THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 15, Issue of April 11, pp. 12920–12928, 2003
Printed in U.S.A.

Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of *Echinococcus granulosus* Thioredoxin Glutathione Reductase*

Received for publication, September 10, 2002
Published, JBC Papers in Press, January 21, 2003, DOI 10.1074/jbc.M209266200

Astrid Agorio‡§, Cora Chalar¶, Soledad Cardozo‡, and Gustavo Salinas‡‖

From the ‡Cátedra de Inmunología, Facultad de Química/Ciencias, Universidad de la República, Avenida Alfredo Navarro 3051, Piso 2, CP 11.600, Montevideo, Uruguay and ¶Sección Bioquímica, Facultad de Ciencias, Universidad de la República, Iguá 4225, CP 11.500, Montevideo, Uruguay

Thioredoxin and glutathione systems are the major thiol-dependent redox systems in animal cells. They transfer via the reversible oxidoreduction of thiols the reducing equivalents of NADPH to numerous substrates and substrate reductases and constitute major defenses against oxidative stress. In this study, we cloned from the helminth parasite *Echinococcus granulosus* two trans-spliced mRNA variants that encode thioredoxin glutathione reductases (TGR). These variants code for mitochondrial and cytosolic selenocysteine-containing isoforms that possess identical glutaredoxin (Grx) and thioredoxin reductase (TR) domains and differ exclusively in their N termini. Western blot analysis of subcellular fractions with specific anti-TGR antibodies showed that TGR is present in both compartments. The biochemical characterization of the native purified TGR suggests that the Grx and TR domains of the enzyme can function either coupled or independently of each other, because the Grx domain can accept electrons from either TR domains or the glutathione system and the TR domains can transfer electrons to either the fused Grx domain or to *E. granulosus* thioredoxin.

The reversible thiol-disulfide reaction is a central theme in biology. On the one hand, this redox exchange is an efficient mechanism of electron transport. On the other hand, this reaction is a molecular device used by nature as a switch to control protein function and localization through the redox state of critical thiol groups (1).

The central role in thiol-disulfide exchange in animal cells is played by the thioredoxin (Trx)[1] and glutathione (GSH) systems. Both systems have overlapping and distinct properties and targets but function in a similar way. They transfer via the reversible oxidoreduction of thiols the reducing equivalents of NADPH to numerous substrates and substrate reductases (2). They maintain the cellular redox homeostasis and constitute major defenses against oxidative stress, acting directly by reducing oxidized compounds and proteins or providing reducing equivalents to the hydrogen peroxide reductases present in animal cells, namely thioredoxin peroxidase and glutathione peroxidase (3). Both systems provide electrons to ribonucleotide reductase and control vital cellular processes such as transcription and signal transduction through the redox regulation of kinases, phosphatases, and transcription factors (4–6).

The animal thioredoxin system comprises the thioredoxin reductase (TR) and Trx, whereas the glutathione system consists of glutathione reductase (GR), glutathione (GSH), and glutaredoxin (Grx). Trx and Grx are thiol-disulfide oxidoreductases that transfer electrons to various substrates and substrates reductases. GSH, another thiol-based reductant, recycles Grx to its reduced state and reduces other protein and non-protein substrates. TR and GR are pyridine nucleotide-disulfide oxidoreductases that transfer the reducing equivalents of NADPH to oxidized Trx and other substrates and to GSH, respectively. Mammalian and *Caenorhabditis elegans* TR possess C-terminal electron transfer centers containing redox active cysteine and selenocysteine (Sec) residues (7–10). The selenol group of Sec is a strong nucleophile (11) that confers a profound reductive capacity to the enzyme. The C-terminal redox center is not present in GR. The equivalent redox link in the GSH system is thought to be provided by GSH (10).

In mammals, both systems are present in mitochondria and the cytosol. This is because of the existence of genes encoding mitochondrial and cytosolic variants for the proteins from both systems and also because of alternative splicing of single genes (12–17). Recently, mitochondrial and cytosolic variants of *Drosophila* TR have also been described to be derived from alternative splicing of a single gene (18).

Recently, a more complex machine exhibiting specificity for both thioredoxin and glutathione systems has been characterized in mammals (19). This enzyme termed thioredoxin glutathione reductase (TGR) is a selenoprotein oxidoreductase that possesses thioredoxin reductase, glutathione reductase, and glutaredoxin activities, achieving this broad substrate specificity by a fusion of TR and Grx domains. This enzyme has been reported to be present in the microsomal fraction of testis cells

* This work was supported in part by University of Uruguay Comision Sectorial de Investigación Científica grant (to C. C. and G. S.), The Wellcome Trust through a Research and Development Award in Tropical Medicine, an International Foundation for Science grant (to G. S.), and a Programa de Desarrollo de las Ciencias Básicas fellowship (to A. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AY147415 and AY147416 for mitochondrial and cytosolic cDNAs respectively.

§ Present address: Instituto de Biología Molecular y Celular de Plantas, CSIC-Universidad Politécnica de Valencia, Avenida de los Naranjos s/n CP46022, Valencia, Spain.

‖ To whom correspondence should be addressed. Tel.: 5982-4801196; Fax: 5982-4874320; E-mail: gsalin@fq.edu.uy.

[1] The abbreviations used are: Trx, thioredoxin; TR, thioredoxin reductase; GR, glutathione reductase; Grx, glutaredoxin; Sec, selenocysteine; TGR, thioredoxin glutathione reductase; PBS, phosphate-buffered saline; SL, splice leader; EgTGR, *E. granulosus* TGR; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); SECIS, selenocysteine incorporation signal; mit, mitochondrial; SE, selenium; TE, 10 mM Tris, 1 mM EDTA, pH 7.8.

from mice. A multifunctional TGR has also been reported in *Schistosoma mansoni,* a platyhelminth organism (20), indicating that this fusion domain has been evolutionarily conserved.

In this article, we report the cloning and characterization of a thioredoxin glutathione reductase of the larval stage of *Echinococcus granulosus*, a cestode parasite that dwells in the liver and lungs of human and cattle hosts. The results indicate that the enzyme possesses glutathione reductase, thioredoxin reductase, and glutaredoxin activities and suggest that there is domain communication between the C-terminal Cys-Sec redox center and the N-terminal glutaredoxin domain. In addition, we provide conclusive evidence that mitochondrial and cytosolic variants of TGR are generated from a single *TGR* gene.

EXPERIMENTAL PROCEDURES

Isolation of Full-length cDNAs Encoding E. granulosus TGR—Fertile hydatid cysts (larval stage of *E. granulosus*, the larva is a fluid-filled cyst bounded by the hydatid cyst wall; the larval worm or protoscoleces are found within the cyst) were collected from the lungs of naturally infected cattle in Uruguay. The hydatid cyst fluid and protoscoleces were aseptically aspirated with a vacuum pump. Once settled by gravity, the protoscoleces were extensively washed in phosphate-buffered saline (PBS) to remove dead protoscolex debris. They were observed under a light microscope for viability (flame cell and vital dye exclusion).

Total RNA was isolated from freshly isolated *E. granulosus* protoscoleces using TriZOL reagent (Invitrogen) and reverse-transcribed at 50 °C using an oligo(dT) primer and Superscript reverse transcriptase (Invitrogen) following the manufacturer's instructions. The cDNA obtained was subsequently used as template for PCR reactions following standard techniques. Sense and antisense oligonucleotides were designed from the mammalian and *C. elegans* thioredoxin reductase-conserved amino acid regions VNVGCIPKKLMH and PNAGEVTQG, respectively. The sense oligonucleotide, 5′-GTGAATGTIGGITGYATY-CCTAARAARYTNATGCA-3′, was used in polymerase chain reactions in combination with antisense oligonucleotides R1 (5′-TTGDATVACYT-CICCNGCGTT-3′) or R2 (5′-TTGDATVACYTCICCNGCATT-3′). A PCR product was obtained with the sense oligonucleotide and R1. This product was isolated (GeneClean, BIO101), cloned into pGEM-T-Easy (Promega), and sequenced using AmpliTaq FS according to manufacturers' and standard protocols on a ABI Prism 377 DNA sequencer, and found to encode the central fragment of a thioredoxin reductase.

The sequence information obtained was used to clone the full-length cDNA. The 3′-end of the gene was PCR-amplified using a gene-specific sense oligonucleotide (5′-CGACAACCGCGTGGTAGGA-3′) in combination with oligo(dT). The 5′-ends of the cDNA were also obtained by reverse transcription-PCR using the gene-specific antisense primer 5′-GGATTTGGCATCCTCCATGTAGTG-3′ and the sense oligonucleotide 5′-CACCGTTAATCGGTCCTTACC-3′ derived from the sequence of the 36 nucleotide splice leader (SL) sequence present at the 5′-end of some *E. granulosus* mRNAs (21). The PCR products obtained were isolated, cloned and sequenced as described above. Since the amplification of the 5′-end of the cDNA yielded two distinct but sequence-related PCR products, subsequent PCRs were carried out to confirm the sequences of the two 5′-end cDNA products. These PCRs were performed using (i) the SL oligonucleotide and two different gene-specific antisense primers and (ii) two different sense oligonucleotides corresponding to the junctions of the SL exon (present in both cDNAs) with the differential downstream nucleotides present in each of the two cDNAs in combination with a gene-specific antisense oligonucleotide (see Fig. 4 for details of cDNA sequences). In all of the cases, sequence analysis was performed using the following programs: (i) Blast 2.0 (www.ncbi.nlm.nih.gov/BLAST) for searching homologies in GenBank™ and the echinobase, a recent EST data base of *E. granulosus* (22) (www.nematodes.org/Lopho/LophDB.php); (ii) SignalP and PSORT for detection of localization signals; and (iii) Mfold to identify secondary mRNA structures (www.bioinfo.rpi.edu/nzukerm/seganal/).

Partial Characterization of EgTGR Gene—Genomic *E. granulosus* DNA was isolated from protoscoleces as described previously (23). This template was subsequently used in PCR reactions to isolate the 5′-end of *EgTGR* gene using the forward oligonucleotide 5′-ATGTTTGGCTGT-CATTGTCT-3′ and the reverse primer 5′-GGATTCTTACGAAGC-ATTTCAACCTG-3′.

Expression and Purification of the Recombinant EgTGR Central Fragment and Recombinant EgTrx—The central fragment of the *EgTGR* gene corresponding to the initial cDNA fragment isolated was cloned in the appropriate reading frame into *Bam*HI/*Hin*dIII sites of pET28a expression vector (Novagen) to produce the His-tagged recombinant fragment. The *Escherichia coli* BL21(DE3) strain was transformed with the construct, and single bacterial colonies were inoculated into LB medium supplemented with 50 μg ml^{-1} kanamycin. Recombinant protein expression was induced at A_{600} of 0.6 with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 3 h at 37 °C. Cells were harvested by centrifugation (8000 × *g*, 30 min), and the pellet was resuspended in 40 ml of lysis buffer (50 mM phosphate buffer, pH 7.5, 10 mM imidazole, 300 mM NaCl, 1 mg ml^{-1} lysozyme). The bacterial suspensions were sonicated on ice and centrifuged (14,000 × *g*, 30 min). The recombinant protein was found entirely in the pelleted fraction. The His-tagged protein was recovered from the inclusion bodies by resuspending the pellet in 40 ml of 10 mM Tris 1 mM EDTA, pH 7.8 (TE) TE and centrifuged for 1 h at 14,000 × *g*. The pellet was resuspended in 20 ml of TE buffer, 8 M urea and incubated for 1 h at 4 °C, and the solution was centrifuged for 1 h at 14,000 × *g*. The solubilized protein recovered from the supernatant was refolded by sequential dialysis against TE buffer containing 2, 1, and 0.4 M urea and a final dialysis against 10 mM phosphate buffer, pH 7.4, containing 150 mM NaCl (PBS). The recombinant protein was analyzed in SDS-PAGE 12% gels under reducing conditions according to standard techniques. Protein concentration was determined using the BCA (Pierce), and the protein was subsequently used to immunize a rabbit. Purification of recombinant EgTrx from a pMal-C2 (New England Biolabs) plasmid carrying the full-length *EgTrx* gene was carried out as described previously (23).

Antiserum against the EgTGR—A rabbit polyclonal serum reactive with EgTGR was obtained by intramuscular immunization with the purified recombinant fragment of EgTGR. The immunization protocol consisted of a priming immunization with 300 μg of EgTGR in Freund's complete adjuvant (Sigma) and two subsequent immunizations on days 30 and 45 with 150 μg of EgTGR in Freund's incomplete adjuvant (Sigma). On day 55, the rabbit was bled and the reactivity of the serum-analyzed by Western blot against the recombinant fragment of EgTGR and total protoscolex extract (see below).

Purification of E. granulosus TGR from Protoscoleces—Protoscoleces were homogenized under liquid nitrogen in PBS containing 1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, 5 μg ml^{-1} leupeptin. Purification was carried out by a combination of salt precipitation, ion-exchange chromatography, and affinity chromatography, and each step was monitored for thioredoxin reductase activity using the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) reduction assay (see below). Ammonium sulfate precipitation of the extract was carried out in three steps of 30, 55, and 85% saturation. The 55% fraction contained most of the thioredoxin reductase activity. The active fraction was dialyzed against 10 mM TE, and applied to a HiTrap Q-Sepharose HP column (Amersham Biosciences). Bound proteins were eluted with increasing salt concentration (50, 100, 150, 200, and 300 mM NaCl steps). All of the thioredoxin reductase activity eluted at 150 mM NaCl. This fraction was dialyzed against TE, applied onto a 2′,5′-ADP-agarose matrix (Sigma), and washed with TE, 20 mM NaCl, and thioredoxin reductase eluted with 200 mM NaCl. The purified protein was analyzed in SDS-PAGE 10% gels under reducing conditions according to standard techniques. Protein concentration was determined using BCA.

Subcellular Fractionation of E. granulosus Protoscoleces—0.5 ml of gravity-decanted protoscoleces were suspended in 2.5 ml of ice-cold 10 mM Tris, pH 7.6, 250 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, 5 μg ml^{-1} leupeptin, and disrupted on ice in a Potter homogenizer. The suspension was settled by gravity for 20 min to remove unbroken cells and small protoscolex fragments. The procedure was repeated twice. Afterward, the supernatant suspension was separated into four fractions by sequential centrifugation essentially as described previously (19). The extract was centrifuged at 900 × *g* for 30 min, the resulting supernatant was centrifuged at 10,000 × *g* for 30 min, and the remaining supernatant was centrifuged at 120,000 × *g* for 1 h. The first, second, and third pellet were considered to be the nuclear, mitochondrial, and microsomal fractions, respectively, whereas the final remaining solution was considered as the cytosolic fraction. The nuclear, mitochondrial, and microsomal fractions were resuspended in the homogenization buffer and recentrifuged twice before use. The cytosolic fraction was recentrifuged once. Pelleted fractions were resuspended in 10 mM Tris, pH 7.6, 1 mM phenylmethylsulfonyl fluoride, 10 mM EDTA, 5 μg/ml leupeptin, sonicated, and stored at −70 °C until use. Cytosolic and mitochondrial markers were used as controls of the subcellular fraction identity (see the next two paragraphs).

Western Blot—Total protoscolex extract and subcellular fractions were probed with the anti-EgTGR serum and with a monoclonal antibody that recognizes the cytosolic parasite protein P-29 (24). Extracts

were diluted in 2× loading buffer (62.5 mM Tris, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 5% (v/v) mercaptoethanol, 0.02% (v/v) bromphenol blue), heated for 5 min at 100 °C, and centrifuged at 10,000 × *g* for 10 min. Supernatants were separated on 10% (w/v) gels and transferred onto nitrocellulose membranes. The nitrocellulose membranes were incubated with blocking buffer (PBS, 1% Tween 20) and subsequently incubated with the rabbit anti-EgTGR serum diluted 1:500 in PBS, 0.1% Tween 20, or with the monoclonal antibody followed by a 2-h incubation with the corresponding secondary antibody coupled to alkaline phosphatase. Protein bands were visualized by revealing the alkaline phosphatase activity using 5-bromo-4-chloro-3-indolyl-phosphate and nitro blue tetrazolium as substrates.

Enzyme Assays—Thioredoxin reductase, glutathione reductase, and glutaredoxin activities were measured in the native purified TGR. Thioredoxin reductase activity was assayed by the DTNB reduction assay (25) and the insulin reduction assay (26). In the DTNB reduction assay, the purified enzyme was added to a reaction mixture containing 100 mM potassium phosphate, pH 7.0, 10 mM EDTA, 0.2 mg ml^{-1} bovine serum albumin, 5 mM DTNB, and 200 μM NADPH. The reaction was carried out at 25 °C and monitored by the increase in absorbance at 412 nm. One enzyme unit is defined as the NADPH-dependent production of 2 μM 2-nitro-5-thiobenzoic acid. For the insulin reduction assay, the purified enzyme was added to a reaction mixture containing 100 mM potassium phosphate, pH 7.0, 1.0 mg ml^{-1} insulin, 20 μM recombinant EgTrx, and 200 μM NADPH. NADPH oxidation was followed at 340 nm. One unit of enzyme was defined as the oxidation of 1 μM/min NADPH/min at 25 °C.

GR activity was assayed as the NADPH-dependent reduction of GSSG determined as the decrease in absorbance at 340 nm at 25 °C in a reaction mixture containing 100 mM potassium phosphate, pH 7.0, 10 mM EDTA, 200 μM NADPH, and 1 mM GSSG. One unit of enzyme was defined as the oxidation of 1 μM/min NADPH/min at 25 °C (27).

The glutaredoxin assay was performed as described previously (28). A reaction mixture containing 1 mM GSH (Sigma), 2 mM EDTA, 0.1 mg ml^{-1} bovine serum albumin, 0.7 mM β-hydroxyethyl disulfide (Acros Organics), and 0.4 units of yeast glutathione reductase (Sigma) was preincubated for 2 min. Afterward, the sample was added and then the reduction of the mixed glutathione-hydroxyethyl disulfide followed by the oxidation of NADPH at 340 nm. One unit of Grx activity was defined as the oxidation of 1 μmol of NADPH/min at 25 °C. Enzyme assays were also carried out as described above but omitting GR or GR and GSH from the reaction mixture as indicated in each case.

Thioredoxin reductase, GR, and Grx assays were carried out in the absence and presence of 10 nM auronofin, a gold compound known to selectively inhibit the redox active selenol group of the enzyme (29). At the concentration used, the activity of control yeast glutathione reductase was not affected. For assays in presence of auronofin, the purified enzyme was preincubated with NADPH. All of the assays were carried out in triplicates.

In addition, thioredoxin reductase and citrate synthase activities were determined in the subcellular fractions of *E. granulosus* protoscoleces. Citrate synthase activity was determined according to Darley-Usmar *et al.* (30).

RESULTS

Cloning of a cDNA (EgTGR) Coding for a Pyridine Nucleotide-disulfide Oxidoreductase Containing Selenocysteine from E. granulosus—Degenerate primers derived from consensus *C. elegans* and mammalian TR amino acid sequences were successfully used to clone the central fragment of a *E. granulosus* thioredoxin reductase cDNA by PCR. This information allowed the cloning of two full-length cDNAs encoding TGR variants that differ in their N-terminal sequences but encode identical Grx and TR domains. The full-length cDNAs sequences have been deposited in GenBank™. The deduced amino acid sequences are shown in Fig. 1. Specific features derived from the sequence analysis are presented and discussed below.

The analysis of the deduced amino acid sequence of the cloned cDNAs indicates the presence of characteristic thioredoxin reductase domains, namely the FAD- and NADPH-binding domains and interface domain as well as the conserved active site sequence CVNVGC and the additional C-terminally located redox active center. This latter electron transfer center displays the molecular signature characteristic of selenium thioredoxin reductases containing the amino acid SEC (U in one-letter code) within the conserved motif GCUG (Fig. 1). The Sec amino acid co-translationally incorporated to the protein synthesis is encoded by an in-frame UGA codon and decoded as a UGA_{Sec} codon by a selenocysteine incorporation signal (SECIS) element present in the 3′-untranslated region of *Echincoccus* cDNA, 180 bp downstream of the UGA_{Sec} codon (Fig. 2). An UAG stop codon is present in EgTGR cDNAs that are two codons downstream from the UGA_{Sec} codon.

The SECIS element present in EgTGR mRNA possesses the characteristic stem loop structure (31–33) containing the functional core with the AUGA sequence in the 5′-arm and the GA in the 3′-arm of the stem and the unpaired AAN bases in the apical loop, both elements spaced by a 11-bp helix. Furthermore, the location of all of the structural features (helix I, internal loop, core, helix II, and apical loop) of the stem loop is well conserved with respect to vertebrate SECIS elements (see Fig. 2). It has recently been proposed that UG-, UC-, and CA-rich sequences around the UGA_{Sec} codon of mRNAs of mammalian selenoproteins may also participate in decoding the UGA_{Sec} codon. Although the location of these regions may vary between mRNAs encoding selenoproteins, they adopt a stem-loop secondary structure (34). Putative UC-, UG-, and CA-decoding regions surrounding the UGA_{Sec} could be identified in EgTGR cDNA. Moreover, the sequence around this codon may fold as a stem-loop-like structure (data not shown).

E. granulosus TGR Possesses TR and Glutaredoxin Domains That Confers the Enzyme the Capacity to Shuttle Electrons to Targets of Both GSH and Trx Systems—In addition to the TR domains, the EgTR cDNAs encode an N-terminal Grx domain. This domain possesses the structural features required for Grx activity: the redox center C*XX*C characteristic of glutaredoxin, a GSH binding motif, and the hydrophobic surface area (Fig. 1). A similar domain fusion has recently been described for a mouse thioredoxin reductase (19). The enzyme was renamed by the authors as TGR, because the isolated enzyme possessed not only TR activity but also glutathione reductase and glutaredoxin activity. More recently, a TGR cDNA encoding a TGR has been isolated from the trematode parasite *S. mansoni* and the enzyme was characterized displaying similar biochemical characteristics to the mouse TGR (20). Similar to the Schistosome TGR and unlike the mammalian TGR, the glutaredoxin domain of EgTGR belongs to the two Cys glutaredoxin subfamilies.

The native purified TGR was purified from protoscolex extracts by sequential salt precipitation, ion-exchange chromatography, and affinity chromatography on an ADP-agarose column. After the affinity chromatography, the preparation contained no visible bands other than a 66-kDa protein on SDS-PAGE (Fig. 3). The native enzyme possessed TR activity toward both the general substrate DTNB and recombinant *E. granulosus* thioredoxin (Table I). NADPH-reduced TR was completely inhibited by near stochiometric concentration of auronofin, giving further confirmatory evidence that EgTGR is a selenoenzyme. Interestingly, no bands in the range of 52–57 kDa typical of isolated vertebrate TRs were observed in the purified active fraction, suggesting that most of the TR activity in the protoscolex extract corresponds to the isolated 66-kDa TGR.

Glutathione reductase assays were also performed with the purified *E. granulosus* TGR. The results indicate that the enzyme is also capable of reducing oxidized glutathione (Table I) with a specific activity similar to mouse TGR (19). The enzyme activity was inhibited by auronofin, indicating that the Cys-Sec redox center participates in the mechanism of electron transfer to oxidized glutathione (Table I).

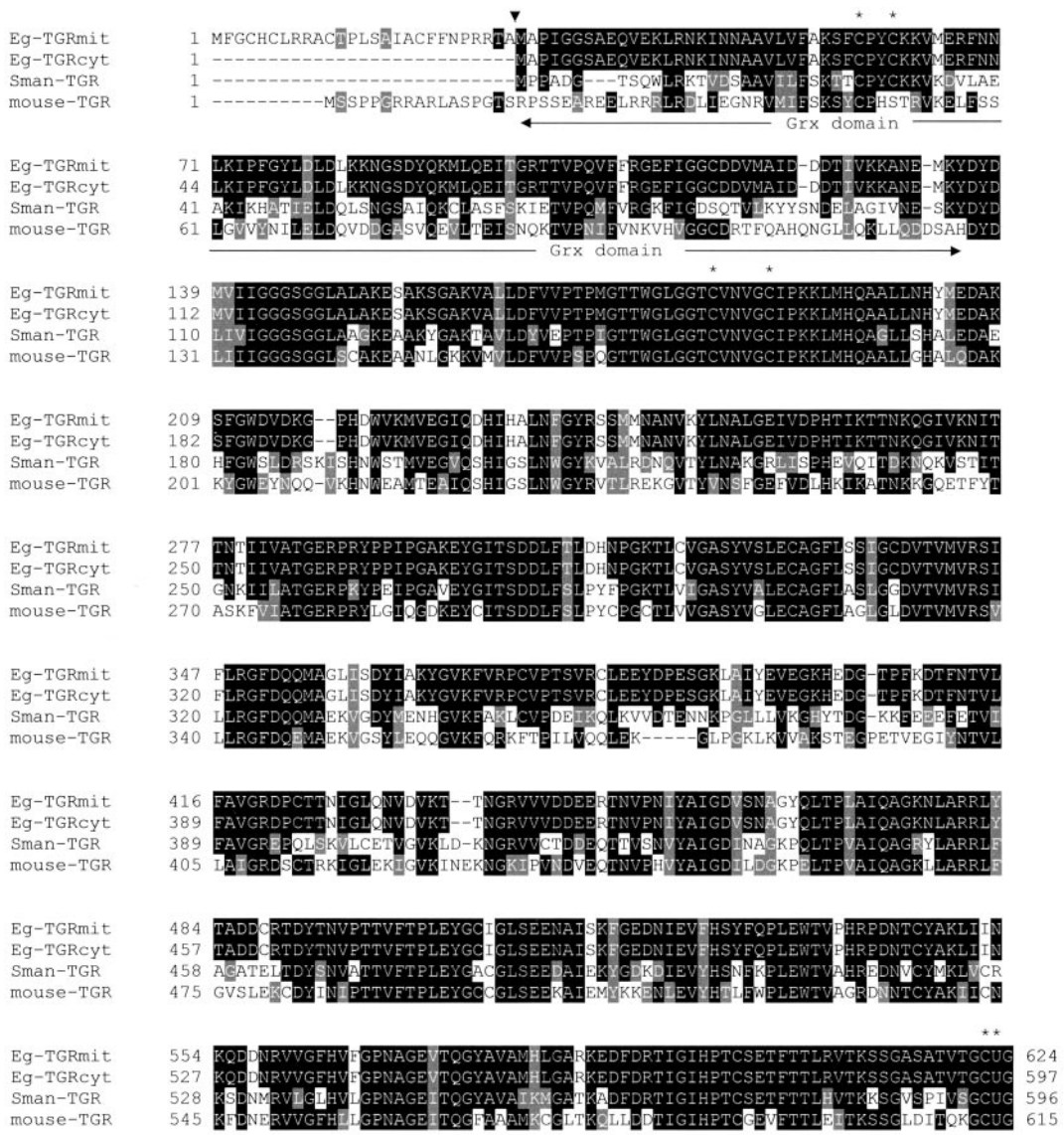

FIG. 1. **Amino acid sequences of *E. granulosus* TGRs and homologues proteins.** Alignment of the deduced amino acid sequences of mitochondrial and cytosolic TGR from *E. granulosus* (*Eg-TGRmit* and *Eg-TGRcyt*, respectively) with sequences of mouse TGR (GenBank™ accession number AAK31172) and *S. mansoni* (GenBank™ accession number AF395822) TGR (*Sman-TGR*). Identical residues are *shaded* in *black*, and conserved substitutions are *shaded* in *gray*. The *arrow* below the sequences indicates the glutaredoxin domain. The predicted cleavage site of the mitochondrial signal peptide of Eg-TGRmit is indicated by a *closed triangle*. The redox active residues of (i) the glutaredoxin domain, (ii) the disulfide center in the pyridine nucleotide-disulfide oxidoreductase domain, and (iii) the C-terminal center containing the amino acid selenocysteine (denoted by *U*) are shown by *asterisks* above sequences. (−) denote gaps introduced to optimize alignment. The cDNA sequences encoding EgTGRmit and EgTGRcyt have been deposited in GenBank™ under accession numbers AY147415 and AY147416, respectively.

Glutaredoxin assays were performed as described previously (28) in a reaction mixture containing GR and GSH; GSH but not GR; and neither GR nor GSH, all of them with or without auronofin. The results are summarized in Table I. The Grx activity in the presence of GR and glutathione was 1.7 units min^{-1} mg^{-1}, similar to the activity reported for mouse TGR. This activity was partially inhibited by auronofin. The purified EgTGR was also capable of reducing glutathione-hydroxyethyl-mixed disulfide in the absence of yeast GR with a Grx-specific activity of 1.0 unit min^{-1} mg^{-1}, and this activity was completely inhibited by auronofin. The β-hydroxyethyl reductase activity in the absence of GSH and yeast GR was reduced to 50% of the activity in the presence of GSH and was negligible when pretreated with auronofin (Table I).

Taken together, all of these results indicate that both Grx and TR domains encoded by the EgTGR cDNA are functionally active in the native enzyme. This domain fusion in EgTGR makes it possible to transfer electrons from NADPH toward the downstream targets of both thioredoxin and glutathione systems.

Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of TGR—Trans-splicing involving a splice leader (SL) mini exon that is added to the 5′-ends of pre-mRNAs has been recently reported to occur in ~20% of *Echinococcus* transcripts (21). Although the function of trans-splicing in these organisms is still unknown, it has already been used to construct *Echinococcus* cDNA libraries from trans-spliced mRNAs (22). The 5′-end of the *EgTGR* gene was cloned by a PCR splice leader strategy using an oligonucleotide derived from the *E. granulosus* SL exon and gene-specific reverse primers (see "Experimental Procedures"). Two PCR bands of similar intensities differing in 85 nucleotides were amplified from protoscolex cDNA (Fig. 4*A*). The PCR products were isolated, cloned, and sequenced. The nucleotide sequence revealed that both cDNAs contained the entire splice leader exon. However, the longer cDNA contained an 85-bp

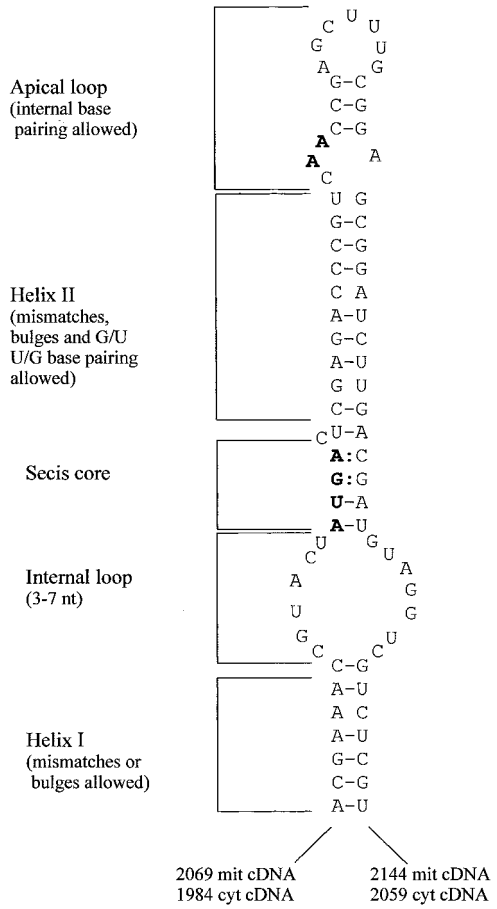

FIG. 2. ***E. granulosus* SECIS element.** Predicted secondary structure of the *E. granulosus* EgTGR SECIS element present in the 3′-end untranslated region of EgTGR cDNAs. (−) indicates Watson Crick base-pairing; *colon* indicates non-Watson Crick interactions at the SECIS core. Consensus SECIS structural features in the stem loop according to Korotkov *et al.* (33) are indicated. Conserved sequences in eukaryotic SECIS elements present in the apical bulge and SECIS core are shown in *boldface*. Nucleotide numbers referring to the cDNA sequences of EgTGR deposited in GenBank™ are indicated.

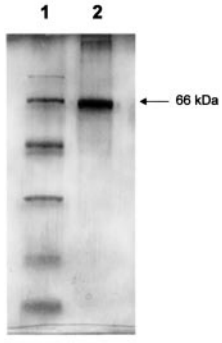

FIG. 3. **Purified *E. granulosus* TGR.** SDS-PAGE analysis of the purified *E. granulosus* TGR under reducing conditions on a 10% resolving gel. *Lane 1*, molecular mass markers (Low molecular mass calibration kit for SDS electrophoresis, Amersham Biosciences, 97, 66, 45, 30, 20.1, and 14.4 kDa); *lane 2*, purified EgTGR (sequential purification by ammonium sulfate precipitation, ion exchange, and ADP-agarose affinity). The gel was silver-stained.

sequence immediately downstream of the SL exon, absent in the shorter cDNA. The downstream 3′-end sequence that follows the extra 85-bp sequence in the longer cDNA and the SL sequence in the shorter cDNA was identical in both cDNAs and encodes the N-terminal Grx domain and C-terminal thioredoxin reductase domains of the TGR (Fig. 4*A*).

The possibility that the two PCR bands were derived from a PCR artifact was ruled out by performing different PCR reactions with the SL forward oligonucleotide and different gene-specific primers. In every case, two bands of equal intensity and differing in 85 bp were obtained (data not shown). In addition, PCR were performed using as forward primers the joining sequences of the SL exon with the two downstream bases of each cDNA. in either case, a single band of the expected size was obtained (data not shown). These independent PCR products were cloned and sequenced and found to be identical to the originally isolated cDNAs. In all of the cases, the PCR results were consistent not only respect to their sizes and sequences but also with regard to band intensity, suggesting that both mRNAs are produced in similar amounts.

The results indicate that both mRNAs contain the trans-spliced leader exon and are derived from a single *TGR* gene, because the coding sequence for the Grx and TR domains is identical in both cDNAs. Two plausible models for generation of the mature trans-spliced mRNAs from the *TGR* gene are depicted in Fig. 4, *B* and *C*. Both of them predict that the longer cDNA contains an extra exon not present in the shorter cDNA and the existence of an intron between the first and second exons of *EgTGR* gene. The model depicted in Fig. 4*B* involves a single primary transcript that is alternatively trans-spliced immediately upstream of a first exon, (*route a*) and immediately upstream of a second exon (*route b*). The former pre-mRNA then would be cis-spliced joining the first and second exons (*route a*). An alternative explanation for the results would be the existence of two alternative primary transcripts from the *TGR* gene (Fig. 4*C*), each of them trans-spliced at the 5′-end. In this latter model, the longer primary transcript would contain exons I and II and an intron between them, whereas the shorter primary transcript would not contain the first exon. This model implies the existence of a transcriptional initiation site within the intron.

Whether trans-splicing occurs on a single primary transcript or on two alternative transcripts, an intron should be located between the first and second exons so that there is an acceptor site for cis/trans-splicing. Thus, we examined the 5′-end genomic organization of *EgTGR* gene (see "Experimental Procedures") and found that the gene encoding EgTGR possesses a first exon that corresponds to the cDNA portion present in the longer cDNA and absent in the shorter cDNA followed by an intron and a downstream exon (Fig. 4*C*). The intron contains a GT-donor and an AG-acceptor site consistent with the models (Fig. 4*D*). In addition, an examination of the intron sequence revealed the presence of a TATA consensus sequence 80 nucleotides upstream of TGR exon II (Fig. 4*D*), consistent with the model depicted in Fig. 4*C*.

Translation of the two EgTGR cDNAs revealed a different first AUG codon for each cDNA in a favorable Kozak consensus sequence for initiation of translation (Fig. 4*A*). The open reading frame encoded by the shorter cDNA is 597 amino acids long, whereas the longer cDNA contains an open reading frame of 624 amino acids coding for an extra 27 amino acid extension at the N terminus of the polypeptide chain, which is absent in the translated sequence of the shorter cDNA. An examination of the sequences with SignalP and PSORT programs indicated the presence of a predicted mitochondrial translocation signal in the N-terminal region of the longer translated product (Fig. 4*A*). Thus, the shorter mRNA would encode a leaderless TGR that by default would localize to the cytosol, and the longer cDNA would encode a mitochondrial TGR variant. The presequence present in the predicted $_{mit}$TGR possesses all the ele-

TABLE I
Specific enzymatic activities of EgTGR

Enzymatic activities were determined as described under "Experimental Procedures" using the native purified TGR. The glutathione reductase used in the glutaredoxin assay was from yeast origin and was not affected by the auronofin concentration used (10 nM) in the assay.

Enzymatic activity	Substrates	Specific activity	Specific activity in presence of 10 nM auronofin
		μmol/min/mg	*μmol/min/mg*
Thioredoxin reductase	NADPH and DTNB	2.3 ± 0.3	0.10 ± 0.04
	NADPH and Trx	2.8 ± 0.3	0.12 ± 0.05
Glutathione reductase	NADPH and GSSG	1.8 ± 0.3	0.10 ± 0.03
Glutaredoxin (glutathione-dependent)	NADPH and HED (in an assay mix containing GR and GSH)	1.7 ± 0.3	0.80 ± 0.14
	NADPH and HED (in an assay mix containing GSH but no GR)	1.0 ± 0.2	0.09 ± 0.03
Hydroxyethyl disulfide reductase	NADPH and HED (in an assay mix without GR and GSH)	0.43 ± 0.08	0.06 ± 0.03

ments to be decoded by the trans-locases of the outer and inner mitochondrial membranes and assures its correct sorting to the mitochondrial matrix (35). It contains several arginines, hydroxylated and hydrophobic residues, and no acidic residues (Fig. 4*A*). In addition, the leader presequence forms an amphipathic α-helix with a positively charged surface on one side and a hydrophobic surface on the other. The presequence of $_{mit}$TGR also contains the critical recognition elements for proteolytic cleavage by the mitochondrial processing peptidases as follows: the distal and proximal arginine residues including one located at the invariant −2 position, proline residues between these regions, and an alanine residue at P1′ site (Fig. 4*A*) (36, 37). Thus, this presequence once in the mitochondrial matrix would be removed by the mitochondrial processing peptidases, giving rise to a mature polypeptide chain of 598 amino acids (Fig. 4*A*) that would be identical to the cytosolic variant with the exception of an extra N-terminal alanine residue present in the $_{mit}$TGR variant. The data also indicate that the mitochondrial trans-location signal coded by the longer cDNA is formed by the joining of the exons I and II (Fig. 4*A*).

Because the gene product variants would localize to different cellular compartments, we examined the presence of TGR in the different subcellular fractions obtained by differential centrifugation of a protoscolex extract (see "Experimental Procedures"). To this end, the central fragment of EgTGR was cloned for expression as a His-tagged fusion and the purified recombinant protein was used to raise a rabbit antiserum. The Western blot showed the presence of a single band of 66 kDa in the total protoscolex extract in the cytosolic fraction, and in the mitochondrial fraction, no band was observed in either the microsomal or nuclear fractions (Fig. 5, *panel A*). Likewise, thioredoxin reductase activity was significant in the cytosolic and mitochondrial fractions (15 and 13 milliunits min^{-1} mg^{-1}, respectively) and negligible in the microsomal and nuclear fractions. A control Western blot performed using a monoclonal antibody raised against p29, a known cytosolic protein present in *E. granulosus* protoscolex (24), revealed a 29-kDa band only in the cytosolic fraction, suggesting that there was no cross-contamination of the other subcellular fractions (Fig. 5, *panel B*). In addition, citrate synthase activity was measured in all of the fractions and found to be significant in the mitochondrial extract exclusively (105 milliunits min^{-1} mg^{-1}), indicating the identity of the mitochondrial fraction and absence of cross-contamination of the other subcellular fractions. These results strongly indicate that the two trans-spliced mRNAs code for a cytosolic and a mitochondrial TGR variant.

The apparent molecular mass of the antibody recognized bands (66 kDa) is consistent with the predicted molecular masses of the cytosolic TGR (65.54 kDa) and the mature $_{mit}$TGR (65.47 kDa) as well as with the band size of the native purified enzyme (Fig. 3). It is worth mentioning that the anti-serum raised against the central fragment of the TGR did not recognized bands of lower molecular masses indicative of TR. These results together with the purification of the native enzyme would suggest that in protoscolex the predominant Se-pyridine nucleotide-disulfide oxidoreductase is the cloned TGR.

DISCUSSION

The characterized TGR cDNA and enzyme indicate that the EgTGR is a selenoenzyme. Indeed, the deduced amino acid sequence contains the C-terminal molecular signature of Se-TR, the cDNA contains the SECIS element needed for decoding the UGA_{Sec} codon, and the activity of the purified enzyme is inhibited by gold compound auronofin. *E. granulosus* is a cestode platyhelminth. The presence of selenium in the C-terminal redox center of thioredoxin reductases and TGR has also been described in the trematode platyhelminth *S. mansoni* (20) and in *C. elegans*, a nematode helminth (9). Thus, this selenium redox center seems to be characteristic not only of mammals but also of helminth phyla, suggesting that it might have appeared before the protostomate/deuterostomate branching. In lower eukaryotic organisms such as *Plasmodium* sp., the C-terminal redox center is present but contains a pair of redox active Cys residues instead of Cys-Sec (38). This finding suggests that Se-piridine-disulfide oxidoreductases might have evolved after the appearance of metazoan organisms. Intriguingly, the C-terminal redox centers of *Drosophila melanogaster* and *Anopheles gambiae* TRs do not possess the Cys-Sec redox pair but rather twin cysteines instead (39). Thus, insect TRs appear to have reverted to the Cys-Cys redox center. A less likely evolutionary scenario would be the independent appearance of Se-TR in deuterostomate and protostomate organisms. Similar to the presence of Sec in the C-terminal redox center of thioredoxin reductases, it is interesting to note that the Grx-TR domain fusion is present in helminth and vertebrates and appears to be absent in *Plasmodium* sp. and insects.

Two trans-spliced TGR mRNA isoforms were isolated from *E. granulosus* protoscoleces. The fact that the sequences of both cDNAs were identical with the exception of the absence of exon I in the shorter cDNA was considered strong evidence for both cDNAs being derived from a single gene. The partial characterization of *EgTGR* gene gave further confirmatory evidence and suggested possible mechanisms for the generation of the two mRNA variants. These alternative mRNAs may arise from trans-splicing on a single primary transcript or on alternative transcripts (see Fig. 4, *B* and *C*). The existence of a TATA consensus sequence in the intron between exons I and II that may serve as an alternative site for transcription initiation would give support to the proposed mechanism that involves two alternative EgTGR transcripts, each of them trans-spliced at the 5′-end. Alternative transcription from a single gene

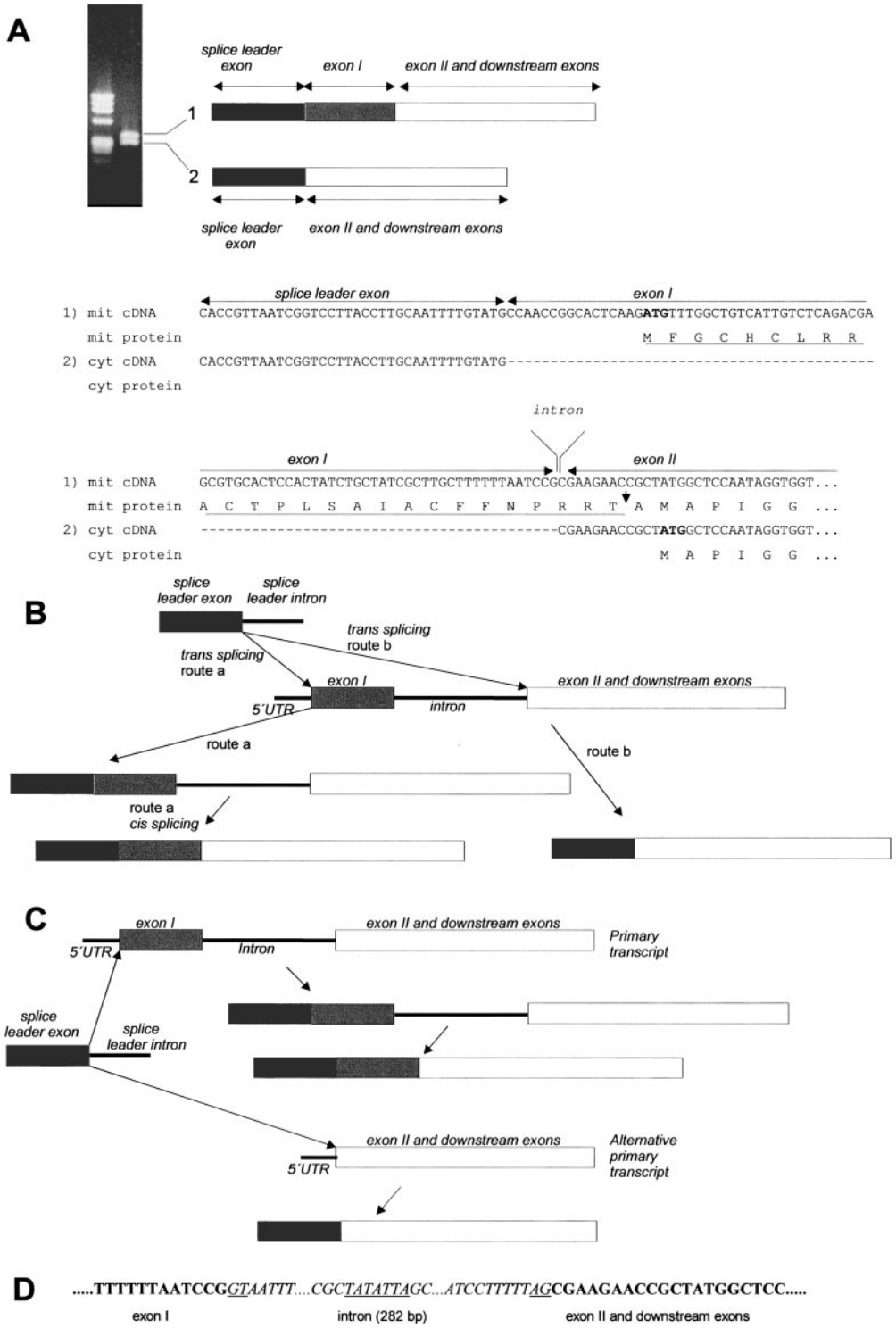

FIG. 4. **Alternative mRNAs arising from trans-splicing encode mitochondrial and cytosolic TGR variants.** *A*, PCR amplification of the 5′-end of TGR cDNA from *E. granulosus* protoscoleces using as forward primer an oligonucleotide derived from the SL exon sequence and a TGR-specific sequence as reverse primer as described under "Experimental Procedures." Two PCR products were amplified (*right track* of the agarose gel and *left track* corresponds to molecular mass markers, ϕX174 Hae). The nucleotide sequences of the PCR products revealed that both cDNAs contained the entire splice leader exon (schematically depicted as a *dark gray box*). The longer cDNA contained an 85-nucleotide exon immediately downstream of the splice leader (*exon I*, *light gray box*) followed by the downstream 3′-end sequence (*exon II* and downstream exons, *white box*). The shorter cDNA did not contain exon I. The SL exon was followed by exon II and downstream exons. Translation of both cDNAs indicates that the longer cDNA encodes a mitochondrial TGR variant, whereas the shorter cDNA codes for a cytosolic TGR variant. The mitochondrial signal peptide is *underlined*, and the predicted cleavage site by the mitochondrial processing peptidase is indicated by a *closed triangle*. *B*, proposed model of alternative trans-splicing on a single primary transcript. *Route a* involves trans-splicing of the SL exon on exon I and then cis-splicing of exon I and exon II. *Route b* involves trans-splicing of the SL exon on exon II. *C*, proposed model of trans-splicing on alternative transcripts. From *EgTGR* gene, two primary transcripts are synthesized from alternative transcription initiation sites. Both transcripts are trans-spliced at the 5′-end, and cis-splicing between exons I and II occurs in the longer transcript only. This model implies the existence of a promoter sequence within the intron between exons I and II. *D*, exon/intron boundaries and TATA consensus sequence. The intron sequence between exons I and II is shown in *italics*. Donor and acceptor splicing sites are *underlined*. A TATA consensus sequence 80 nucleotides upstream of exon II that may serve as alternative transcription initiation site is also *underlined*.

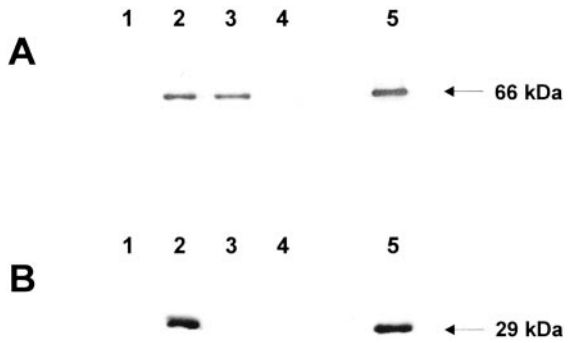

FIG. 5. **Subcellular localization of TGR by immunoblot.** Western blot of a total *E. granulosus* larval worm extract (*lane 5*) and subcellular fractions of the larval worm (*lanes 1–4*, microsomal, cytosolic, mitochondrial, and nuclear fractions, respectively) probed with an antiserum raised against a recombinant fragment of EgTGR (*panel A*) and with a monoclonal antibody raised against P29, a known cytosolic protein of *E. granulosus* (*panel B*). The total larval worm extract and subcellular fractions obtained as described under "Experimental Procedures," were resolved on 10% gels under reducing conditions and then subjected to immunoblot. Single bands of 66 kDa were recognized in the total extract and mitochondrial and cytosolic fractions by anti-TGR antibodies. Single bands of 29 kDa were recognized in the total extract and the cytosolic fraction by anti-P29 antibodies.

followed by trans-splicing has also been proposed to occur in *E. granulosus elp* gene (21).

Accumulated recent evidence suggests that alternative splicing and alternative primary transcripts from single genes represent generalized processes that increase the diversity of gene expression. Recent studies on expression of mammalian TR and Grx genes illustrate this phenomenon. Mammals possess three different *TR* genes, namely *TR1*, *TR3*, and *TGR*. Whereas TGR codes for a thioredoxin glutathione reductase, *TR1* and *TR3* were originally described as cytosolic and mitochondrial enzymes, respectively (40). However, it has been recently demonstrated that both *TR1* and *TR3* exhibit extensive heterogeneity because of differential transcript splicing (15, 16). Alternative first exon splicing in human *TR3* gene results in the generation of mitochondrial and cytosolic proteins. A comparison among mouse, rat, and human revealed that the multiple isoforms are conserved in mammals (16). Similar to what has been described in mammals, a recent report (41) has revealed that one of the two *Drosophila TR* genes codes for two isoforms of the enzyme, a cytosolic and a mitochondrial one. Likewise, alternative splicing forms of human, mouse, and rat Grx mRNA do occur. Two recent reports (17, 42) indicate extensive variations in the 5′-sequences of Grx mRNAs because of alternative first exon splicing. So far, the generation of gene product variants that localize to different compartments from a single *TGR* gene has not been previously reported. Indeed, mouse TGR originally isolated from the testis has been reported to be present exclusively in the microsomal fraction (19). The recently characterized TGR cDNA from *S. mansoni* encodes a cytosolic TGR (20). In addition, our results suggest that alternative transcription and trans-splicing constitute mechanisms capable of contributing to increase in gene product diversity for the thioredoxin and glutathione systems and possibly more general mechanisms that enriches and regulates the expression of the genomes from organisms employing RNA trans-splicing.

The two cDNA isoforms encode a mitochondrial and a cytosolic TGR variant. The immunoblot of subcellular fractions of *E. granulosus* protoscoleces revealed the presence of TGR in both compartments. As already mentioned, the $_{mit}$TGR variant contains all of the elements to be trans-located to this compartment. However, there remains an intriguing or at least curious feature of the N-terminal presequence of $_{mit}$TGR, the presence of four Cys residues. In this regard, it is interesting to note that localization of proteins has been reported to be dependent on the redox state of thiol groups (43, 44). It is possible to speculate that the mitochondrial presequence may act as a localization switch, becoming available to the protein import machinery of the mitochondria only when the Cys residues of the presequence are oxidized or reduced.

The cytosolic and the mitochondrial processing peptidases processed mitochondrial TGR variants are expected to be almost identical regarding their biochemical properties, because they have the same amino acid sequence with the exception of an extra N-terminal Ala residue in the mitochondrial variant. Parasite material is the major drawback of studying this organism and precluded purification and further characterization of each TGR variant. We are currently attempting to clone both variants for expression in a eukaryotic vector.

As expected, the purified native enzyme possesses TR activity. In addition, the purified TGR possesses Grx activity in a reaction mixture containing yeast GR and glutathione. This activity was only partially inhibited by 10 nM auronofin, a concentration that completely inhibited TR activity, and did not affect yeast GR. This finding suggests that the glutaredoxin domain of TGR can accept electrons from both the Cys-Sec redox center and the glutathione system. In other words, the Grx domain of TGR can also function independently of TR domains. The purified enzyme also possesses Grx activity in the absence of yeast GR, this activity being completely inhibited by auronofin, indicating that the Cys-Sec redox center participates in the electron transfer toward oxidized glutathione. Because this assay is driven by the reduction of oxidized glutathione, this result implies that the enzyme also possesses GR activity. Indeed, in GR assays, the purified enzyme was capable of reducing oxidized glutathione, an activity inhibited by auronofin, a known selective inhibitor of selenocysteine containing thioredoxin reductases. Because conventional thioredoxin reductases (*i*.e. without a Grx domain) cannot reduce oxidized glutathione, it is reasonable to speculate that the transfer of electrons from the Cys-Sec redox center toward oxidized glutathione occurs through the Grx domain. Thus, in this situation, the electron flow in TGR would include a step in the opposite direction to the flow in the GSH system. Because the standard redox potentials of GSH (−240 mV) and Grx (−233 to −198 mV) have near values (45), the reduction of GSH by the Grx domain would be thermodynamically favorable when the concentrations of reduced Grx and oxidized glutathione increase. The interaction of the C-terminal redox center with the Grx domain has been predicted based on molecular modeling of TGR structure. This together with biochemical data has led us to suggest that the direction of electron transfer in TGR is from the Cys-Sec redox center toward the Grx domain and downstream electron acceptor (19). Although not very efficient, EgTGR was also capable of reducing β-hydroxyethyldisulfide independently of GSH (*i.e.* in the absence of exogenous GSH).

Thus, taken together, the biochemical characterization of TGR indicates that the Grx and TR domains of the enzyme can function either coupled or independently of each other, because the Grx domain can accept electrons from either TR domains or the glutathione system and the TR domains can transfer electrons to either the fused Grx domain or to *E. granulosus* thioredoxin.

In addition to the biochemical data, the results presented in this article suggest that EgTGR seems to be the major Se-pyridine-disulfide oxidoreductase present in *E. granulosus* protoscolex. Indeed, conventional TR seems to be absent in the larval worm. As already mentioned, the only protein recognized

by an antiserum raised against the central fragment of EgTGR was the 66-kDa band that corresponds to TGR. Consistently, purification of TR activity from protoscolex extracts resulted in the exclusive isolation of a 66-kDa band. It is interesting to note that for the helminth parasite *S. mansoni*, it has been proposed that both GR and conventional TR may be absent in the adult worm and that these functions would be carried out exclusively by TGR (20).

Together, the thioredoxin and glutathione systems constitute mechanisms of electron transfer to numerous substrates and substrate reductases, participate in many cellular and metabolic processes, and play an important role as a primary line of defense against oxidative damage. The role of the thioredoxin and GSH systems in the mitochondria is not fully understood. In yeast, the absence of mitochondrial Grx (Grx5) leads to constitutive oxidative damage, causing iron accumulation in the cell and inactivation of enzymes requiring iron/sulfur clusters for their activity (46). *E. granulosus* is a parasite; thus, it is subjected not only to endogenous oxidants but also to the oxidative stress imposed by the cells of the immune system of its hosts. Activated macrophages and neutrophils can release on the parasite surface enormous amounts of superoxide anion and nitric oxide that give rise to hydrogen peroxide and potent oxidants such as hydroxyl radical and peroxynitrite. Thus, oxidative stress control is central to parasite survival. We have previously reported the existence of *E. granulosus* genes encoding leaderless thioredoxin and thioredoxin peroxidase and the capacity of the larval worm to be metabolically competent toward high hydrogen peroxide concentrations (23) (47). An examination of a recent *E. granulosus* EST data base (www.nematodes.org/Lopho/LophDB.php) (22) seems to reinforce the idea that antioxidant defenses are also critical in the mitochondria. Both a thioredoxin and a thioredoxin peroxidase with putative mitochondrial trans-location signal were identified. Therefore, *E. granulosus* would possess in the cytosolic and mitochondrial compartments not only TGR but also Trx and thioredoxin peroxidase.

Thus, the results presented in this article indicate that *E. granulosus* thioredoxin glutathione reductase is a multifunctional parasite enzyme that possesses a functional Grx and TR domain fusion capable of shuttling electrons to targets of both thioredoxin and glutathione systems and that mitochondrial and cytosolic variants of the enzyme are generated from a single *TGR* gene.

Acknowledgments—We thank Professor Ricardo Ehrlich (Department of Biochemistry, Universidad de la República, Montevideo, Uruguay); Professor Murray Selkirk (Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, United Kingdom); Dr. Cecilia Fernández, Dr. Álvaro Díaz, Florencia Irigoín (Cátedra de Inmunología, Universidad de la República, Montevideo, Uruguay); and Dr. Klaus Brehm (Institut fur Hygiene und Mikrobiologie, Universität Würzburg, Würzburg, Germany) for helpful discussions and suggestions.

REFERENCES

1. Arner, E. S., and Holmgren, A. (2000) *Eur. J. Biochem.* **267,** 6102–6109
2. Halliwell, B. (1999) *Free Radical Res.* **31,** 261–272
3. Nordberg, J., and Arner, E. S. (2001) *Free Radical Biol. Med.* **31,** 1287–1312
4. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., and Yodoi, J. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 3633–3638
5. Rhee, S. G. (1999) *Exp. Mol. Med.* **31,** 53–59
6. Finkel, T. (2000) *FEBS Lett.* **476,** 52–54
7. Gladyshev, V. N., Jeang, K. T., and Stadtman, T. C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 6146–6151
8. Zhong, L., Arn-er, E. S., Ljung, J., Aslund, F., and Holmgren, A. (1998) *J. Biol. Chem.* **273,** 8581–8591
9. Gladyshev, V. N., Krause, M., Xu, X. M., Korotkov, K. V., Kryukov, G. V., Sun, Q. A., Lee, B. J., Wootton, J. C., and Hatfield, D. L. (1999) *Biochem. Biophys. Res. Commun.* **259,** 244–249
10. Zhong, L., Arner, E. S., and Holmgren, A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 5854–5859
11. Stadtman, T. C. (1996) *Annu. Rev. Biochem.* **65,** 83–100
12. Watabe, S., Makino, Y., Ogawa, K., Hiroi, T., Yamamoto, Y., and Takahashi, S. Y. (1999) *Eur. J. Biochem.* **264,** 74–84
13. Lee, S. R., Kim, J. R., Kwon, K. S., Yoon, H. W., Levine, R. L., Ginsburg, A., and Rhee, S. G. (1999) *J. Biol. Chem.* **274,** 4722–4734
14. Miranda-Vizuete, A., Damdimopoulos, A. E., and Spyrou, G. (2000) *Antioxid. Redox Signal* **2,** 801–810
15. Osborne, S. A., and Tonissen, K. F. (2001) *BMC Genomics* **2,** 10
16. Sun, Q. A., Zappacosta, F., Factor, V. M., Wirth, P. J., Hatfield, D. L., and Gladyshev, V. N. (2001) *J. Biol. Chem.* **276,** 3106–3114
17. Lundberg, M., Johansson, C., Chandra, J., Enoksson, M., Jacobsson, G., Ljung, J., Johansson, M., and Holmgren, A. (2001) *J. Biol. Chem.* **276,** 26269–26275
18. Missirlis, F., Ulschmid, J. K., Hirosawa-Takamori, M., Gronke, S., Schafer, U., Becker, K., Phillips, J. P., and Jackle, H. (2002) *J. Biol. Chem.* **277,** 11521–11526
19. Sun, Q. A., Kirnarsky, L., Sherman, S., and Gladyshev, V. N. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 3673–3678
20. Alger, H. M., and Williams, D. L. (2002) *Mol. Biochem. Parasitol.* **121,** 129–139
21. Brehm, K., Jensen, K., and Frosch, M. (2000) *J. Biol. Chem.* **275,** 38311–38318
22. Fernandez, C., Gregory, W. F., Loke, P., and Maizels, R. M. (2002) *Mol. Biochem. Parasitol.* **122,** 171–180
23. Chalar, C., Martinez, C., Agorio, A., Salinas, G., Soto, J., and Ehrlich, R. (1999) *Biochem. Biophys. Res. Commun.* **262,** 302–307
24. Gonzalez, G., Spinelli, P., Lorenzo, C., Hellman, U., Nieto, A., Willis, A., and Salinas, G. (2000) *Mol. Biochem. Parasitol.* **105,** 177–184
25. Luthman, M., Eriksson, S., Holmgren, A., and Thelander, L. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 2158–2162
26. Holmgren, A. (1979) *J. Biol. Chem.* **254,** 9627–9632
27. Carlberg, I., and Mannervik, B. (1985) *Methods Enzymol.* **113,** 484–490
28. Holmgren, A., and Aslund, F. (1995) *Methods Enzymol.* **252,** 283–292
29. Gromer, S., Arscott, L. D., Williams, C. H., Jr., Schirmer, R. H., and Becker, K. (1998) *J. Biol. Chem.* **273,** 20096–20101
30. Darley-Usmar, V., Rickwood, D., and Wilson, M. T. (1987) in *Mitochondria: a Practical Approach* (Darley-Usmar, V., Rickwood, D. H., and Wilson, M. T., eds) 1st Ed., pp. 160–161, IRL Press, Oxford, United Kingdom
31. Low, S. C., and Berry, M. J. (1996) *Trends Biochem. Sci.* **21,** 203–208
32. Grundner-Culemann, E., Martin, G. W., III, Harney, J. W., and Berry, M. J. (1999) *RNA* **5,** 625–635
33. Korotkov, K. V., Novoselov, S. V., Hatfield, D. L., and Gladyshev, V. N. (2002) *Mol. Cell. Biol.* **22,** 1402–1411
34. Goto, C., Osaka, T., and Mizutani, T. (2001) *Biofactors* **14,** 25–35
35. Pfanner, N., and Geissler, A. (2001) *Nat. Rev. Mol. Cell. Biol.* **2,** 339–349
36. Moriwaki, K., Ogishima, T., and Ito, A. (1999) *J. Biochem.* **126,** 874–878
37. Ito, A. (1999) *Biochem. Biophys. Res. Commun.* **265,** 611–616
38. Kanzok, S. M., Schirmer, R. H., Turbachova, I., Iozef, R., and Becker, K. (2000) *J. Biol. Chem.* **275,** 40180–40186
39. Kanzok, S. M., Fechner, A., Bauer, H., Ulschmid, J. K., Muller, H. M., Botella-Munoz, J., Schneuwly, S., Schirmer, R., and Becker, K. (2001) *Science* **291,** 643–646
40. Miranda-Vizuete, A., Damdimopoulos, A. E., Pedrajas, J. R., Gustafsson, J. A., and Spyrou, G. (1999) *Eur. J. Biochem.* **261,** 405–412
41. Missirlis, F., Phillips, J. P., and Jackle, H. (2001) *Curr. Biol.* **11,** 1272–1277
42. Gladyshev, V. N., Liu, A., Novoselov, S. V., Krysan, K., Sun, Q. A., Kryukov, V. M., Kryukov, G. V., and Lou, M. F. (2001) *J. Biol. Chem.* **276,** 30374–30380
43. Kuge, S., Jones, N., and Nomoto, A. (1997) *EMBO J.* **16,** 1710–1720
44. Kuge, S., Arita, M., Murayama, A., Maeta, K., Izawa, S., Inoue, Y., and Nomoto, A. (2001) *Mol. Cell. Biol.* **21,** 6139–6150
45. Aslund, F., Berndt, K. D., and Holmgren, A. (1997) *J. Biol. Chem.* **272,** 30780–30786
46. Rodriguez-Manzaneque, M. T., Tamarit, J., Belli, G., Ros, J., and Herrero, E. (2002) *Mol. Biol. Cell* **13,** 1109–1121
47. Salinas, G., Fernandez, V., Fernandez, C., and Selkirk, M. E. (1998) *Exp. Parasitol.* **90,** 298–301

ENZYME CATALYSIS AND REGULATION:

Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of *Echinococcus granulosus* Thioredoxin Glutathione Reductase

Astrid Agorio, Cora Chalar, Soledad Cardozo and Gustavo Salinas

J. Biol. Chem. 2003, 278:12920-12928.
doi: 10.1074/jbc.M209266200 originally published online January 21, 2003