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Linked thioredoxin-glutathione systems in platyhelminths

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The thioredoxin and glutathione systems play a central role in thiol-disulfide redox homeostasis in many organisms by providing electrons to essential enzymes, and defence against oxidative stress. These systems have recently been characterized in platyhelminth parasites, and the emerging biochemical scenario is the existence of linked processes with the enzyme thioredoxin glutathione reductase supplying reducing equivalents to both pathways. In contrast to their hosts, conventional thioredoxin reductase and glutathione reductase enzymes appear to be absent. Analysis of published data and expressed-sequence tag databases indicates the presence of linked thioredoxin–glutathione systems in the cytosolic and mitochondrial compartments of these parasites.

The reversible thiol-disulfide reaction is a central chemical theme in biology. It is used as a general strategy to accept and donate electrons, and as a molecular device to control protein function. The thioredoxin (Trx) and glutathione (GSH) systems are the major thiol-dependent redox pathways present in cells. Both systems have overlapping yet distinct properties, but function in a similar manner, maintaining cellular redox homeostasis and providing defence against oxidative stress by: (i) reducing oxidized compounds; and (ii) supplying reducing equivalents to peroxide and sulfoxide reductases (Figure 1a) [1]. A molecular link has recently been described in thioredoxin glutathione reductase (TGR), a complex enzyme which is capable of shuttling electrons from NADPH to targets of both systems, via the fusion of glutaredoxin (Grx) and thioredoxin reductase (TrxR) domains into a single protein [2].

Parasites that cause chronic infections, such as helminths, are model organisms to study antioxidant defences because they are not only subjected to endogenous oxidants, but can also be placed under oxidative stress by host cells [3]. Recently, the existence of linked Trx–GSH systems has been demonstrated in platyhelminths [4–6]. Selenocysteine (Sec, see Box 1)-containing TGR appears to be the major oxido-reductase, which transfers electrons to glutathione disulfide (GSSG) and Trx in these organisms, functionally replacing both TrxR and glutathione reductase (GR).

The thioredoxin system

The Trx system comprises the oxido-reductases Trx and TrxR (Figure 1a). Trx contains a redox-active site with cysteine residues in close proximity (CXXC). Trx is a potent reductase of protein disulfides that transfers electrons to a variety of cellular proteins [7]. One essential function of Trx is to provide reducing equivalents to enzymes containing redox-active cysteine residues as part of their catalytic cycle, such as ribonucleotide reductase, the catalyst for the synthesis of deoxynucleotides from ribonucleotides, and thioredoxin peroxidase (TPx), a hydrogen peroxide reductase that belongs to the 2-Cys peroxiredoxin family [8]. Trx also regulates the activities of various transcription factors, kinases and phosphatases in mammalian cells, and serves specific functions in different organisms [9,10].

Reduction of disulfide bonds in various substrates leads to oxidation of the Trx-active site. TrxR (EC 1.8.1.9) catalyzes the NADPH-dependent reduction of the redox-active disulfide of Trx. Unlike Trx, two forms of TrxR, low-M_r TrxR and high-M_r TrxR (in reference to their relative molecular mass), which differ in their catalytic mechanisms, have evolved by convergent evolution [11–13]. Low-M_r TrxRs are typical of prokaryotes, plants, fungi and some protists, whereas high-M_r TrxRs are typical of metazoans, but are also present in some protists [14,15]. High-M_r TrxRs are homodimeric proteins with monomers oriented in a head-to-tail manner [12]. Each monomer has: (i) a domain that binds FAD and contains the CXXXXC redox-active site; (ii) a domain that binds NADPH; and (iii) an interface domain with a C-terminal extension containing an additional redox-active centre. The C-terminal extension possesses redox-active cysteines in Diptera [16] and *Plasmodium* spp. [17], or Cys and Sec in mammals [18]. Electrons flow from NADPH to FAD, the adjacent CXXXXC redox centre, and to the peripheral C-terminal redox centre of the second subunit. This C-terminal centre is within a flexible arm that, once reduced, transfers the electrons to oxidized Trx [12,19] (Figure 1b).

High-M_r TrxRs have a broad range of substrate specificities as a result of the structural flexibility of the

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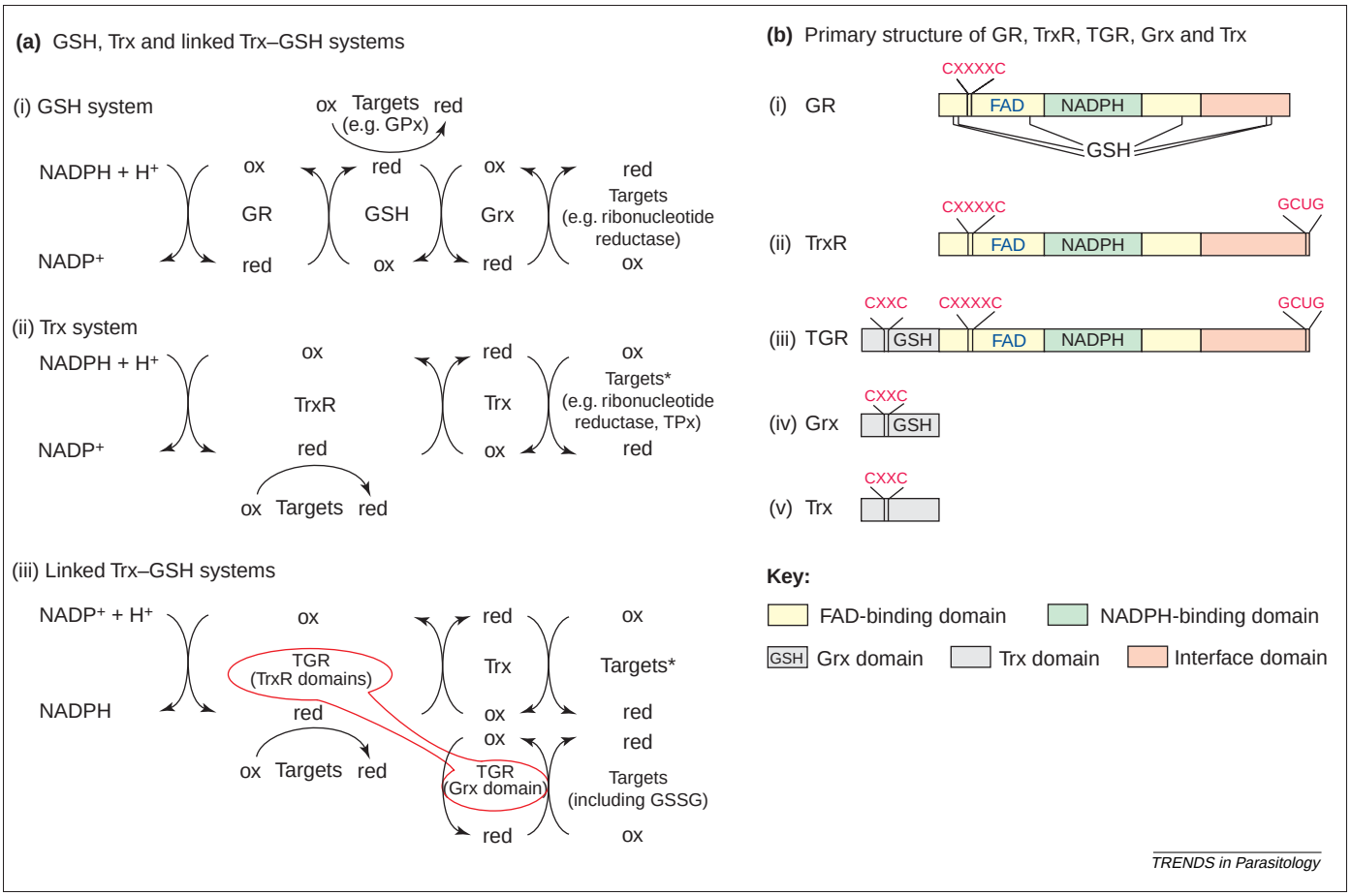

Figure 1. GSH, Trx and linkec Trx–GSH systems. **(a)** Electron flow in the Trx, GSH and linked Trx–GSH systems. The GSH system (i) comprises GR, GSH and Grx, whereas the thioredoxin system (ii) comprises TrxR and Trx. In linked Trx–GSH systems (iii), TGR functionally replaces TrxR, GR and Grx, and provides reducing equivalents to targets of both systems. The fusion between Grx and TrxR domains in TGR is highlighted in red. In all systems, NADPH is the upstream donor of reducing equivalents. *Targets of reduced Trx include GSSG, which might play a role in cells with increased GSSG levels, but inadequate GR activity [46]. **(b)** Primary structure of GR, TrxR, TGR, Grx and Trx. Redox centres of GR, TrxR, TGR, Grx and Trx are indicated in red, the FAD prosthetic group is indicated in blue, and the ligands NADPH and GSH are indicated in black. The GSH-binding site is discontinuous in GR. TrxR and TGR possess a C-terminal extension missing in GR, which contains the GCUG redox-active motif (U denotes selenocysteine). TGR possesses an N-terminal Grx domain, which is absent in TrxR and GR. The Grx and Trx domains belong to the Trx fold, and contain the CXXC redox centre. Grx, unlike Trx, binds GSH. GR, TrxR, and TGR are homodimers, with monomers oriented in a head-to-tail manner. For GR, electrons flow from NADPH to FAD, to the CXXXXC redox centre, and finally to GSSG (the binding site of which is formed by residues of both subunits). For TrxR, electrons flow from NADPH to FAD, to the C-terminal GCUG redox centre of the second subunit via the CXXXXC redox centre, and finally to TrxR targets (e.g. Trx) [12]. Important details of electron transfer within TGR have not yet been resolved but, based on molecular modelling, the GCUG redox centre of one subunit has been proposed to transfer electrons from the CXXXXC redox centre to the CXXC Grx domain of the second subunit and *vice versa*, and that the Grx domain would have a certain degree of flexibility to allow Trx to bind to the same binding site as Grx [2]. This model is consistent with the biochemical data described in the text. Abbreviations: GPx, glutathione peroxidase; GR, glutathione reductase, Grx, glutaredoxin; GSH, glutathione; GSSG, glutathione disulfide; ox, oxidized; red, reduced; TGR, thioredoxin glutathione reductase; TPx, thioredoxin peroxidase; Trx, thioredoxin; TrxR, thioredoxin reductase.

C-terminal redox centre and of the presence of Sec. The selenol group of Sec is a stronger nucleophile than the thiol group of Cys [20]. In addition to Trx, they can reduce several biologically relevant antioxidant molecules including lipid hydroperoxides, dehydroascorbate (the oxidized form of vitamin C), vitamin K3, lipoic acid, ubiquinone and selenium-containing compounds [10,21]. Thus, Sec-containing TrxRs are thought to be central enzymes for protection against oxidative stress, acting directly on oxidized substrates, in addition to providing electrons to Trx and TPx. TrxR and Trx are both present in the cytosol, and have been described in the mitochondria of eukaryotic cells [22,23].

The glutathione system

The other major thiol redox pathway widely distributed in Nature is the GSH system, which comprises GR, GSH and Grx (Figure 1a). Grx is a small thiol-disulfide oxido-reductase that belongs to the Trx family; it has a similar active site, and transfers electrons to its substrates and substrate reductases (e.g. ribonucleotide reductase) [24]. Unlike Trx, Grx contains a binding site for GSH, which recycles Grx to its reduced state. In addition to Grx, GSH reduces other proteins such as glutathione peroxidase (GPx), which in many organisms contains Sec at the active site. GSH also acts as an antioxidant *per se*, reducing oxidized substrates (e.g. dehydroascorbate) and reacting with pro-oxidant radical species such as peroxynitrite, redirecting their reactivity [25]. Furthermore, GSH reacts with hydrophobic electrophiles (a conjugation reaction catalyzed by glutathione *S*-transferase) rendering them less reactive and more hydrophilic, thus facilitating their excretion [26]. GSSG is recycled by GR (EC 1.8.1.7) via the transfer of reducing equivalents from NADPH (Figure 1a and 1b). Unlike high-M_r TrxRs, GRs do not possess a C-terminal redox-active centre (Figure 1b),

Box 1. Selenocysteine: the 21st amino acid

The amino acid selenocysteine (Sec) is co-translationally inserted into proteins at an in-frame UGA codon by a selenocysteinyl-charged transfer RNA (tRNA) [52]. Decoding of UGA for Sec requires a selenocysteine-insertion sequence (SECIS) element, a *cis*-acting stem–loop structure present in the selenoprotein mRNA, which is located at the 3′-untranslated region in eukaryotes [53]. Decoding of Sec in eukaryotes also involves a Sec-binding protein (SBP2) and a Sec-specific elongation factor (Ef-Sec, also known as mSelB) [54].

Sec incorporation into protein synthesis occurs in the three domains of life (archaea, bacteria and eukarya), although many organisms appear to have lost the decoding machinery. Co-translational incorporation of Sec is believed to have evolved once, before the division of the major domains, and has subsequently been lost in several lineages. Another intriguing fact is that, apart from selenophosphate synthetase, the sets of selenoproteins in prokaryotes and eukaryotes do not intersect [55]. Nevertheless, irrespective of their origin, in selenoproteins of known function, Sec is part of the active site and is usually involved in redox reactions [56].

Mammals express 25 different selenoproteins, 12 of unknown function [57]. In platyhelminths, the only selenoproteins of known function are glutathione peroxidase and thioredoxin glutathione reductase (see main text and Table I).

Table I. cDNAs and ESTs corresponding to components and targets of the glutathione and thioredoxin systems in platyhelminths[a]

	Echinococcus granulosus				*Echinococcus multilocularis*				*Schistosoma mansoni*				*Schistosoma japonicum*		
	cDNA	ESTs (n = 1754)[b]			cDNA	ESTs (n = 353)[b]			cDNA	ESTs (n = 139 064)[b]			cDNA	ESTs (n = 45902)[b]	
	Accession no.	Reads	Stages	EGC cluster[c]	Accession no.	Reads	Stages	EMC cluster[c]	Accession no.	Reads	Stages	SmAE cluster[c]	Accession no.	Reads	Stages
cTrx	AF034637	5	P,PP	00470	–	1	W	00215	AF473536	6	C,S,M,G	607213.1	AF091538	26	A,M,F,E
									–	1	G	713622.1	–	1	F
mtTrx	–	1	PP	01178	–	–	–	–	–	15	A,L,G	608648.1	–	3	M,F
cTPx	AF034959	11	W,P,PP	00084	AB071135	2	W	00176	AF121199[d]	12	S,A	606067.1[d]	–	29	A,M,F,E
									AF157561[d]	30	S,A,E,L,G	610878.1[d]	–	20	A,M,F,E
mtTpx	–	1	W	00918	–	–	–	–	AF301001[d]	16	A,M,F,G	606260.1[d]	–	10	M,F,E
CGPx	–	2	P	00127	–	–	–	–	M86510	39	C,S,A,M,E,L,G	610266.1	–	22	M,F,E
sGPx	–	–	–	–	–	–	–	–	–	10	A,F,L	601555.1	–	1	F
cTGR	AY147416	–	–	–	–	–	–	–	AF395822	38[e]	C,S,A,M,F,E,G	608522.1[f]	–	20[e]	M,F,L
												602609.1[f]			
												709646.1[f]			
mtTGR	AY147415	–	–	–	–	–	–	–	–	2	S,E	608522.1	–	–	–
cGrx	–	–	–	–	–	–	–	–	–	2?[g]	L	610382.1[g]	–	–	–
mtGrx	–	2	P,W	00387	–	–	–	–	–	8	A,E,G	606035.1	–	–	–

[a]The table summarizes the information available in GenBank as of 5 March 2004. Some information regarding EST initiatives in these platyhelminths has been published [58–61], additional data can be retrieved from LophDB (http://nema.cap.ed.ac.uk/Lopho/LophDB.php), *S. mansoni* database (http://bioinfo.iq.usp.br/schisto), and *S. japonicum* database (http://schistosoma.chgc.sh.cn). Abbreviations: A, mixed adults; c, cytosolic; C, cercariae; E, eggs; F, females; G, sporocyst germ balls; GPx, glutathione peroxidase; GRx, glutaredoxin; L, miracidia; M, males; mt, mitochondrial; P, protoscoleces; PP, HCl/pepsin-treated protoscoleces; s, secreted; TGR; thioredoxin glutathione reductase; TPx, thioredoxin peroxidase; Trx, thioredoxin; W, cyst wall (germinal layer).
[b]Total number of entries in dbEST.
[c]EGC and EMC cluster identification in LophDB. Assembled EST sequence (SmAE) number in the *S. mansoni* database; these clusters were derived from 124 640 ESTs [59].
[d]AF121199 and SmAE 606067.1 correspond to TPx1; AF157561 and SmAE 610878.1 to TPx2; AF301001 and SmAE 602260.1 to TPx3. An additional SmAE (716844.1; 1 read/435 bp) is annotated as a mtTPx; Blastx reveals high identity with rodent mtTPx (94% identity with rat TPx3), most likely indicating host contamination.
[e]Some ESTs might correspond to the mtTGR variant because mitochondrial and cytosolic isoforms are derived from a single gene, and most ESTs do not include the cDNA 5′-end.
[f]SmAEs corresponding to partial sequences of cTGR cDNA; they contain the regions coding for amino acids 1-234 (608522.1); 258-598 (602609.1), 3-293 (709646.1).
[g]Blastx of this SmAE (461 bp/2 reads) reveals similarity to prokaryotic Grx (highest with *Yersinia pestis* Grx3; 61% identity), probably reflecting bacterial contamination.

and the equivalent redox link is provided by GSH. Like Trx and TrxR, GSH, Grx and GR are also present in the cytosol and mitochondria [27–29].

Platyhelminths possess linked thioredoxin–glutathione systems

Paradoxically, targets rather than components of the GSH and Trx systems were first described in platyhelminths as a result of searching for hydrogen peroxide detoxification enzymes. A complementary DNA (cDNA) from *Schistosoma mansoni* encoding a Sec-containing GPx was cloned 12 years ago [30], and the corresponding protein was demonstrated to reduce hydrogen peroxide and phospholipid hydroperoxides [31]. Later studies identified the existence of TPx cDNAs in *Fasciola hepatica*, *Echinococcus granulosus* and *S. mansoni* [32–34], and subsequent work showed that recombinant TPx reduced hydrogen peroxide and protected cellular components from metal-catalyzed oxidations [34–36]. The Trx was then cloned and shown to be the electron donor for TPx in *F. hepatica*, *E. granulosus* and *S. mansoni* [37–39].

The characterization of these thiol-disulfide redox pathways has revealed a common biochemical scenario for platyhelminths in which the GSH and Trx systems are linked by the Sec-containing enzyme TGR. TGR is an oxido-reductase that possesses TrxR, GR and Grx activities, achieving its broad range of substrate specificities by a fusion between Grx and TrxR domains [2] (Figure 1b). Originally described as a gene expressed in mammalian testes, TGR homologues have been detected in *S. mansoni* [4], *E. granulosus* [5] and *Taenia crassiceps* [6], where TGR appears to be the single enzyme responsible for recycling both Trx and GSH.

Treatment of *S. mansoni* adult worm extracts with auranofin, a potent inhibitor of Sec-containing TrxR and some other Sec-containing enzymes, resulted in complete inhibition of TrxR and GR activities [4]. Antibodies raised against *S. mansoni* TGR bound a single protein in parasite extracts and removed >90% of TrxR and GR activities in immunoprecipitation assays [4]. In addition, recent data showed that TGR was the single protein isolated from

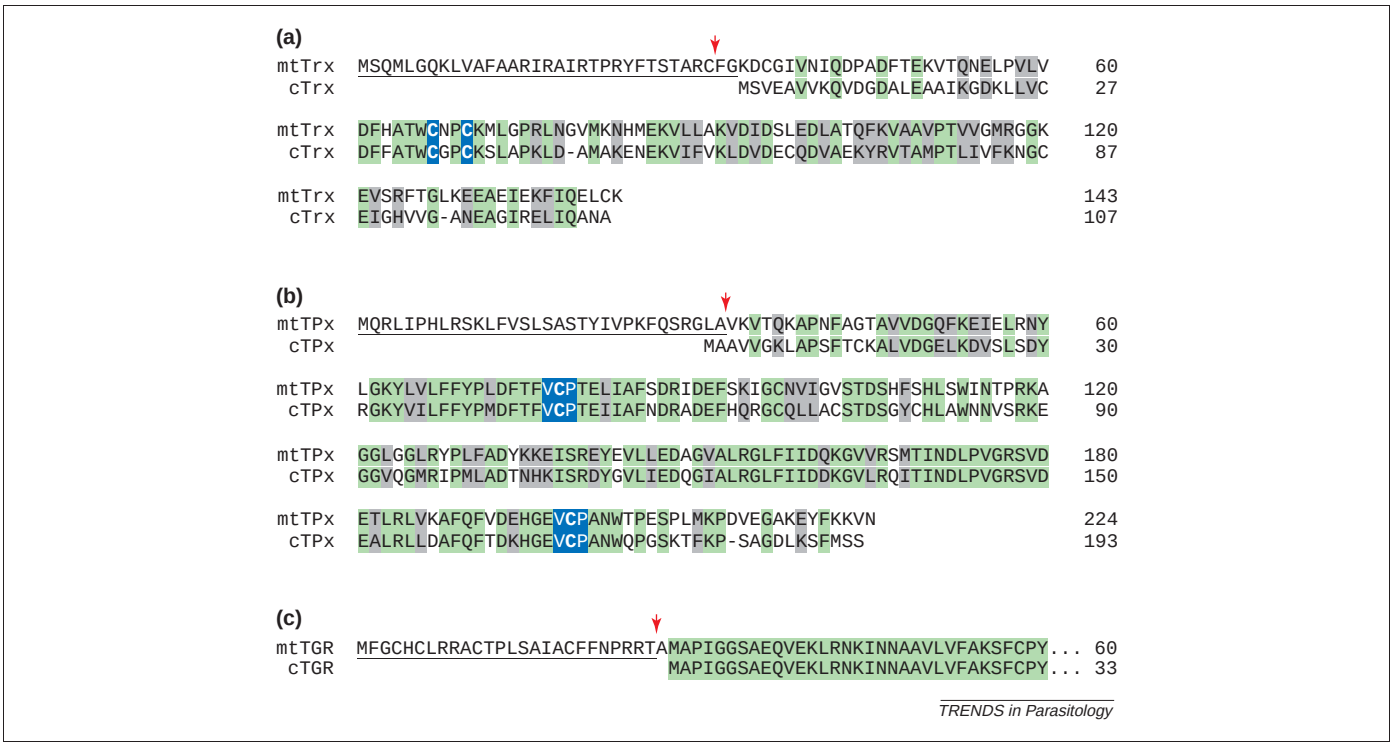

Figure 2. Mitochondrial and cytosolic thioredoxins, thioredoxin peroxidases and thioredoxin glutathione reductases from *Echinococcus granulosus*. Alignments of the amino acid sequences predicted for *E. granulosus* Trx **(a)** and TPx **(b)** variants are shown, with an alignment of the N-terminal regions of the TGR isoforms **(c)**. The cTrx, cTPx, cTGR and mtTGR were translated from corresponding complementary DNA (AF034637, AF034959, AY147416 and AY147415), whereas mtTrx and mtTPx were defined by ESTs from EGC01178 and EGC00918 clusters in LophDB (http://nema.cap.ed.ac.uk/Lopho/LophDB.php). Identical amino acids are indicated in green, conservative substitutions in grey, and the active sites of Trx and TPx in blue. Underlined sequences contain elements typical of mitochondrial import signals; their predicted cleavage sites are indicated by red arrows (http://www.cbs.dtu.dk/services/TargetP). Abbreviations: cDNA, complementary DNA; cTGR, cytosolic thioredoxin glutathione reductase; cTPx, cytosolic thioredoxin peroxidase; cTrx, cytosolic thioredoxin; EST, expressed-sequence tag; mtTGR, mitochondrial thioredoxin glutathione reductase; mtTPx, mitochondrial thioredoxin peroxidase; mtTrx, mitochondrial thioredoxin.

T. crassiceps extracts as a result of tracing GR and TrxR activities [6].

Studies on *E. granulosus* TGR indicated that the native enzyme shuttles electrons from NADPH to oxidized Trx (TrxR activity), GSSG (GR activity) and glutathione–mixed disulfides (Grx activity) [5]. A stoichiometric amount of auranofin inhibited TrxR, GR and Grx activities, suggesting that the Sec-containing C-terminal redox centre participates in electron transfer to the different substrates. The TrxR domains and the Grx domain can function either coupled to each other or independently because the Grx domain can accept electrons from the TrxR domains or from exogenous GR and GSH. Likewise, the TrxR domains can donate electrons to the fused Grx domain or to Trx. Conventional TrxRs do not bind GSH or possess GR activity. Thus, it is reasonable to speculate that reduction of GSSG is carried out by the N-terminal Grx domain of TGR, which accepts electrons from the Sec-containing C-terminal redox centre [5].

Collectively, the results from cestodes and trematodes strongly suggest that TGR is the main pyridine-nucleotide thiol-disulfide oxido-reductase in platyhelminths. This proposition appears to be reinforced by examination of expressed-sequence tags (ESTs) from these organisms (see Table I). cDNAs encoding conventional TrxR or GR have not been identified to date in *S. mansoni, Schistosoma japonicum, E. granulosus* or *Echinococcus multilocularis*. TGR has also been found to be expressed in *F. hepatica* (C. Carmona, pers. commun.).

Thioredoxin–glutathione systems in the cytosol and mitochondria

The molecular characterization of TGR in *E. granulosus* revealed two *trans*-spliced cDNAs derived from a single gene [5]. These variants code for mitochondrial (mtTGR) and cytosolic (cTGR) TGRs, which contain identical Grx and TrxR domains, but differ in their N-termini. Examination of the recent *S. mansoni* EST database (http://bioinfo.iq.usp.br/schisto/) identified mtTGR and cTGR variants also derived from a single gene (Table I).

The presence of TGR in both compartments raises the question of the existence of targets in these sites. The previously characterized *E. granulosus* Trx and TPx lack N-terminal signal peptides and are thus presumed to be cytosolic [33,37]. Examination of the *E. granulosus* EST database (http://nema.cap.ed.ac.uk/Lopho/pg_anno.php) revealed additional cDNAs corresponding to Trx and TPx variants with putative N-terminal mitochondrial sorting signals (Figure 2). In the case of *S. mansoni*, cDNAs coding for three TPx variants have been reported (Table I): (i) TPx1, the gene product of which has been characterized; (ii) TPx2 which is predicted to be cytosolic; and (iii) TPx3 which contains a N-terminal mitochondrial sorting signal. Scrutiny of *S. japonicum* ESTs identified three TPx sequences orthologous to those from *S. mansoni*. ESTs coding for three Trx are also present in *S. mansoni* and *S. japonicum* transcriptomes. Two are predicted to be cytosolic and the third as mitochondrial (Table I). The closest identity of platyhelminth mtTrx and mtTPx is with

mammalian mitochondrial proteins and not with parasite cytosolic variants. Thus, despite the lack of studies on subcellular localization, these parasites most likely possess Trx and TPx, in addition to functional TGR, in the cytosol and mitochondria.

Parasite databases were also searched for cytosolic and mitochondrial variants of Grx and GPx. ESTs corresponding to a putative Grx with a mitochondrial translocation signal and a C-terminal extension were identified in *E. granulosus* and *Schistosoma* spp. (Table I). Concerning GPx, ESTs with highest similarity to the previously characterized *S. mansoni* cGPx were found in *S. japonicum* and *E. granulosus*. Inspection of GPx cDNAs with an algorithm allowing *in silico* identification of selenoproteins (http://genome.unl.edu/SECISearch.html) [40] identified a putative SECIS (see Box 1), predicting that these cDNAs encode Sec-containing GPx.

The physiological role of the Trx and GSH systems in mitochondria is most likely related to neutralization of reactive oxygen species (ROS) generated from leaks in the respiratory chain, in addition to reduction of oxidized cysteines. In yeast, mtGrx (Grx5) is essential for coordination of iron–sulfur clusters in electron transport complexes [41].

Secreted variants of the components and their targets

A search for GPx variants in dbEST identified a second Sec-containing GPx (GPx2) in *S. mansoni* and *S. japonicum* (Table I). This GPx2 possesses an N-terminal signal peptide that would target it to the lumen of the endoplasmic reticulum, topologically equivalent to the xtracellular compartment. Both Trx and TPx have been detected in platyhelminth secretions [34,35,38,39,42], suggesting that they might also provide protection against host-derived ROS. In the case of *F. hepatica*, the major secretory products of the adult worms are cathepsins L1 and L2 [43], and it is possible that Trx and TPx could help to stabilize the active-site cysteine residues of these enzymes.

Human lymphocytes and fibroblasts secrete Trx which, among other properties, is chemotactic for blood mononuclear cells [44]. If parasite Trx has a similar effect, it might not be simple to reconcile its protective and pro-inflammatory roles. In the case of *Schistosoma* spp., however, granuloma formation has been proposed to be required for expulsion of eggs from the host [45], and thus secreted Trx and TPx could have a dual role, promoting granuloma formation while helping to maintain the integrity of parasite eggs.

Future directions

The characterization of Trx–GSH systems in platyhelminths raises several interesting questions. TGR is a complex enzyme and several aspects of its catalytic mechanism remain to be addressed. Elucidation of the structure of TGR will aid understanding of the electron transfer events, whereas determination of the redox potentials of TGR centres could help to define under which condition reduction of GSSG would be thermodynamically favorable.

The presence of TGR and the absence of GR and TrxR raises the issue of whether reduction of GSSG is achieved solely by TGR. Trx has the inherent capacity to reduce GSSG, and this has been proposed to play a role in cells with inadequate GR activity [46]. Although primarily carried out by TGR, reduction of GSSG in schistosome extracts can be enhanced by the addition of recombinant Trx [4].

Data acquired thus far reinforce the concept that linked Trx–GSH systems play a central role in platyhelminth antioxidant defences. Both adult and larval worms metabolize high concentrations of hydrogen peroxide, and appear to rely exclusively on these systems. Indeed, where examined, catalase activity has been undetectable, and information available from the transcriptomes of *Schistosoma* and *Echinococcus* spp. strengthens this proposition. The capacity of parasite TPx, GPx and TGR to catalytically reduce peroxynitrite, as shown by ahpC (bacterial TPx) [47] and Sec-containing GPx [48], is another direction for future studies.

The presence of Trx, TPx and probably GPx in secretions also merits further investigation. No cDNAs have yet been isolated from these parasites, which predict Trx and TPx with N-terminal signal peptides. It is unclear whether the secreted isoforms are encoded by the genes described to date and, if so, how they are released. Mammalian cTrx lacks a signal peptide and is secreted via a non-conventional mechanism [49]. It is also not known whether the role of these products is primarily related to their antioxidant activity, or whether they have alternative functions. Answers to these questions should be provided in the near future.

Concluding remarks

The characterization of Trx and GSH systems in platyhelminths has revealed that these systems are linked by the key enzyme TGR. Although the presence of functional GR and TrxR genes in these organisms cannot be ruled out, it seems unlikely and, if present, their expression must be very low. *Drosophila* lack GR and TGR, and effectively use the Trx system to reduce GSSG [16], whereas *Caenorhabditis* contain GR, TrxR and TGR genes [50] (http://www.wormbase.org). In other nematodes, reduction of GSSG is carried out mainly by GR [51]. This illustrates that the evolutionary history of these systems is complex and interrelated, and suggests that gene duplication and/or fusion gave rise to functional redundancy that resulted in the loss of specific genes in different lineages.

The linked Trx–GSH systems of platyhelminths contrast with the Trx and GSH systems of their mammalian hosts, in which GSSG and Trx are primarily reduced by GR and TrxR respectively, with TGR expression only identified in the testis to date. The apparent lack of redundant mechanisms to provide reducing equivalents to essential enzymes raises the possibility of TGR as a pharmacological target for these organisms because inhibition of this enzyme could lead to impaired synthesis of DNA and antioxidant defences, thus compromising parasite survival. The validity of TGR as a drug target would rely on TGR being an essential enzyme and sufficiently different from the host homologue(s) so it can be inhibited selectively. Although not formally proven, the available data suggest that TGR is probably essential for these

organisms, but additional work is needed to address the possibility of selective inhibition.

Note added in proof

The three thioredoxin peroxidase (TPx) isoforms of *Schistosoma mansoni* are now biochemically characterized [62]. Recombinant TPx2 and TPx3 were found to accept reducing equivalents not only from thioredoxin (Trx), but also from glutathione (GSH). This suggests an additional molecular link between the Trx and GSH systems in the cytosol and mitochondria of *S. mansoni* cells.

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