2936.
- Boshoff, H. I., Reed, M. B., Barry, C. E., 3rd & Mizrahi, V. (2003). DnaE2 polymerase contributes to in vivo survival and the emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell* 113(2): 183-193.
- Brigelius-Flohe, R. & Flohe, L. (2011). Basic Principles and Emerging Concepts in the Redox Control of Transcription Factors. *Antioxid Redox Signal*.
- Bryk, R., Griffin, P. & Nathan, C. (2000). Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* 407(6801): 211-215.

- Bryk, R., Lima, C. D., Erdjument-Bromage, H., Tempst, P. & Nathan, C. (2002). Metabolic enzymes of mycobacteria linked to antioxidant defense by a thioredoxin-like protein. *Science* 295(5557): 1073-1077.
- Bzymek, K. P., Newton, G. L., Ta, P. & Fahey, R. C. (2007). Mycothiol import by *Mycobacterium smegmatis* and function as a resource for metabolic precursors and energy production. *J Bacteriol* 189(19): 6796-6805.
- Clifford, D. P. & Repine, J. E. (1982). Hydrogen peroxide mediated killing of bacteria. *Mol Cell Biochem* 49(3): 143-149.
- Cole, S. T. & Barrell, B. G. (1998). Analysis of the genome of *Mycobacterium tuberculosis* H37Rv. *Novartis Found Symp* 217: 160-172; discussion 172-167.
- Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S., Barry, C. E., 3rd, Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gentles, S., Hamlin, N., Holroyd, S., Hornsby, T., Jagels, K., Krogh, A., McLean, J., Moule, S., Murphy, L., Oliver, K., Osborne, J., Quail, M. A., Rajandream, M. A., Rogers, J., Rutter, S., Seeger, K., Skelton, J., Squares, R., Squares, S., Sulston, J. E., Taylor, K., Whitehead, S. & Barrell, B. G. (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393(6685): 537-544.
- Covert, B. A., Spencer, J. S., Orme, I. M. & Belisle, J. T. (2001). The application of proteomics in defining the T cell antigens of *Mycobacterium tuberculosis*. *Proteomics* 1(4): 574-586.
- Cox, A. G., Peskin, A. V., Paton, L. N., Winterbourn, C. C. & Hampton, M. B. (2010). Correction to Redox Potential and Peroxide Reactivity of Human Peroxiredoxin 3. *Biochemistry* 49 (44):9677-9677.
- Chae, H. Z., Chung, S. J. & Rhee, S. G. (1994). Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem* 269(44): 27670-27678.
- Chang, T. S., Jeong, W., Choi, S. Y., Yu, S., Kang, S. W. & Rhee, S. G. (2002). Regulation of peroxiredoxin I activity by Cdc2-mediated phosphorylation. *J Biol Chem* 277(28): 25370-25376.
- Chang, T. S., Jeong, W., Woo, H. A., Lee, S. M., Park, S. & Rhee, S. G. (2004). Characterization of mammalian sulfiredoxin and its reactivation of hyperoxidized peroxiredoxin through reduction of cysteine sulfinic acid in the active site to cysteine. *J Biol Chem* 279(49): 50994-51001.
- Chauhan, R. & Mande, S. C. (2002). Site-directed mutagenesis reveals a novel catalytic mechanism of *Mycobacterium tuberculosis* alkylhydroperoxidase C. *Biochem J* 367(Pt 1): 255-261.
- Chen, L., Xie, Q. W. & Nathan, C. (1998). Alkyl hydroperoxide reductase subunit C (AhpC) protects bacterial and human cells against reactive nitrogen intermediates. *Mol Cell* 1(6): 795-805.
- Darwin, K. H., Ehrt, S., Gutierrez-Ramos, J. C., Weich, N. & Nathan, C. F. (2003). The proteasome of *Mycobacterium tuberculosis* is required for resistance to nitric oxide. *Science* 302(5652): 1963-1966.
- De Groote, M. A., Ochsner, U. A., Shiloh, M. U., Nathan, C., McCord, J. M., Dinauer, M. C., Libby, S. J., Vazquez-Torres, A., Xu, Y. & Fang, F. C. (1997). Periplasmic superoxide dismutase protects *Salmonella* from products of phagocyte NADPH-oxidase and nitric oxide synthase. *Proc Natl Acad Sci U S A* 94(25): 13997-14001.

- Denicola, A., Freeman, B. A., Trujillo, M. & Radi, R. (1996). Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys* 333(1): 49-58.
- Denicola, A., Rubbo, H., Rodriguez, D. & Radi, R. (1993). Peroxynitrite-mediated cytotoxicity to *Trypanosoma cruzi*. *Arch Biochem Biophys* 304(1): 279-286.
- Diaz, G. A. & Wayne, L. G. (1974). Isolation and characterization of catalase produced by *Mycobacterium tuberculosis*. *Am Rev Respir Dis* 110(3): 312-319.
- Dietz, K. J., Jacob, S., Oelze, M. L., Laxa, M., Tognetti, V., de Miranda, S. M., Baier, M. & Finkemeier, I. (2006). The function of peroxiredoxins in plant organelle redox metabolism. *J Exp Bot* 57(8): 1697-1709.
- Dosanji, N. S., Rawat, M., Chung, J. H. & Av-Gay, Y. (2005). Thiol specific oxidative stress response in *Mycobacteria*. *FEMS Microbiol Lett* 249(1): 87-94.
- Dussurget, O., Stewart, G., Neyrolles, O., Pescher, P., Young, D. & Marchal, G. (2001). Role of *Mycobacterium tuberculosis* copper-zinc superoxide dismutase. *Infect Immun* 69(1): 529-533.
- Edwards, K. M., Cynamon, M. H., Voladri, R. K., Hager, C. C., DeStefano, M. S., Tham, K. T., Lakey, D. L., Bochan, M. R. & Kernodle, D. S. (2001). Iron-cofactored superoxide dismutase inhibits host responses to *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med* 164(12): 2213-2219.
- Ehrt, S. & Schnappinger, D. (2009). Mycobacterial survival strategies in the phagosome: defence against host stresses. *Cell Microbiol* 11(8): 1170-1178.
- Evans, M. V., Turton, H. E., Grant, C. M. & Dawes, I. W. (1998). Toxicity of linoleic acid hydroperoxide to *Saccharomyces cerevisiae*: involvement of a respiration-related process for maximal sensitivity and adaptive response. *J Bacteriol* 180(3): 483-490.
- Ferrer-Sueta, G., Manta, B., Botti, H., Radi, R., Trujillo, M. & Denicola, A. (2011). Factors affecting protein thiol reactivity and specificity in peroxide reduction. *Chem Res Toxicol* 24(4): 434-450.
- Ferrer-Sueta, G. & Radi, R. (2009). Chemical biology of peroxynitrite: kinetics, diffusion, and radicals. *ACS Chem Biol* 4(3): 161-177.
- Festa, R. A., McAllister, F., Pearce, M. J., Mintseris, J., Burns, K. E., Gygi, S. P. & Darwin, K. H. (2010). Prokaryotic ubiquitin-like protein (Pup) proteome of *Mycobacterium tuberculosis* [corrected]. *PLoS One* 5(1): e8589.
- Flohe, L., Jaeger, T., Pilawa, S. & Sztajer, H. (2003). Thiol-dependent peroxidases care little about homology-based assignments of function. *Redox Rep* 8(5): 256-264.
- Flohe, L., Toppo, S., Cozza, G. & Ursini, F. (2010). A Comparison of Thiol Peroxidase Mechanisms. *Antioxid Redox Signal*. 15(3):763-80.
- Fridovich, I. (1997). Superoxide anion radical (O₂⁻), superoxide dismutases, and related matters. *J Biol Chem* 272(30): 18515-18517.
- Gandotra, S., Schnappinger, D., Monteleone, M., Hillen, W. & Ehrt, S. (2007). In vivo gene silencing identifies the *Mycobacterium tuberculosis* proteasome as essential for the bacteria to persist in mice. *Nat Med* 13(12): 1515-1520.
- Gerasimov, O. V. & Lyman, S. V. (1999). The yield of hydroxyl radical from the decomposition of peroxynitrous acid. *Inorg Chem* 38 (19):4317-4321.
- Ghiladi, R. A., Medzihradszky, K. F. & Ortiz de Montellano, P. R. (2005). Role of the Met-Tyr-Trp cross-link in *Mycobacterium tuberculosis* catalase-peroxidase (KatG) as revealed by KatG(M255I). *Biochemistry* 44(46): 15093-15105.

- Goldstein, S. & Czapski, G. (1995). Direct and indirect oxidations by peroxynitrite. *Inorg. Chem.* 34: 4041-4048.
- Groemping, Y. & Rittinger, K. (2005). Activation and assembly of the NADPH oxidase: a structural perspective. *Biochem J* 386(Pt 3): 401-416.
- Gu, S., Chen, J., Dobos, K. M., Bradbury, E. M., Belisle, J. T. & Chen, X. (2003). Comprehensive proteomic profiling of the membrane constituents of a *Mycobacterium tuberculosis* strain. *Mol Cell Proteomics* 2(12): 1284-1296.
- Guimaraes, B. G., Souchon, H., Honore, N., Saint-Joanis, B., Brosch, R., Shepard, W., Cole, S. T. & Alzari, P. M. (2005). Structure and mechanism of the alkyl hydroperoxidase AhpC, a key element of the *Mycobacterium tuberculosis* defense system against oxidative stress. *J Biol Chem* 280(27): 25735-25742.
- Hall, A., Karplus, P. A. & Poole, L. B. (2009). Typical 2-Cys peroxiredoxins--structures, mechanisms and functions. *FEBS J* 276(9): 2469-2477.
- Hall, A., Parsonage, D., Poole, L. B. & Karplus, P. A. (2010). Structural evidence that peroxiredoxin catalytic power is based on transition-state stabilization. *J Mol Biol* 402(1): 194-209.
- Hillas, P. J., del Alba, F. S., Oyarzabal, J., Wilks, A. & Ortiz De Montellano, P. R. (2000). The AhpC and AhpD antioxidant defense system of *Mycobacterium tuberculosis*. *J Biol Chem* 275(25): 18801-18809.
- Hofmann, B., Hecht, H. J. & Flohe, L. (2002). Peroxiredoxins. *Biol Chem* 383(3-4): 347-364.
- Hu, Y. & Coates, A. R. (2009). Acute and persistent *Mycobacterium tuberculosis* infections depend on the thiol peroxidase TpX. *PLoS One* 4(4): e5150.
- Huang, C. H., Chuang, M. H., Wu, Y. H., Chuang, W. C., Jhuang, P. J. & Chiou, S. H. (2010). Characterization of site-specific mutants of alkylhydroperoxide reductase with dual functionality from *Helicobacter pylori*. *J Biochem* 147(5): 661-669.
- Hugo, M., Turell, L., Manta, B., Botti, H., Monteiro, G., Netto, L. E., Alvarez, B., Radi, R. & Trujillo, M. (2009). Thiol and sulfenic acid oxidation of AhpE, the one-cysteine peroxiredoxin from *Mycobacterium tuberculosis*: kinetics, acidity constants, and conformational dynamics. *Biochemistry* 48(40): 9416-9426.
- Hurst, J. K. & Lyman, S. V. (1997). Toxicity of peroxynitrite and related reactive nitrogen species toward *Escherichia coli*. *Chem Res Toxicol* 10(7): 802-810.
- Iglesias-Baena, I., Barranco-Medina, S., Lazaro-Payo, A., Lopez-Jaramillo, F. J., Sevilla, F. & Lazaro, J. J. (2010). Characterization of plant sulfiredoxin and role of sulphinic form of 2-Cys peroxiredoxin. *J Exp Bot* 61(5): 1509-1521.
- Iglesias-Baena, I., Barranco-Medina, S., Sevilla, F. & Lazaro, J. J. (2011). The dual-targeted plant sulfiredoxin retroreduces the sulfinic form of atypical mitochondrial peroxiredoxin. *Plant Physiol* 155(2): 944-955.
- Jaeger, T., Budde, H., Flohe, L., Menge, U., Singh, M., Trujillo, M. & Radi, R. (2004). Multiple thioredoxin-mediated routes to detoxify hydroperoxides in *Mycobacterium tuberculosis*. *Arch Biochem Biophys* 423(1): 182-191.
- Jakopitsch, C., Ivancich, A., Schmuckenschlager, F., Wanasinghe, A., Poltl, G., Furtmuller, P. G., Ruker, F. & Obinger, C. (2004). Influence of the unusual covalent adduct on the kinetics and formation of radical intermediates in *synechocystis* catalase peroxidase: a stopped-flow and EPR characterization of the MET275, TYR249, and ARG439 variants. *J Biol Chem* 279(44): 46082-46095.

- Johnsson, K., Froland, W. A. & Schultz, P. G. (1997). Overexpression, purification, and characterization of the catalase-peroxidase KatG from *Mycobacterium tuberculosis*. *J Biol Chem* 272(5): 2834-2840.
- Jungblut, P. R., Schaible, U. E., Mollenkopf, H. J., Zimny-Arndt, U., Raupach, B., Mattow, J., Halada, P., Lamer, S., Hagens, K. & Kaufmann, S. H. (1999). Comparative proteome analysis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG strains: towards functional genomics of microbial pathogens. *Mol Microbiol* 33(6): 1103-1117.
- Kissner, R., Nauser, T., Bugnon, P., Lye, P. G. & Koppenol, W. H. (1997). Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. *Chem Res Toxicol* 10(11): 1285-1292.
- Koppenol, W. H. & Kissner, R. (1998). *Chem Res Toxicol* 11: 87-90.
- Koshkin, A., Knudsen, G. M. & Ortiz De Montellano, P. R. (2004). Intermolecular interactions in the AhpC/AhpD antioxidant defense system of *Mycobacterium tuberculosis*. *Arch Biochem Biophys* 427(1): 41-47.
- Krause, G., Lundstrom, J., Barea, J. L., Pueyo de la Cuesta, C. & Holmgren, A. (1991). Mimicking the active site of protein disulfide-isomerase by substitution of proline 34 in *Escherichia coli* thioredoxin. *J Biol Chem* 266(15): 9494-9500.
- Latimer, W. M., *Oxidation Potentials*, second ed. (1952). Prentice-Hall, New York.
- Lawn, S. D. & Zumla, A. I. (2011). Tuberculosis. *Lancet* 378(9785):57-72.
- Lee, W. L., Gold, B., Darby, C., Brot, N., Jiang, X., de Carvalho, L. P., Wellner, D., St John, G., Jacobs, W. R., Jr. & Nathan, C. (2009). *Mycobacterium tuberculosis* expresses methionine sulphoxide reductases A and B that protect from killing by nitrite and hypochlorite. *Mol Microbiol* 71(3): 583-593.
- Li, S., Peterson, N. A., Kim, M. Y., Kim, C. Y., Hung, L. W., Yu, M., Lakin, T., Segelke, B. W., Lott, J. S. & Baker, E. N. (2005). Crystal Structure of AhpE from *Mycobacterium tuberculosis*, a 1-Cys peroxiredoxin. *J Mol Biol* 346(4): 1035-1046.
- Lim, J. C., Choi, H. I., Park, Y. S., Nam, H. W., Woo, H. A., Kwon, K. S., Kim, Y. S., Rhee, S. G., Kim, K. & Chae, H. Z. (2008). Irreversible oxidation of the active-site cysteine of peroxiredoxin to cysteine sulfonic acid for enhanced molecular chaperone activity. *J Biol Chem* 283(43): 28873-28880.
- Lymar, S. V. & Hurst, J. K. (1995). Rapid reaction between peroxynitrite anion and carbon dioxide: implication for biological activity. *J Am Chem Soc* 117: 8867-8868.
- MacMicking, J., Xie, Q. W. & Nathan, C. (1997). Nitric oxide and macrophage function. *Annu Rev Immunol* 15: 323-350.
- MacMicking, J. D., Taylor, G. A. & McKinney, J. D. (2003). Immune control of tuberculosis by IFN-gamma-inducible LRG-47. *Science* 302(5645): 654-659.
- Malen, H., Berven, F. S., Fladmark, K. E. & Wiker, H. G. (2007). Comprehensive analysis of exported proteins from *Mycobacterium tuberculosis* H37Rv. *Proteomics* 7(10): 1702-1718.
- Malen, H., Pathak, S., Softeland, T., de Souza, G. A. & Wiker, H. G. (2010). Definition of novel cell envelope associated proteins in Triton X-114 extracts of *Mycobacterium tuberculosis* H37Rv. *BMC Microbiol* 10: 132.
- Manta, B., Hugo, M., Ortiz, C., Ferrer-Sueta, G., Trujillo, M. & Denicola, A. (2009). The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2. *Arch Biochem Biophys* 484(2): 146-154.

- Martin, E., Nathan, C. & Xie, Q. W. (1994). Role of interferon regulatory factor 1 in induction of nitric oxide synthase. *J Exp Med* 180(3): 977-984.
- Master, S. S., Springer, B., Sander, P., Boettger, E. C., Deretic, V. & Timmins, G. S. (2002). Oxidative stress response genes in *Mycobacterium tuberculosis*: role of *ahpC* in resistance to peroxynitrite and stage-specific survival in macrophages. *Microbiology* 148(Pt 10): 3139-3144.
- Mawuenyega, K. G., Forst, C. V., Dobos, K. M., Belisle, J. T., Chen, J., Bradbury, E. M., Bradbury, A. R. & Chen, X. (2005). *Mycobacterium tuberculosis* functional network analysis by global subcellular protein profiling. *Mol Biol Cell* 16(1): 396-404.
- Meena, L. S. & Rajni (2010). Survival mechanisms of pathogenic *Mycobacterium tuberculosis* H37Rv. *FEBS J* 277(11): 2416-2427.
- Miller, C. C., Rawat, M., Johnson, T. & Av-Gay, Y. (2007). Innate protection of *Mycobacterium smegmatis* against the antimicrobial activity of nitric oxide is provided by mycothiol. *Antimicrob Agents Chemother* 51(9): 3364-3366.
- Mollenkopf, H. J., Jungblut, P. R., Raupach, B., Mattow, J., Lamer, S., Zimny-Arndt, U., Schaible, U. E. & Kaufmann, S. H. (1999). A dynamic two-dimensional polyacrylamide gel electrophoresis database: the mycobacterial proteome via Internet. *Electrophoresis* 20(11): 2172-2180.
- Moon, J. C., Hah, Y. S., Kim, W. Y., Jung, B. G., Jang, H. H., Lee, J. R., Kim, S. Y., Lee, Y. M., Jeon, M. G., Kim, C. W., Cho, M. J. & Lee, S. Y. (2005). Oxidative stress-dependent structural and functional switching of a human 2-Cys peroxiredoxin isotype II that enhances HeLa cell resistance to H₂O₂-induced cell death. *J Biol Chem* 280(31): 28775-28784.
- Murphy, D. J. & Brown, J. R. (2007). Identification of gene targets against dormant phase *Mycobacterium tuberculosis* infections. *BMC Infect Dis* 7: 84.
- Nathan, C. (2009). Taming tuberculosis: a challenge for science and society. *Cell Host Microbe* 5(3): 220-224.
- Nathan, C. & Shiloh, M. U. (2000). Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. *Proc Natl Acad Sci U S A* 97(16): 8841-8848.
- Nelson, K. J., Knutson, S. T., Soito, L., Klomsiri, C., Poole, L. B. & Fetrow, J. S. (2011). Analysis of the peroxiredoxin family: using active-site structure and sequence information for global classification and residue analysis. *Proteins* 79(3): 947-964.
- Nelson, K. J., Parsonage, D., Hall, A., Karplus, P. A. & Poole, L. B. (2008). Cysteine pK(a) values for the bacterial peroxiredoxin AhpC. *Biochemistry* 47(48): 12860-12868.
- Newton, G. L., Av-Gay, Y. & Fahey, R. C. (2000). A novel mycothiol-dependent detoxification pathway in mycobacteria involving mycothiol S-conjugate amidase. *Biochemistry* 39(35): 10739-10746.
- Newton, G. L. & Fahey, R. C. (2002). Mycothiol biochemistry. *Arch Microbiol* 178(6): 388-394.
- Ogusucu, R., Rettori, D., Munhoz, D. C., Netto, L. E. & Augusto, O. (2007). Reactions of yeast thioredoxin peroxidases I and II with hydrogen peroxide and peroxynitrite: rate constants by competitive kinetics. *Free Radic Biol Med* 42(3): 326-334.
- Parsonage, D., Karplus, P. A. & Poole, L. B. (2008). Substrate specificity and redox potential of AhpC, a bacterial peroxiredoxin. *Proc Natl Acad Sci U S A* 105(24): 8209-8214.

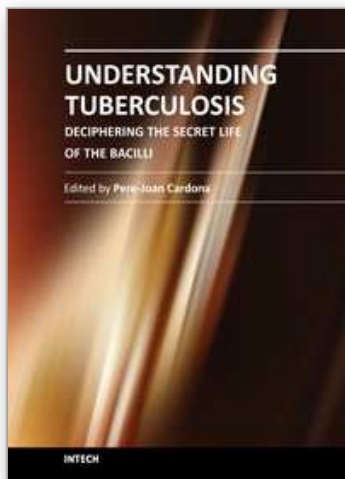
- Parsonage, D., Youngblood, D. S., Sarma, G. N., Wood, Z. A., Karplus, P. A. & Poole, L. B. (2005). Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. *Biochemistry* 44(31): 10583-10592.
- Pascual, M. B., Mata-Cabana, A., Florencio, F. J., Lindahl, M. & Cejudo, F. J. (2010). Overoxidation of 2-Cys peroxiredoxin in prokaryotes: cyanobacterial 2-Cys peroxiredoxins sensitive to oxidative stress. *J Biol Chem* 285(45): 34485-34492.
- Passardi, F., Theiler, G., Zamocky, M., Cosio, C., Rouhier, N., Teixeira, F., Margis-Pinheiro, M., Ioannidis, V., Penel, C., Falquet, L. & Dunand, C. (2007). PeroxiBase: the peroxidase database. *Phytochemistry* 68(12): 1605-1611.
- Patel, M. P. & Blanchard, J. S. (2001). Mycobacterium tuberculosis mycothione reductase: pH dependence of the kinetic parameters and kinetic isotope effects. *Biochemistry* 40(17): 5119-5126.
- Pearce, M. J., Arora, P., Festa, R. A., Butler-Wu, S. M., Gokhale, R. S. & Darwin, K. H. (2006). Identification of substrates of the Mycobacterium tuberculosis proteasome. *EMBO J* 25(22): 5423-5432.
- Poole, L. B. (2007). The catalytic mechanism of peroxiredoxins. *Subcell Biochem* 44: 61-81.
- Prutz, W. A., Monig, H., Butler, J. & Land, E. J. (1985). Reactions of nitrogen dioxide in aqueous model systems: oxidation of tyrosine units in peptides and proteins. *Arch Biochem Biophys* 243(1): 125-134.
- Pryor, W. A. & Squadrito, G. L. (1995). The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268(5 Pt 1): L699-722.
- Raman, S., Song, T., Puyang, X., Bardarov, S., Jacobs, W. R., Jr. & Husson, R. N. (2001). The alternative sigma factor SigH regulates major components of oxidative and heat stress responses in Mycobacterium tuberculosis. *J Bacteriol* 183(20): 6119-6125.
- Rawat, M., Newton, G. L., Ko, M., Martinez, G. J., Fahey, R. C. & Av-Gay, Y. (2002). Mycothiol-deficient Mycobacterium smegmatis mutants are hypersensitive to alkylating agents, free radicals, and antibiotics. *Antimicrob Agents Chemother* 46(11): 3348-3355.
- Reyes, A. M., Hugo, M., Trostchansky, A., Capece, L., Radi, R. & Trujillo, M. (2011). Oxidizing substrate specificity of Mycobacterium tuberculosis alkyl hydroperoxide reductase E: kinetics and mechanisms of oxidation and overoxidation. *Free Radic Biol Med* 51(2):464-473.
- Rhee, S. G., Chae, H. Z. & Kim, K. (2005). Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med* 38(12): 1543-1552.
- Rhee, S. G. & Woo, H. A. (2011). Multiple Functions of Peroxiredoxins: Peroxidases, Sensors and Regulators of the Intracellular Messenger H₂O₂, and Protein Chaperones. *Antioxid Redox Signal* 15(3):781-794.
- Rho, B. S., Hung, L. W., Holton, J. M., Vigil, D., Kim, S. I., Park, M. S., Terwilliger, T. C. & Pedelacq, J. D. (2006). Functional and structural characterization of a thiol peroxidase from Mycobacterium tuberculosis. *J Mol Biol* 361(5): 850-863.
- Rosenkrands, I., King, A., Weldingh, K., Moniatte, M., Moertz, E. & Andersen, P. (2000). Towards the proteome of Mycobacterium tuberculosis. *Electrophoresis* 21(17): 3740-3756.
- Sasseti, C. M., Boyd, D. H. & Rubin, E. J. (2003). Genes required for mycobacterial growth defined by high density mutagenesis. *Mol Microbiol* 48(1): 77-84.

- Seebeck, F. P. (2010). In vitro reconstitution of Mycobacterial ergothioneine biosynthesis. *J Am Chem Soc* 132(19): 6632-6633.
- Sevanian, A., Muakkassah-Kelly, S. F. & Montestruque, S. (1983). The influence of phospholipase A2 and glutathione peroxidase on the elimination of membrane lipid peroxides. *Arch Biochem Biophys* 223(2): 441-452.
- Sherman, D. R., Mdluli, K., Hickey, M. J., Arain, T. M., Morris, S. L., Barry, C. E., 3rd & Stover, C. K. (1996). Compensatory *ahpC* gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. *Science* 272(5268): 1641-1643.
- Sherman, D. R., Voskuil, M., Schnappinger, D., Liao, R., Harrell, M. I. & Schoolnik, G. K. (2001). Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding α -crystallin. *Proc Natl Acad Sci U S A* 98(13): 7534-7539.
- Shi, S. & Ehrt, S. (2006). Dihydrolipoamide acyltransferase is critical for *Mycobacterium tuberculosis* pathogenesis. *Infect Immun* 74(1): 56-63.
- Shiloh, M. U. & Nathan, C. F. (2000). Reactive nitrogen intermediates and the pathogenesis of *Salmonella* and mycobacteria. *Curr Opin Microbiol* 3(1): 35-42.
- Sohling, B., Parther, T., Rucknagel, K. P., Wagner, M. A. & Andreessen, J. R. (2001). A selenocysteine-containing peroxiredoxin from the strictly anaerobic organism *Eubacterium acidaminophilum*. *Biol Chem* 382(6): 979-986.
- Soito, L., Williamson, C., Knutson, S. T., Fetrow, J. S., Poole, L. B. & Nelson, K. J. (2011). PREX: PeroxiRedoxin classification indEX, a database of subfamily assignments across the diverse peroxiredoxin family. *Nucleic Acids Res* 39(Database issue): D332-337.
- Springer, B., Master, S., Sander, P., Zahrt, T., McFalone, M., Song, J., Papavinasasundaram, K. G., Colston, M. J., Boettger, E. & Deretic, V. (2001). Silencing of oxidative stress response in *Mycobacterium tuberculosis*: expression patterns of *ahpC* in virulent and avirulent strains and effect of *ahpC* inactivation. *Infect Immun* 69(10): 5967-5973.
- Stehr, M., Hecht, H. J., Jager, T., Flohe, L. & Singh, M. (2006). Structure of the inactive variant C60S of *Mycobacterium tuberculosis* thiol peroxidase. *Acta Crystallogr D Biol Crystallogr* 62(Pt 5): 563-567.
- Stone, J. R. (2004). An assessment of proposed mechanisms for sensing hydrogen peroxide in mammalian systems. *Arch Biochem Biophys* 422(2): 119-124.
- Storz, G., Christman, M. F., Sies, H. & Ames, B. N. (1987). Spontaneous mutagenesis and oxidative damage to DNA in *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* 84(24): 8917-8921.
- Sturgill-Koszycki, S., Schlesinger, P. H., Chakraborty, P., Haddix, P. L., Collins, H. L., Fok, A. K., Allen, R. D., Gluck, S. L., Heuser, J. & Russell, D. G. (1994). Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 263(5147): 678-681.
- Ta, P., Buchmeier, N., Newton, G. L., Rawat, M. & Fahey, R. C. (2011). Organic hydroperoxide resistance protein and ergothioneine compensate for loss of mycothiol in *Mycobacterium smegmatis* mutants. *J Bacteriol* 193(8): 1981-1990.
- Tartaglia, L. A., Storz, G. & Ames, B. N. (1989). Identification and molecular analysis of *oxyR*-regulated promoters important for the bacterial adaptation to oxidative stress. *J Mol Biol* 210(4): 709-719.

- Tian, J., Bryk, R., Shi, S., Erdjument-Bromage, H., Tempst, P. & Nathan, C. (2005). *Mycobacterium tuberculosis* appears to lack alpha-ketoglutarate dehydrogenase and encodes pyruvate dehydrogenase in widely separated genes. *Mol Microbiol* 57(3): 859-868.
- Trindade, D. F., Cerchiaro, G. & Augusto, O. (2006). A role for peroxymonocarbonate in the stimulation of biothiol peroxidation by the bicarbonate/carbon dioxide pair. *Chem Res Toxicol* 19(11): 1475-1482.
- Trujillo, M., Clippe, A., Manta, B., Ferrer-Sueta, G., Smeets, A., Declercq, J. P., Knoop, B. & Radi, R. (2007). Pre-steady state kinetic characterization of human peroxiredoxin 5: taking advantage of Trp84 fluorescence increase upon oxidation. *Arch Biochem Biophys* 467(1): 95-106.
- Trujillo, M., Ferrer-Sueta, G., Thomson, L., Flohe, L. & Radi, R. (2007). Kinetics of peroxiredoxins and their role in the decomposition of peroxynitrite. *Subcell Biochem* 44: 83-113.
- Trujillo, M., Mauri, P., Benazzi, L., Comini, M., De Palma, A., Flohe, L., Radi, R., Stehr, M., Singh, M., Ursini, F. & Jaeger, T. (2006). The mycobacterial thioredoxin peroxidase can act as a one-cysteine peroxiredoxin. *J Biol Chem* 281(29): 20555-20566.
- Vandal, O. H., Pierini, L. M., Schnappinger, D., Nathan, C. F. & Ehrt, S. (2008). A membrane protein preserves intrabacterial pH in intraphagosomal *Mycobacterium tuberculosis*. *Nat Med* 14(8): 849-854.
- Wang, G., Hong, Y., Johnson, M. K. & Maier, R. J. (2006). Lipid peroxidation as a source of oxidative damage in *Helicobacter pylori*: protective roles of peroxiredoxins. *Biochim Biophys Acta* 1760(11): 1596-1603.
- Weldingh, K., Rosenkrands, I., Jacobsen, S., Rasmussen, P. B., Elhay, M. J. & Andersen, P. (1998). Two-dimensional electrophoresis for analysis of *Mycobacterium tuberculosis* culture filtrate and purification and characterization of six novel proteins. *Infect Immun* 66(8): 3492-3500.
- Wengenack, N. L., Jensen, M. P., Rusnak, F. & Stern, M. K. (1999). *Mycobacterium tuberculosis* KatG is a peroxynitritase. *Biochem Biophys Res Commun* 256(3): 485-487.
- Wilson, T. M. & Collins, D. M. (1996). *ahpC*, a gene involved in isoniazid resistance of the *Mycobacterium tuberculosis* complex. *Mol Microbiol* 19(5): 1025-1034.
- Woo, H. A., Yim, S. H., Shin, D. H., Kang, D., Yu, D. Y. & Rhee, S. G. (2010). Inactivation of peroxiredoxin I by phosphorylation allows localized H₂O₂ accumulation for cell signaling. *Cell* 140(4): 517-528.
- Wood, Z. A., Poole, L. B. & Karplus, P. A. (2003). Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling. *Science* 300(5619): 650-653.
- Wood, Z. A., Schroder, E., Robin Harris, J. & Poole, L. B. (2003). Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 28(1): 32-40.
- Xie, Q. W., Whisnant, R. & Nathan, C. (1993). Promoter of the mouse gene encoding calcium-independent nitric oxide synthase confers inducibility by interferon gamma and bacterial lipopolysaccharide. *J Exp Med* 177(6): 1779-1784.
- Yamamoto, Y., Ritz, D., Planson, A. G., Jonsson, T. J., Faulkner, M. J., Boyd, D., Beckwith, J. & Poole, L. B. (2008). Mutant AhpC peroxiredoxins suppress thiol-disulfide redox deficiencies and acquire deglutathionylating activity. *Mol Cell* 29(1): 36-45.
- Yang, K. S., Kang, S. W., Woo, H. A., Hwang, S. C., Chae, H. Z., Kim, K. & Rhee, S. G. (2002). Inactivation of human peroxiredoxin I during catalysis as the result of the oxidation

- of the catalytic site cysteine to cysteine-sulfinic acid. *J Biol Chem* 277(41): 38029-38036.
- Zahrt, T. C. & Deretic, V. (2002). Reactive nitrogen and oxygen intermediates and bacterial defenses: unusual adaptations in *Mycobacterium tuberculosis*. *Antioxid Redox Signal* 4(1): 141-159.
- Zahrt, T. C., Song, J., Siple, J. & Deretic, V. (2001). Mycobacterial FurA is a negative regulator of catalase-peroxidase gene *katG*. *Mol Microbiol* 39(5): 1174-1185.
- Zhang, Y., Heym, B., Allen, B., Young, D. & Cole, S. (1992). The catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 358(6387): 591-593.
- Zhao, X., Suarez, J., Khajo, A., Yu, S., Metlitsky, L. & Magliozzo, R. S. (2010). A radical on the Met-Tyr-Trp modification required for catalase activity in catalase-peroxidase is established by isotopic labeling and site-directed mutagenesis. *J Am Chem Soc* 132(24): 8268-8269.

IntechOpen



Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli

Edited by Dr. Pere-Joan Cardona

ISBN 978-953-307-946-2

Hard cover, 334 pages

Publisher InTech

Published online 17, February, 2012

Published in print edition February, 2012

Mycobacterium tuberculosis, as recent investigations demonstrate, has a complex signaling expression, which allows its close interaction with the environment and one of its most renowned properties: the ability to persist for long periods of time under a non-replicative status. Although this skill is well characterized in other bacteria, the intrinsically very slow growth rate of Mycobium tuberculosis, together with a very thick and complex cell wall, makes this pathogen specially adapted to the stress that could be generated by the host against them. In this book, different aspects of these properties are displayed by specialists in the field.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Martín Hugo, Rafael Radi and Madia Trujillo (2012). Thiol-Dependent Peroxidases in Mycobacterium tuberculosis Antioxidant Defense, Understanding Tuberculosis - Deciphering the Secret Life of the Bacilli, Dr. Pere-Joan Cardona (Ed.), ISBN: 978-953-307-946-2, InTech, Available from:
<http://www.intechopen.com/books/understanding-tuberculosis-deciphering-the-secret-life-of-the-bacilli/thiol-dependent-peroxidases-in-mycobacterium-tuberculosis-antioxidant-defense>

INTech
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen