June 2006

Selenoproteins in parasites

Gustavo Salinas
Universidad de la República, Montevideo, Uruguay

Alexei V. Lobanov
University of Nebraska - Lincoln

Vadim N. Gladyshev
University of Nebraska-Lincoln, vgladyshev1@unl.edu

Published in *Selenium: Its molecular biology and role in human health*, *Second Edition,* edited by Dolph L. Hatfield, Marla J. Berry, and Vadim N. Gladyshev. Springer Science+Business Media LLC, 2006. Pages 355–366.

CHAPTER 31

Selenoproteins in parasites

Gustavo Salinas

Cátedra de Inmunología, Facultad de Química-Facultad de Ciencias, Universidad de la República. Instituto de Higiene, Avda. A. Navarro 3051, Montevideo, CP 11600, Uruguay

Alexey V. Lobanov & Vadim N. Gladyshev

Department of Biochemistry, University of Nebraska–Lincoln, Lincoln, NE 68588-0664, USA

Summary: Parasites, which cause an enormous burden in the population of the third world, are a diverse group of organisms, many of which are sensitive to oxidative stress imposed by their hosts. In recent years, several selenoprotein families, some with antioxidant properties, have been described and characterized in metazoan parasites. Glutathione peroxidase and thioredoxin glutathione reductase (TGR) appear to be essential selenoproteins in flatworms (phylum *Platyhelminthes).* TGR is the single enzyme that provides reducing equivalents to both thioredoxin and glutathione pathways, in contrast to hosts, which evolve parallel pathways. In roundworms (phylum *Nematoda),* selenoproteins have recently been described, revealing species differences in the Sec/Cys protein sets and the presence of an unusual SECIS element. *Plasmodium* sp, one of the most important protozoan parasites that affect humans, also decode Sec. The selenoprotein families encoded by Plasmodial genomes have neither Sec nor Cys homologs in their hosts, raising the possibility that targeting their selenoproteomes may provide new treatment strategies.

Introduction

Although significant research efforts have been made to study selenoproteins and selenocysteine insertion systems in humans and various model organisms, little has been reported in the literature regarding the utilization of selenium in eukaryotic parasitic organisms. This chapter focuses on the progress made in the characterization of selenoenzyme families in flatworms, the recent advances in the synthesis and utilization of selenoproteins in roundworms and protozoan parasites, and discusses why selenoproteins of platyhelminths and plasmodia may represent interesting targets for chemo- or immune-prophylaxis.

Parasites: diverse organisms that face similar oxidative stress challenges

Parasites live at least part of their lifecycle inside another organism (the host), which they exploit for their own survival and reproduction. This definition includes different types of infectious agents (viruses, bacteria, fungi, protozoa, helminths). However, for historical reasons, the term is most often reserved for 'protozoa' and 'helminths' organisms. Indeed, parasitology was identified as a separate research field during the exploration of the tropics and the establishment of 'tropical medicine' [1]. Both 'protozoa' and 'helminths' also include free-living organisms, and neither 'protozoa' nor 'helminths' are monophyletic; on the contrary, both groups are represented by highly divergent phyla. Nonetheless, this historical classification is not useless. These two groups of parasites are very different: protozoan are unicellular protists, which multiply quickly within the host, and are, in most cases, intracellular in habitat; in contrast, helminths are metazoan organisms with complex multicellular organization (with nervous system and reproductive organs), which undergo complex metamorphoses and migrations within the host. Table 1 presents the main features of the major human parasitic infections.

In spite of the diversity of parasites, all face similar biological problems that relate to their parasitic lifestyle. Among them, the neutralization of the effector mechanisms deployed by the host immune system is of paramount importance. Resident macrophages and inflammatory-site phagocytic leukocytes (mostly neutrophils, but also monocytes and eosinophils, depending of the type of infection) are cells equipped to kill foreign organisms. They possess an oxidase system located in their plasma membrane, which becomes activated upon certain stimuli, for example, by interaction of cell receptors with antibodies bound to the foreign organism or with parasite molecular motifs (Figure la) [3]. Subsequently, 'respiratory burst' (increase in oxygen uptake not linked to respiration) takes place and produces superoxide anion and additional reactive oxygen species (ROS) [4]. Large amounts of nitric oxide ($^{\cdot}NO$) are also produced by macrophages (and to a lesser extent by neutrophils) activated by a variety of immunological stimuli, such as y-interferon and tumor necrosis factor. $^{\cdot}NO$ reacts with superoxide to produce peroxynitrite and other reactive nitrogen species (RNS) (Figure 1b) [5]. In addition, activated neutrophils and eosinophils release myeloperoxidase and eosinophil peroxidase, respectively, that catalyze the conversion of hydrogen peroxide and halides into hypohalous acids that are powerful oxidants and can form further damaging species [4].

Collectively, ROS and RNS are powerful oxidants and nitrating species: they can inactivate enzymes and initiate the process of lipid peroxidation and nitration, which leads to radical chain reactions that further damage membranes, nucleic acids and proteins (Figure 1c). These processes (and an additional arsenal of the host effector cells, such as hydrolytic enzymes) may ultimately lead to killing parasitic organisms. Yet, well-adapted parasites cope

Table 1. Major human parasites (Source: [2])

Protozoan parasites[b]		
Species (Disease)	**Phylum**	**Death per year/DALYs[a]**
Plasmodium sp (Malaria[c])	*Apicomplexa*	1,124,000/42,280,000
Trypanosoma brucei (sleeping sickness[d])	*Kinetoplastida*	50,000/1,590,000
Trypanosoma cruzi (Chagas disease[e])	*Kinetoplastida*	13,000/649,000
Leishmania sp (Leishmaniasis[f])	*Kinetoplastida*	59,000/2,357,000
Helminths parasites[g]		
Species/Disease	**Phylum**	**Death per year/DALYs**
Schistosoma sp (Schistosomiasis or bilharzia[g])	*Plathyhelminthes*	15,000/1,760,000
Onchocerca volvulus (Onchocerciasis or river blindness[h])	*Nematoda*	0/987,000
Filariidae family (Lymphatic filariasis[i])	*Nematoda*	0/5,644,000

[a] DALYs: Disability Adjusted Life Years (the number of healthy years of life lost due to premature death and disability).

[b] Protozoan parasites include many diverse phyla, among them *Apicomplexa* and *Kinetoplastida.*

[c] Distribution: mainly confined to poorer tropical areas of Africa, Asia and Latin America. More than 90% of malaria cases and the great majority of malaria deaths occur in tropical Africa. *Plasmodium falciparum* is the main cause of severe clinical malaria and death.

[d] Distribution: 36 countries in sub-Saharan Africa

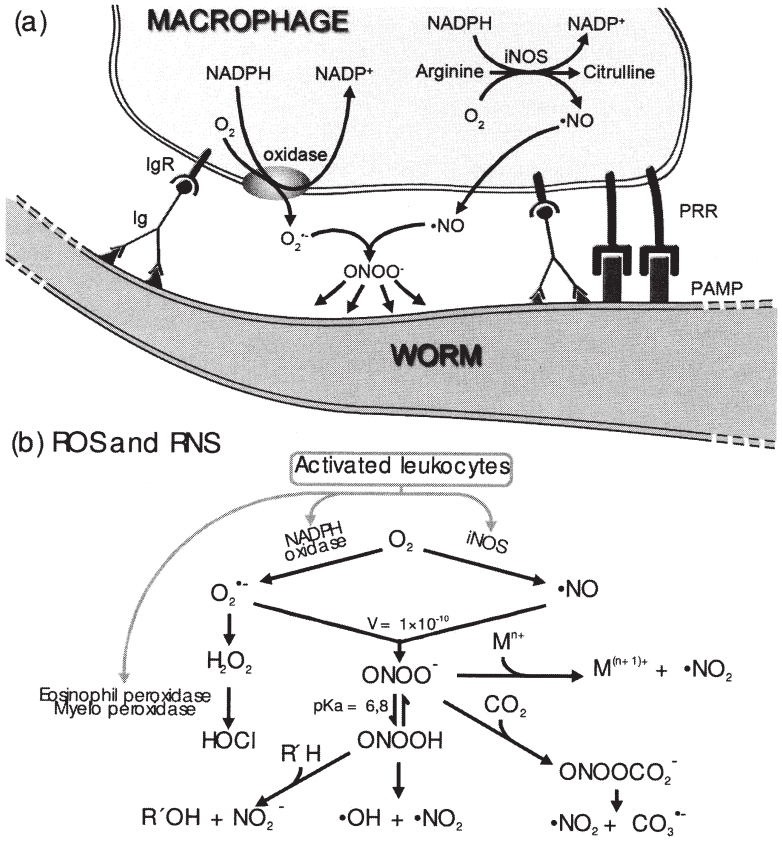
[e] Distribution: Latin America

[f] Distribution: Endemic in 88 countries on 4 continents. Two forms of the disease: cutaneous (caused by *Leishmania major),* and visceral (caused by *L. donovani)*

[g] Helminth parasites are contained in three phyla: *Nematoda* (roundworms), *Platyhelminthes* (flatworms) and *Acantocephala* (spiny-headed worms). Helminth infections are rarely fatal, but pose an enormous burden to human population in the tropics Distribution: endemic in 74 developing countries with more than 80% of infected people living in sub-Saharan Africa

[h] Distribution: 35 countries in total. 28 in tropical Africa, where 99% of infected people live. Isolated foci in Latin America and Yemen.

[i] Distribution: Endemic in over 80 countries in Africa, Asia, South and Central America and the Pacific Islands. Three species are of significance, *Wuchereria bancrofti, Brugia malayi,* and *Brugia timori.*



with the oxidative stress imposed by the host's immune response by a series of cellular chemicals and antioxidant enzymes that directly neutralize ROS and RNS (Figure Id), and constitute important model organisms to study antioxidant defense. Several antioxidant enzymes found in parasites belong to selenoprotein families.

Glutathione peroxidase: the first selenoenzyme described in parasites

Glutathione peroxidase was the first selenoenzyme to be characterized from a parasite. A cDNA from the platyhelminth *Schistosoma mansoni* encoding a GPx with a TGA in-frame at the active site was cloned in the early 1990s [6]. The protein encoded by this gene has biochemical properties similar to mammalian phospholipid hydroperoxide glutathione peroxidase (PHGPx); its activity being higher with phosphatidyl choline hydroperoxide and other phospholipid hydroperoxides than with hydroperoxide substrates, such as cumene hydroperoxide and hydrogen peroxide [7]. GPx and superoxide dismutase, another antioxidant enzyme, co-localize in the tegument and gut epithelium of adult worms, which are the exposed interfaces of the parasite towards the host [8]. Additional evidence suggests that antioxidant enzymes, and GPx in particular, are vital for ROS neutralization and parasite survival within the host. Indeed, expression of GPx is developmentally regulated,

Figure 1. Reactive oxygen and nitrogen species generated by the host immune response and antioxidant defenses, (a) Recognition of parasites by host leukocytes (such as macrophages, neutrophils and eosinophils) occurs by pattern recognition receptors (PRR) that bind to pathogen-associated molecular patterns (PAMPs), or through antibodies (Ig), and leads to activation of host immune cells. Upon activation, these cells produce superoxide ($^{\cdot}O_2^-$) and nitric oxide ($^{\cdot}NO$) radicals. $^{\cdot}NO$ is produced in the cytosol (but can cross membranes) by inducible nitric oxide synthase (iNOS); $^{\cdot}O_2^-$ is produced by a multi-component, membrane-associated NADPH oxidase. Superoxide is released towards the extracellular space in the case of non-phagocytosable parasites *(e.g.,* worms), or towards the phagosome (topologically equivalent to the extracellular space) in the case of intracellular parasites *(e.g.,* protozoans). (b) $^{\cdot}NO$ and $^{\cdot}O_2^-$ react at diffusible controlled rate to produce peroxynitrite ($ONOO^-$). Peroxynitrite can react in one-electron oxidations *(e.g.,* with transition metal centers), two electrons oxidations (of a given target), or with CO_2, redirecting its reactivity. It also decomposes spontaneously into other ROS and RNS such as $^{\cdot}OH$ and $^{\cdot}NO_2$. In addition, activated neutrophils and eosinophils release myeloperoxidase and eosinophil peroxidases, respectively, which catalyze the conversion of hydrogen peroxide and halides into hypohalous acids. (c) Collectively, these products can inactivate enzymes, damage membranes and nucleic acids, and ultimately kill the parasitic organisms. (D) Parasites' defenses include antioxidant enzymes that directly scavenge superoxide, decreasing peroxynitrite formation (superoxide dismutases), and hydrogen and organic peroxide reductases (GPx and TPx). Some TPx have also been shown to reduce peroxynitrite catalytically. Repair mechanisms include methionine sulfoxide reductase, thioredoxin, and sulfiredoxin among others. *R'H denotes a hydrocarbon chain, or alcohol (R'H = ROH), or a thiol R'H = RSH)

with the highest levels present in the adult worm [8], the stage most resistant to oxidative stress and immune elimination [9]. In addition, GPx expression is upregulated by hydrogen peroxide and xanthine/xanthine oxidase generated ROS [10].

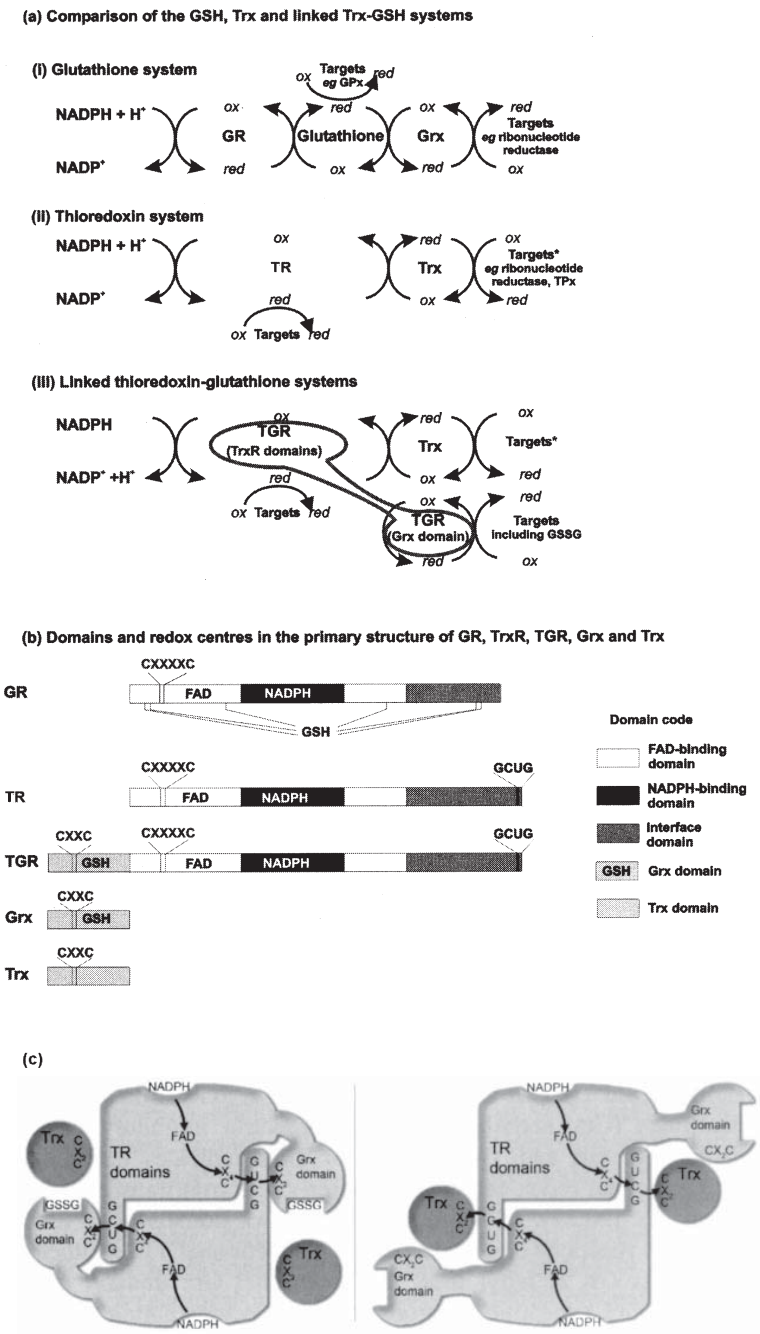
Recently, a search for GPx in Expressed Sequence Tag databases (dbEST) of platyhelminths identified a second GPx (GPx2) in *S. mansoni* and *S. japonicum* [11]. GPx2 also encodes a Sec residue at the active site and possesses an N-terminal signal peptide, which targets this isoform to the extracellular compartment, suggesting that this secreted variant would be important for extracellular hydroperoxide removal, helping to protect the parasite in its immediate environment. In this study, a GPx1 ortholog whose 3'-untranslated region revealed the presence of a SECIS element was also identified in *Echinococcus granulosus* (another flatworm) transcriptome using the SECISearch algorithm (Chapter 9 and http://genome.unl.edu/SECISearch.html) [12].

In contrast to platyhelminths, the corresponding Cys-containing enzymes appear to occur in nematodes [13], as reviewed in [14]. Nevertheless, recent data-mining of nematode dbEST revealed some exceptions (see below) [15]. Free-living nematode *Caenorhabditis elegans* has no Sec-containing GPx encoded in its genome [15].

GSH- and Trx-reduction pathways in platyhelminth parasites are controlled by a single selenoenzyme

In most living organisms, there are two analogous and mutually supporting enzymic systems that provide antioxidant defense to cells: the glutathione (GSH) and the thioredoxin (Trx) systems (Figure 2) [16,17]. These systems have overlapping yet distinct targets. GSH, due to its reactivity and intracellular concentration, is one of the most important cellular antioxidants, being efficient in rescuing small disulfide molecules and in reacting directly with ROS. The major function of Trx is to maintain cysteine residues in substrate proteins in the reduced form. In addition to their direct function as antioxidants, GSH and Trx provide electrons to GPx and Trx peroxidase (TPx), respectively, which reduce hydrogen peroxide and organic hydroperoxides, and to methionine sulfoxide reductase, which is also an important antioxidant repair enzyme. GSH and Trx are usually reduced by GSH and Trx reductases (GR and TR), respectively, at the expense of NADPH oxidation.

Recent characterization of these systems in platyhelminth parasites has shown that 'conventional' GR and TR are absent; instead, the GSH and Trx systems are intermingled with the enzyme thioredoxin glutathione reductase (TGR), which provides reducing equivalents to both pathways (Figure 2).



This protein is a second selenoenzyme family that has been characterized in platyhelminth parasites (reviewed in [11]). TGR is an oxidoreductase shown to possess TR, GR and Grx activities, achieving its broad substrate specificity by a fusion between Grx and TR domains (Figure 2b); this domain fusion was originally described in a mouse testis TGR [18].

Experimental and *in silico* data support the proposition that TGR is the single enzyme responsible for recycling both oxidized Trx and GSH in platyhelminth parasites. Treatment of *S. mansoni* adult worm extracts with auranofin, a known inhibitor of Sec-containing TRs, resulted in complete inhibition of TR and GR activities [19]. In addition, TGR was the single protein isolated from *Taenia crassiceps* (also a flatworm) extracts as a result of tracing GR and TR activities [20]. Examination of EST databases from *Schistosoma* species, which covers more than 90% of the gene content of this organism [21], revealed cDNAs encoding TGR, but not conventional TR or GR [11]. The biochemical characterization of *E. granulosus* and *T. crassiceps* TGR indicated that the native enzyme shuttles electrons from NADPH to oxidized Trx (TR activity), GSSG (GR activity) and glutathione-mixed disulfides (Grx activity). The stoichiometric inhibitory effect of auranofin on both GR and TR activities of TGR indicates that the Sec-containing C-terminal redox center participates in electron transfer to GSSG and oxidized Trx [20,22]. In addition, TR and Grx domains can function either in coupled reactions or independently. Conventional TRs neither bind GSH nor possess GR activity; thus, the N-terminal Grx domain of TGR would reduce GSSG, accepting electrons

Figure 2. Linked thioredoxin-glutathione systems, (a) Comparison of thioredoxin, glutathione and linked thioredoxin-glutathione systems. The glutathione system comprises (i) GR, GSH and Grx, whereas the thioredoxin system consists of (ii) TR and Trx. In linked Trx-GSH systems (iii), TGR functionally replaces TR, GR and Grx, providing reducing equivalents to targets of both systems. In all systems, NADPH is the upstream donor of reducing equivalents. (b) Components of the thioredoxin and glutathione systems. Redox centers of GR, TR, TGR, Grx and Trx are indicated, as well as the FAD prosthetic group and the ligands NADPH and GSH. TR and TGR possess a C-terminal extension missing in GR, which contains the C-terminal GCUG redox-active motif. TGR possesses an N-terminal Grx domain that is absent in TR and GR. The Grx and Trx domains contain the CXXC redox center. Grx, unlike Trx, binds GSH. (c) Schematic representation of electron flow in TGR. TGR, like GR and TR, is a homodimer, with monomers oriented in a head-to-tail manner. Electrons flow from NADPH to FAD, to the CX_4C redox center, to the C-terminal GCUG redox center of the second subunit, to the CX_2C redox center of the Grx domain of the first subunit, and to targets, including GSSG (left scheme). Alternatively, electrons can flow, presumably directly, from the GCUG redox center to Trx (right scheme). The model proposes a flexible hinge, which connects the TR and Grx domains. This organization allows electrons to flow to the 'in built' Grx domain or to Trx. Parts (a) and (b) in the figure reprinted with modifications from [11] with copyright with permission from Elsevier.

from the Sec-containing C-terminal redox center. The idea that the C-terminal redox center donates electrons to the fused Grx domain implies that the Grx domain of TGR would be linked to the TR domains by a flexible hinge to allow reduction of the oxidized Trx (Figure 2c). It is interesting to note that *T. crassiceps* TGR showed a hysteretic behavior in enzymatic assays with GSSG at high concentrations; this observation led the authors to propose a model in which TGR would possess high and low affinity sites for glutathione [20]. Clearly, further biochemical characterization and structural data on this multifunctional enzyme are needed that will shed light on the mechanism of catalysis. In addition, molecular characterization of the corresponding gene could also provide clues regarding the mechanism of generation of isoforms. Indeed, the analysis of TGR in *E. granulosus* revealed two trans-spliced cDNAs derived from a single gene [22]. These variants code for mitochondrial (mt) and cytosolic (c) TGRs, containing identical Grx and TrxR domains, but differing in their N-termini. These variants derive from alternative initiation of transcription, followed by trans-splicing. Similarly, mtTGR and cTGR variants also derived from a single gene have been identified in *S. mansoni* [11].

Collectively, the results from platyhelminth studies strongly suggest that TGR is the main pyridine-nucleotide thiol-disulfide oxidoreductase in these organisms, in contrast to their hosts, where there is some redundancy of mechanisms for recycling oxidized Trx and GSH.

Very little has been published about these pathways in the other phylum of helminth parasites (*Nematoda*), and to the best of our knowledge, nothing is known about Sec/Cys-containing TR or TGR in parasitic nematodes. However, no single genome has yet been completed from metazoan parasites.

Selenoproteins of nematode parasites: old families, unusual SECIS

An *in silico* analysis of *Caenorhabditis elegans* and *Caenorhabditis briggsae* (free-living nematodes) genomes revealed that these organisms encode a single a selenoprotein, TR [15], corroborating earlier experimental data [23]. However, no experimental studies have yet been performed with Selenoproteins from parasitic nematodes. Nevertheless, in a recent study [15], the existing nematode ESTs were searched for selenoprotein genes using SECISearch and by screening for homologs of known Selenoproteins. These analysis identified selenoprotein homologs of selK, selT, selW, Sep15, selenophosphate synthetase and GPx. Two interesting points were noted from these analyses. First, various nematodes encode different selenoproteins, and the distribution of selenoprotein families within this phylum is mosaic. Second, it was found that all detected nematode selenoprotein genes contained an unusual form of SECIS element, with G rather than a canonical A at the conserved position preceding the quartet of non-Watson-Crick base pairs [15].

Selenoproteins of protozoan parasites: waiting for surprises?

Very little is known about selenoproteins from protozoan parasites. Recently, the presence of $tRNA^{Sec}$ was described in several species of the phylum *Apicomplexa* [24] (Lobanov et al., submitted). *Plasmodium falciparum,* which is the causative agent of malaria—the most overwhelming human parasitic infection, belongs to this phylum. The finding of $tRNA^{Sec}$ was consistent with the presence of putative EFsec and selenophosphate synthetase in *P. falciparum* and other Plasmodia. In addition, $tRNA^{Sec}$ was observed in *Toxoplasma,* but not in *Cryptosporidium* parasites. Genome-wide searches for SECIS elements in the six *Plasmodium* genomes revealed four selenoprotein genes. Interestingly, homology analyses of these proteins identified no hits outside Apicomplexa, suggesting that these selenoproteins do not exist in the apicomplexan hosts. These properties make the new selenoproteins attractive targets for anti-malaria drug development.

The other reference in the literature to a parasite Sec-decoding protozoan is the description of a Cys-containing selenophosphate synthetase from *Leishmania major* [25]. *Leishmania* belongs to *trypanosomatidae* family, which also includes *Trypanosoma brucei,* and *T. cruzi* (Table 1), which are causative agents of disabling and fatal diseases in the poorest rural population of the third world [26]. Consistent with the finding of selenophosphate synthetase, recent bioinformatics analyses revealed three selenoprotein genes in several *Trypanosoma* genomes (Lobanov and Gladyshev, unpublished).

Finally, no single reference could be found in the literature regarding a Sec-decoding amoebae, a traditional group of protozoa that include the parasitic amoebae of humans, *Entamoebae histolytica.*

Parasite selenoproteins: drug or vaccine candidates?

From a global perspective, the control of parasitic infections, which are a major cause of disability and mortality in many developing countries, remains as one of the most important challenges for medicine in the 21st century [2]. Although there are safe and effective drugs to control some parasitic diseases, parasites can develop resistance to drugs rendering them ineffective, as it has been the case of certain antimalarial drugs [27]. Thus, effective vaccines and new drugs against parasitic organisms are needed. The task ahead is enormous considering that parasite and hosts are eukaryotic organisms; as yet, there is not a single vaccine for a human parasitic infection. Whether selenoproteins can be drug targets or generate immunity depends on premises that are not necessarily different from those for any other target protein: the validity of a drug target would rely on it being an essential protein, and sufficiently different from the host homolog(s) as to be selectively inhibited. Likewise, a good vaccine candidate should generate an appropriate and selective immune response against the parasite, without inducing pathology to the host.

In platyhelminths, TGR is an attractive pharmacological target because of the lack of redundant mechanisms (i.e., TR and GR) to provide reducing equivalents to essential enzymes. Inhibition of this enzyme could lead to impaired synthesis of DNA and antioxidant defenses, compromising parasite survival. TGR may also be a good vaccine candidate, since it is a large protein with a degree of identity to host enzymes below 60%. However, there are no studies regarding TGR as an immunogen. Contrary to TGR, there are promising studies on the use of GPx as a vaccine candidate. Vaccination of mice (not a natural host) against the platyhelminth *S. mansoni* with naked DNA constructs containing Sec-containing GPx showed significant levels of protection compared to a control group [28]. In this context, it is important to emphasize not only the fact that GPx appears to be important at the host parasite interface, but also that platyhelminth lack catalase and rely exclusively on GSH and Trx peroxidases for hydrogen peroxide removal.

In the case of protozoan parasites, further studies are needed to identify and functionally characterize their selenoproteins. Nevertheless, it is highly significant that the four selenoproteins identified in *Plasmodium sp* have neither Sec nor Cys homologs in humans. Considering that Sec is usually located at the redox-active sites of enzymes, the selenol- and thiol-based redox systems may play vital an important role in the survival of protozoan parasites [29].

Finally, selenoproteins may be different to other proteins in one respect: electrophilic drugs, such as gold or platinum compounds, or alkylating agents that react preferentially with Sec over Cys may affect the parasite and the host to a different extent, depending on the relative importance of selenoproteins for the two organisms, and the presence/absence of Cys-containing enzymatic back up systems.

Acknowledgements

This work has been supported by Fogarty International Research Collaboration Award TW006959 and Ministry of Education, Uruguay, PDT 29/171.

References

1. K Warren 1988 *The Biology of Parasitism* PT Englund, A Sher (Ed) Alan R. Riss Inc New York 3
2. WHO The world health report – changing history. 2004 (http://www.who.int/whr/2004/en/report04_en.pdf)
3. DH McGuinness, PK Dehal, RJ Pleass 2003 *Trends Parasitol* 19:312
4. BG Halliwell, JMC Gutteridge 1999 *Free Radicals in Biology and Medicine* Oxford University Press Inc New York
5. R Radi, G Peluffo, MN Alvarez, M Naviliat, A Cayota 2001 *Free Radic Biol Mod* 30:463

6. DL Williams, RJ Pierce, E Cookson, A Capron 1992 *Mol Biochem Parasitol* 52:1277. M Maiorino, C Roche, M Kiess, K Koenig, D Gawlik, M Matthes, E Naldini, R Pierce, L Flohe 1996 *Eur J Biochem* 238:838

8. H Mei, PT LoVerde 1997 *Exp Parasitol* 86:69

9. GM Mkoji, JM Smith, RK Prichard 1988 *Int J Parasitol* 18:661

10. UE Zeick, B Von Janowsky 2004 *Parasitology* 128:493

11. G Salinas, ME Selkirk, C Chalar, RM Maizels, C Femandez 2004 *Trends Parasitol* 20:340

12. GV Kryukov, VM Kryukov, VN Gladyshev 1999 *J Biol Chem* 274:33888

13. L Tang, K Gounaris, C Griffiths, ME Selkirk 1995 *J Biol Chem* 270:18313

14. K Henkle-Duhrsen. A Kampkotter 2001 *Mol Biochem Parasitol* 114:129

15. K Taskov, C Chappie, GV Kryukov, S Castellano, AV Lobanov, KV Korotkov, R Guigo, VN Gladyshev 2005 *Nucleic Acids Res 2005* 33:2227

16. A Holmgren 2000 *Antioxid Redox Signal* 2:811

17. PG Winyard, CJ Moody, C Jacob 2005 *Trends Biochem Sci* 30:453

18. QA Sun, L Kimarsky, S Sherman, VN Gladyshev 2001 *Proc Natl Acad Sci USA 2001* 98:3673

19. HM Alger, AA Sayed, MJ Stadecker, DL Williams 2002 *Int J Parasitol* 32:1285

20. JL Rendon, IP del Arenal, A Guevara-Flores, A Uribe, A Plancarte, G Mendoza-Hernandez 2004 *Mol Biochem Parasitol* 133:61

21. S Verjovski-Almeida, R DeMarco, EA Martins, PE Guimaraes, EP Ojopi, AC Paquola, JP Piazza, MY Nishiyama, Jr., JP Kitajima, RE Adamson, PD Ashton, MF Bonaldo, PS Coulson, GP Dillon, LP Farias, SP Gregorio, PL Ho, RA Leite, LC Malaquias, RC Marques, PA Miyasato, AL Nascimento, FP Ohiweiler, EM Reis, MA Ribeiro, RG Sa, GC Stukart, MB Scares, C Gargioni, T Kawano, V Rodrigues, AM Madeira, RA Wilson, CF Menck, JC Setubal, LC Leite, E Dias-Neto 2003 *Nat Genet 2003* 35:148

22. A Agorio, C Chalar, S Cardozo, G Salinas 2003 *J Biol Chem* 2003 Apr 11 278: 12920

23. VN Gladyshev, M Krause, XM Xu, KV Korotkov, GV Kryukov, QA Sun, BJ Lee, JC Wootton, DL Hatfield 1999 *Biochem Biophys Res Commun* 259:244

24. T Mourier, A Pain, B Barrell, S Griffiths-Jones 2005 *RNA* 11:119

25. PC Jayakumar, VV Musande, YS Shouche, MS Patole 2004 *DNA Seq* 15:66

26. CM Morel, T Acharya, D Broun, A Dangi, C Elias, NK Ganguly, CA Gardner, RK Gupta, J Haycock, AD Heher, PJ Hotez, HE Kettler, GT Keusch, AF Krattiger, FT Kreutz, S Lall, K Lee, R Mahoney, A Martinez-Palomo, RA Mashelkar, SA Matlin, M Mzimba, J Oehler, RG Ridley, P Senanayake, P Singer, M Yun 2005 *Science* 309:401

27. TE Mansour *Chemotherapeutic Targets in Parasites: Contemporary strategies* 2002 T Mansour (ed) Cambridge University Press Cambridge 4

28. KA Shalaby, L Yin, A Thakur, L Christen, EG Niles, PT LoVerde 2003 *Vaccine* 22:130

29. S Muller, E Liebau, RD Walter, RL Krauth-Siegel 2003 *Trends Parasitol* 19:320