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FORUM EDITORIAL

An Update on Redox Biology of Parasites

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Abstract

Parasite redox biology is vital for understanding parasite-host interactions and adaptations. Studies in this area are leading to discoveries regarding drug targets and drug leads to treat infections caused by protozoan and worm parasites for which there are few effective drugs. Parasite unique and nonredundant core redox enzymes are choke points of metabolism and pharmacological targets. This Forum revises this concept and proposes new drug targets. It also highlights recent studies using genetically manipulated and natural strains that reveal emerging regulatory functions of antioxidant enzymes in parasite differentiation, apoptosis, virulence, acute infection, and disease progression and outcome. The challenge ahead is to understand the redox changes linked to differentiation and drastic transitions between environments that take place during parasitic complex life cycles. The combined use of new tools and techniques, such as genetically-manipulated parasites, live imaging, redox sensors, and proteomics, allow the challenge to be undertaken. Some of these methodologies, for example, transgenic parasites encoding redox biosensors, can also be applied to drug high throughput screening and to assess the effect of currently known drugs that affect redox homeostasis. *Antioxid. Redox Signal.* 19, 661–664.

Parasites are organisms that live, at least part of their lifecycle inside another organism (the host), which they exploit for their own benefit. Despite the broad definition, the term is restricted (in Medicine and in this Forum) to protozoan (unicellular, protists) and helminth (multicellular metazoan) parasites. Infections by parasites are highly prevalent in vast and expanding regions of the world, and cause a wide variety of highly disabling and mortal diseases in humans, particularly in developing countries. Parasitic infections are one of the most important challenges for human medicine in the 21st century. They also cause an enormous economic burden, since many parasite species infect livestock. Paradoxically, the tools to control parasitic infections are scarce. There is no single vaccine available for a human parasite. For the most important parasitic human diseases, such as malaria, caused by *Plasmodium* spp., sleeping sickness, and Chagas disease caused by *Trypanosoma* spp., and schistosomiasis or bilharzia (caused by *Schistosoma* spp.), there are just one or two drugs available for massive treatment and increasing concern about the emergence and spread of drug resistance. Except for malaria, there is limited interest from the pharmaceutical industry in research and development on new drugs for these neglected diseases.

The interest in redox biology of parasites is fully justified. The maintenance of intracellular redox homeostasis is of paramount importance for parasite viability inside their hosts. For parasites, the host is "the environment"; even for those parasites with a free-living stage, this simply represents a necessary step for movement from host to host. However, despite this environment provides survival advantages for the parasite, it also entails potential risks: the host can react against the pathogen *via* innate or adaptive defense mechanisms. Indeed, the host-imposed immune response includes an induced oxidative attack that parasites should neutralize and control to survive. Another important reason for the study of parasite redox biology is the fact that many parasitic lineages possess distinct and streamlined redox systems in comparison to their hosts. Therefore, parasites expand our knowledge on the repertoire of redox pathways. The investigation of key redox enzymes and pathways of parasites opens up very interesting possibilities for pharmacologically targeting infectious diseases. This concept is illustrated in the accompanying Figure 1A. This Forum is also timely because evidence is accruing regarding the importance of proteins involved in redox homeostasis as virulence factors and determiners of infection, disease progression and outcome (see accompanying Fig. 1C). Finally, parasites are also very

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interesting organisms to investigate and understand redox adaptive mechanisms and responses that occur during their complex life cycles, which involve sudden transitions between phylogenetically unrelated hosts, intra-/extracellular stages, and dividing/nondividing forms. In this regard, the continuous development of new methodologies and tools, such as redox proteomics, redox sensors and probes, and deep sequencing, will continue to fuel new discoveries in the field.

The present ARS Forum on "Redox biology of parasites" continues the previous excellent homonymous one edited by Katja Becker and Stefan Rahlfs (2). The present one covers new topics, including helminth parasites. Five reviews and two original articles by leading groups working in the field of parasite redox biology are presented.

Trypanosomatids are the causative agents of serious diseases which affect millions of people in Africa and America and are spreading at an increasing pace, due to migration of infected hosts and insect vectors. Trypanosomatids rely on a unique low molecular mass thiol, trypanothione (a bis-glutathionyl spermidine conjugate), for many redox and cellular processes. The mono- and dithiol glutaredoxins (1C-Grxs and 2C-Grxs, respectively) in Trypanosomatids are revised in depth by Comini *et al.* (3). This revision is timely, since the use of trypanothione by trypanosomatid Grxs has established novel roles for the lineage-specific low molecular mass thiol. 2C-Grxs catalyze the oxidized glutathione and glutathione-mixed disulfides reduction by trypanothione. The evidence and working models of iron-sulfur cluster (ISC) assembly by 1C-Grxs with glutathione, monoglutathionylspermidine and trypanothione are reviewed. The interplay between the trypanothione system and Grx is carefully revised and critically discussed on the basis that these organisms are devoid of glutathione reductase. In a back-to-back original article, Manta *et al.* (5) presents the biochemical basis of iron-sulfur coordination of 1C-Grx-1 and its 3D structure, the first reported for a pathogenic protozoan. The results suggest that 1C-Grx-1 utilizes a new mechanism for ISC binding. This article also shows that trypanothione forms stable protein-free ISC species that are incorporated *in vitro* into Grxs. In addition, the *in vivo* relevance of *Trypanosoma brucei* 1C-Grx-1 is assessed: this mitochondrial protein facilitates host infection and disease progression, since overexpression of the lack-of-function C104S 1C-Grx1 mutant impairs parasite survival in mice. Overall, these two contributions emphasize the specialization of the trypanosomal proteins in using trypanothione as cofactor for redox reactions and ISC binding, which constitutes a remarkable evolutionary adaptation of Grxs to the unique thiol-redox metabolism of these parasites.

Piacenza *et al.* revise several aspects of redox control in *Trypanosoma cruzi* infections (7). The mechanisms used by the parasite to cope with nitrooxidative stress are thoroughly reviewed. The concept that the success of infection in the acute phase depends on parasite antioxidant enzyme levels is addressed and the emerging evidence regarding key components of *T. cruzi* antioxidant network as virulence factors in naturally occurring strains is discussed. Recent progress on how modulation of mitochondrial superoxide radical levels influences parasite programmed cell death and Chagas' disease progression is also reviewed.

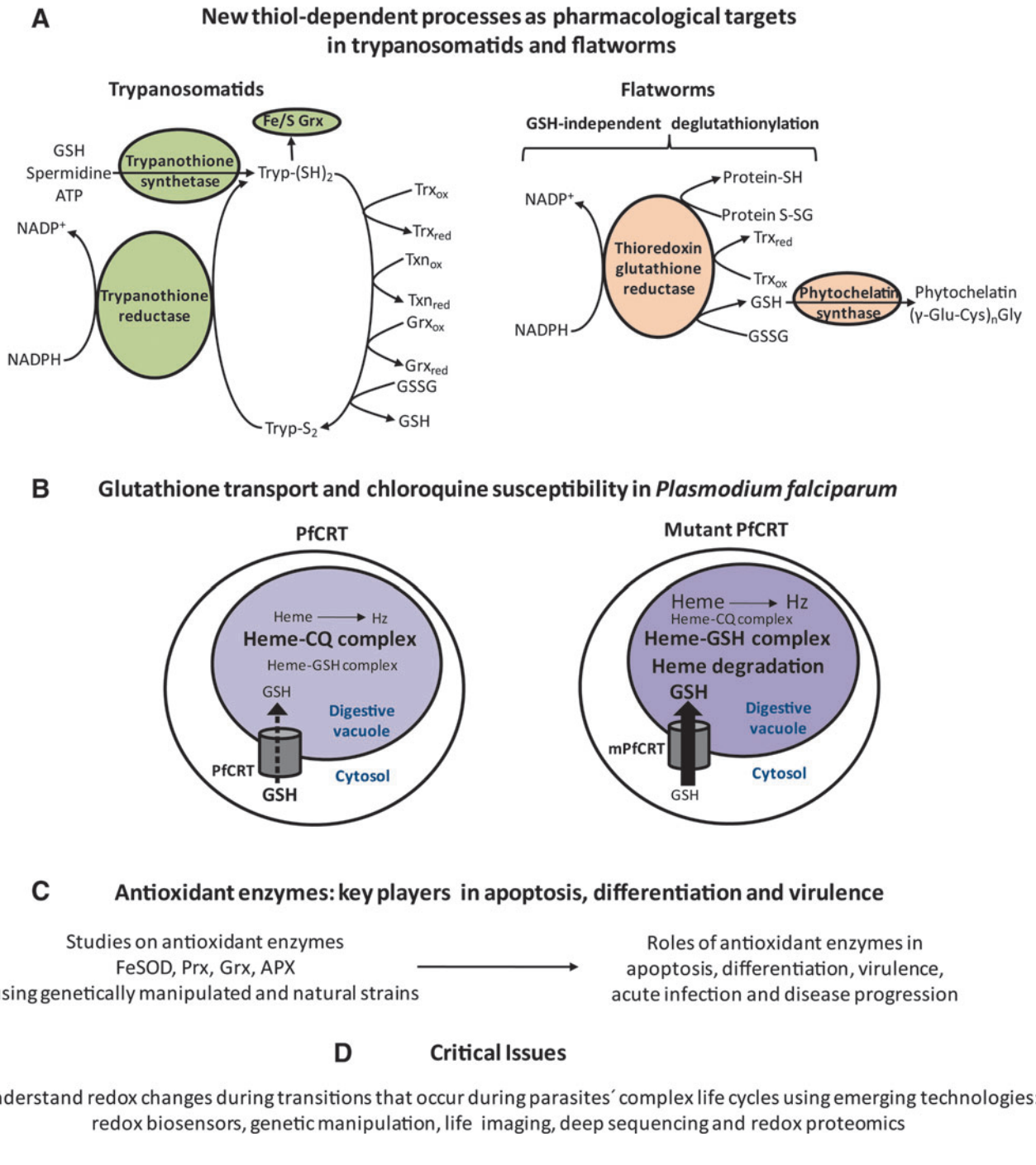
Mitochondrial redox processes in trypanosomatids are revised by Tomás and Castro (8). The sources of reactive oxygen species (ROS), particularly in response to *in vivo* conditions and physiological stimuli are addressed, and the mechanisms of ROS elimination are covered in detail. The review also stresses the need to address, more precisely, the generation and quantification of some intramitochondrial ROS as well as of potential key players for ROS removal, such as trypanothione.

Adak and Pal review what is known of a unique heme peroxidase (APX), which is a hybrid cytochrome c/ascorbate peroxidase of plant origin that localizes in the intermembrane space of single-celled *Leishmania* parasites (1). This enzyme is

FIG. 1. Key advances and critical areas of consideration on redox biology of parasites. (A) Parasites possess distinct and streamlined redox pathways that can be pharmacologically targeted to treat infections. The upper scheme illustrates the unique thiol-dependent redox pathway present in trypanosomatids (unicellular, protist parasites). These organisms lack thioredoxin reductase and glutathione reductase: instead they rely on trypanothione reductase, which reduces the unique low molecular mass thiol trypanothione (Tryp-S_2, oxidized form; Tryp-$(SH)_2$, reduced form). Tryp-$(SH)_2$ can reduce thioredoxin (Trx), tryparedoxin (Txn), glutaredoxin (Grx) and glutathione (GSSG) and also be a cofactor for iron-sulfur cluster coordination by Grxs. Known as well as new (proposed in this Forum) pharmacological targets are indicated in green. The lower scheme illustrates the simplified redox pathways of flatworm parasites (multicellular, metazoan parasites). These organisms also lack conventional Trx reductase and glutathione reductase: they possess a single Trx glutathione reductase (TGR), a natural fusion of a conventional Trx reductase to a Grx domain, which supports both pathways. In addition, flatworm parasites lack metallothionein for metal detoxification. Instead, they rely on phytochelatin, a GSH-derived polymer absent in their hosts, whose synthesis is catalyzed by phytochelatin synthase. TGR, a validated and promising drug target, and phyhochelatin synthase, a potential one, are highlighted in orange. ox: oxidized; red: reduced. **(B)** New clues in *Plasmodium falciparum* chloroquine (CQ) resistance. Heme from the parasitized red cells is normally sequestered in *P. falciparum* digestive vacuole where it forms insoluble crystals called hemozoin (Hz); therefore, avoiding heme toxicity. The drug CQ exerts its toxic effect by binding heme and interfering with this biocrystallization process, leading to heme prooxidant effects (*left panel*). Mutations in the *P. falciparum* CQ resistance transporter (Pf*CRT*) are associated with CQ resistance. Mutant PfCRTs (mPfCRT, *right panel*), but not wild-type PfCRT (*left panel*), facilitate the transport of GSH to the digestive vacuole, where it is postulated to form complex with CQ, outcompeting heme binding to CQ, thereby leading to heme degradation, Hz formation, and CQ resistance. **(C)** Antioxidant enzymes regulate key physiological processes. Work performed with natural as well as genetically manipulated (knock out, mutant and overexpressing) parasite strains have changed the classic view of antioxidant enzymes as defense mechanisms. Key roles in several physiological processes, such as apoptosis, differentiation, infection, and in virulence and disease outcome are beginning to be elucidated. The roles of iron superoxide dismutase (FeSOD), peroxiredoxin (Prx), Grx and hybrid cytochrome c/ascorbateperoxidase (APX) are revised in this Forum. **(D)** Critical Issues. The advent of new tools and methods should be used to fully address the redox regulation during the sudden and drastic environmental changes that parasite face during their complex life cycles. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

a key player in protecting the intracellular form of these trypanosomatids, which reside inside macrophage phagolysosomes. The spectral characteristics, catalytic mechanism and a 3D-homology modeling of the enzyme are reviewed. The effects on parasite's defense and signaling for *Leishmania major* cells overexpressing *APX* and for null *APX* mutants is revised and discussed. Interestingly, the crystal structure of the *L. major* peroxidase-cytochrome c complex has recently been solved by Jasion *et al.* (4) adding value to early biochemical evidences and structural models.

Malaria is the most devastating parasitic disease, causing more than a million deaths per year. The antimalarial chloroquine was one of the most important drugs ever developed, until the spreading of resistance. Chloroquine resistance is associated with mutations in the digestive vacuole membrane protein *Plasmodium falciparum* chloroquine resistance transporter (PfCRT). Yet, the molecular basis of chloroquine resistance is not fully elucidated. In an original article, Patzewitz *et al.* shed new light on this phenomenon (6). They revealed a previously unconsidered role for Pf*CRT* chloroquine resistant alleles. The authors demonstrate that Pf*CRT* mutants facilitate the transport of glutathione from the cytosol to the digestive vacuole. The data generated allowed the authors to postulate a chloroquine resistance mechanism in which GSH is selectively transferred into the digestive vacuole *via* a mutant PfCRT, where it competes with chloroquine for heme binding,



results in destruction of heme, and thereby mediates protection of the parasites from the prooxidant activity of the chloroquine–heme complex (see accompanying Fig. 1B).

Flatworm parasites include flukes (*e.g., Schistosoma* spp.) and tapeworms (*e.g., Echinococcus* spp.) that cause chronic infections and debilitating diseases. Williams *et al.* (9) review the linked thioredoxin glutathione system of these parasites, which relies on the selenoenzyme thioredoxin glutathione reductase (TGR), a validated drug target, as the sole enzyme that supports both pathways. Recent structural and biochemical studies that elucidated how TGR functions are carefully revised, stressing the ability of TGR to catalyze GSH-independent deglutathionylation (see accompanying Fig. 1A). The TGR-dependent redox network is revisited considering recent genomic information, highlighting the differences between free-living and parasitic flatworms. The efforts that have led to the identification of TGR inhibitors that kill flatworm parasites are also reviewed.

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References

1. Adak S and Pal S. Ascorbate peroxidase acts as a novel determiner of redox homeostasis in *Leishmania*. *Antioxid Redox Signal* 19: 746–754, 2013.
2. Becker K and Rahlfs S. Redox biology of parasites I. *Antioxid Redox Signal* 19: 661–664, 2013.
3. Comini MA, Krauth-Siegel RL, and Bellanda M. Mono- and dithiol glutaredoxins in the trypanothione-based in the redox metabolism of pathogenic *Trypanosomes*. *Antioxid Redox Signal* 19: 708–722, 2013.
4. Jasion VS, Doukov T, Pineda SH, Li H, and Poulos TL. Crystal structure of the *Leishmania major* peroxidase-cytochrome c complex. *Proc Natl Acad Sci U S A* 109: 18390–18394, 2012.
5. Manta M, Pavan C, Sturlese M, Medeiros A, Crispo M, Berndt C, Krauth-Siegel RL, Bellanda M, and Comini MA. Iron–sulfur cluster binding by mitochondrial monothiol glutaredoxin-1 of *Trypanosoma brucei*: molecular basis of iron–sulfur cluster coordination and relevance for parasite infectivity. *Antioxid Redox Signal* 19: 665–682, 2013.
6. Patzewitz EM, Salcedo-Sora JE, Wong EH, Sethia S, Stocks PA, Maughan SC, Murray JAH, Krishna S, Bray PG, Ward SA, and Müller S. Glutathione transport: a new role for PfCRT in chloroquine resistance. *Antioxid Redox Signal* 19: 683–695, 2013.
7. Piacenza L, Peluffo G, Alvarez MN, Martínez A, and Radi R. *Trypanosoma cruzi* antioxidant enzymes as virulence factors in Chagas disease. *Antioxid Redox Signal* 19: 723–734, 2013.
8. Tomás AM and Castro H. Redox metabolism in mitochondria of trypanosomatids. *Antioxid Redox Signal* 19: 696–707, 2013.
9. Williams DL, Bonilla M, Gladyshev VN, and Salinas G. Thioredoxin glutathione reductase-dependent redox networks in platyhelminth parasites. *Antioxid Redox Signal* 19: 735–745, 2013.

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Abbreviations Used

1C-Grx = monothiol glutaredoxin
2C-Grx = dithiol glutaredoxin
APX = hybrid cytochrome c/ascorbate peroxidase
CQ = chloroquine
FeSOD = iron superoxide dismutase
Hz = hemozoin
ISC = iron-sulfur cluster
mPfCRT = mutant *Plasmodium falciparum* chloroquine resistance transporter
PfCRT = *Plasmodium falciparum* chloroquine resistance transporter
Prx = peroxiredoxin
ROS = reactive oxygen species
TGR = thioredoxin glutathione reductase
Tryp-S_2 = trypanothione, oxidized form
Tryp-$(SH)_2$, = trypanothione, reduced form
Trx = thioredoxin
Txn = tryparedoxin