Molecular and functional characterization and tissue localization of 2 glucose transporter homologues (TGTP1 and TGTP2) from the tapeworm *Taenia solium*

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SUMMARY

Tapeworms absorb and consume large quantities of glucose through their syncytial tegument, storing the excess as glycogen. Although some studies on the metabolism of glucose in several tapeworms are available, the proteins that mediate its uptake and distribution in their tissue have not been identified. We describe the isolation and characterization of cDNA clones encoding 2 facilitated diffusion glucose transporters (TGTP1 and TGTP2) from *Taenia solium*, the causal agent of human and porcine cysticercosis. Radio-isotope labelled hexose uptake mediated by TGTP1 expressed in *Xenopus* oocytes is inhibited by the natural stereoisomers D-glucose and D-mannose but not by L-glucose. Transport by TGTP1 is sensitive to classical inhibitors of facilitated diffusion such as phloretin and cytochalasin B, and insensitive to ouabain. TGTP2 did not function in *Xenopus* oocytes. Localization studies using specific anti-TGTP1 and anti-TGTP2 antibodies show that TGTP1 is abundant in a number of structures underlying the tegument in adult parasites and larvae, whereas TGTP2 appears to be localized only on the tegumentary surface of the larvae and is not detected in adults.

Key words: facilitated diffusion glucose transporter, tapeworm parasites, *Taenia solium*, cysticercosis, cDNA, localization, tegument.

INTRODUCTION

Tapeworms lack a digestive tract and carry out metabolic exchange with their host through the syncytial tegument. When glucose is available, the parasites absorb and consume large quantities, storing the excess as glycogen (Roberts, 1983). Adult worms live in the intestine of vertebrates and require carbohydrates in the host diet for their normal development and reproduction (Read & Simmons, 1963). The larval stages, or metacestodes, lodge in the tissues of their intermediate hosts where surrounding fluids provide nourishment. Their maturation can be accelerated *in vitro* by culturing in a glucose-rich medium (Graham & Berntzen, 1970).

Substantial research on the acquisition, storage and catabolism of carbohydrates in tapeworms has been previously reported. For example, uptake of glucose through a mediated system has been demonstrated in several species of cestodes (Pappas & Read, 1975). Uptake by adult worms has been reported to be driven by a Na^+-dependent active transport system, similar to counterparts in the vertebrate intestine (Pappas & Read, 1975; Pappas, 1983). Metacestodes appear to take up carbohydrates by passive as well as active transport (von Brand *et al.* 1964; Arme, Middleton & Scott, 1973; Pappas, Uglem & Read, 1973; Pappas, 1983; Rosen & Uglem, 1988). In *H. diminuta*, both types of transport have been localized in different tissues of the infective cysticercoid; facilitated diffusion is associated primarily with the cyst wall and is lost when the larva excysts in the vertebrate host, whereas Na^+-dependent active transport is associated with the external plasma membrane of the scolex (Rosen & Uglem, 1988).

The molecules that mediate the uptake and distribution of carbohydrate in the tissue of the cestodes have not previously been identified. In this report, we describe the isolation and characterization of 2 cDNA clones containing the complete coding sequence for 2 glucose transporter homologues of *T. solium* (TGTP1 and TGTP2). TGTP1 has been functionally expressed in *Xenopus* oocytes and shown to have properties typical of facilitated diffusion

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glucose transporters. Immunolocalization studies indicate that TGTP1 is abundant in a number of structures underlying the tegument of larval and adult *T. solium*; its homologues are also present in *T. saginata* and *T. crassiceps*. In contrast, TGTP2 appears preferentially on the external surface of larval-stage parasites.

MATERIALS AND METHODS

Isolation and characterization of T. solium *glucose transporters* (*TGTP*) *cDNA*

A probe was obtained by PCR amplification of *T. solium* cysticerci cDNA, using degenerate primers designed from conserved amino acid sequence motifs of human (GLUT1) and *Schistosoma mansoni* (SGTP1, 2 and 4) facilitated diffusion glucose-transporters (see Fig. 2 in Skelly *et al*. 1994). The sense primer TGTP-2cg (5′-CAGCAGTTCTCC-GGCAT(A/C/T)AA(C/T)G-3′) was designed from amino acid positions 281–288 in SGTP1, and the antisense primer TGTP-3cg (5′-TCGTCGAA-GGTGCGGCCCTT(A/C/G/T)GT(T/C)TC-3′) from position 460–468. PCR utilized 25 cycles, annealing at 50 °C/60 sec, extension at 72 °C/60 sec and denaturation at 94 °C/60 sec. The product was ethanol precipitated and run on a 1% agarose gel, and the region of the gel containing DNA of approximately 550 bp was excised, purified by glass affinity and used as a template in a second PCR under the same conditions. The now apparent amplified 550 bp DNA fragment was radio-isotope labelled with the Random Primed DNA Labelling Kit (U.S.B. Corp., Ohio) and [α^{32}-P]dATP (Amersham International plc) and used to screen about 80000 plaques of a *T. solium* cysticerci cDNA library prepared as previously described (Landa *et al*. 1993). Hybridization was carried out at high stringency (65 °C, 2 × SSC), and several positively hybridizing plaques were isolated, each representing isolates of TGTP2 cDNA.

A second strategy for isolating glucose transporter clones from the cysticerci cDNA library was to use a 1 kb DNA probe containing the amino-terminal coding end of the *S. mansoni* glucose transporter SGTP1 as a probe (Skelly *et al*. 1994). The amplified DNA fragment was radio-isotope labelled and used to screen the cDNA library at low stringency (50 °C, 6 × SSC). This strategy resulted in the isolation of TGTP1 cDNA clones.

The inserts in all selected clones were subcloned into M13 vectors and sequenced by the dideoxy chain-termination method (Sanger, 1977). The complete sequences were obtained from both strands of the cDNA using synthetic oligonucleotides. Sequences included in this paper have been submitted to the GeneBank with accession numbers: U39197 (for TGTP1) and U62917 (for TGTP2).

Functional expression of TGTPs in Xenopus *oocytes*

Functional expression of the 2 TGTP cDNA clones was characterized essentially as described by Skelly *et al*. (1994). Briefly, the complete coding regions of the 2 glucose transporters were amplified by PCR and ligated into the *Xenopus* expression vector, pSP64T. Linear DNA was prepared from the resulting plasmids and used to synthesize RNA *in vitro*. Approximately 50 ng of RNA were injected into each *Xenopus* oocyte. For analysis of glucose uptake, groups of 5–6 oocytes were incubated at room temperature in a buffer containing 0·1 mM 2-deoxyglucose, 1 μCi/ml [1,2-^{3}H]2-deoxyglucose for 1 h. Individual oocytes were washed, solubilized with 0·2 ml of 2% SDS and the incorporated radioactivity was evaluated by liquid scintillation counting. The effects of several sugars and inhibitors (at 10 mM and 1 mM, respectively) on the transport of 2-deoxyglucose were studied. When assaying the effects of the inhibitors, the oocytes were pre-incubated with the compounds 30 min prior to the start of the transport assay. To evaluate the effect of sodium on glucose transport, choline chloride (100 mM) replaced NaCl in the buffer. Experiments were undertaken twice, and the data are presented as the mean ± the standard deviation of uptake by at least 4 individual oocytes. Data were compared by Student's two-tailed *t*-test ($P < 0{\cdot}05$).

Localization studies

T. solium cysts were dissected from skeletal muscles of infected pigs obtained in Mexico City. *T. crassiceps* cysticerci (ORF strain) were obtained from the peritoneal cavity of BALB/c mice and maintained in the laboratory. *T. saginata* adult worms were kindly donated by A. Aluja, Facultad de Medicina Veterinaria y Zootecnia, UNAM, and *T. solium* adult worms were obtained from experimentally infected hamsters (kindly provided by J. Ambrosio and A. Flisser, Facultad de Medicina, UNAM). All larvae and adult worms were washed 3 times with sterile phosphate-buffered saline, pH 7·2 (PBS) and used immediately for localization studies or stored at −70 °C until use.

For the preparation of a specific antiserum, 2 peptides were synthesized (C. Dahl, Harvard Medical School), based on the extrapolated amino acid sequence at the carboxyl-terminus of both TGTP1 (NH2-CEAATALRRSDEEDAKVDA-COOH) and TGTP2 (NH2-CRSLPSENGENMTKSDR-VKF-COOH), with a cysteine added to the amino terminus of each peptide for coupling to a carrier. Peptides were coupled to BSA and to ovalbumin, and the BSA-coupled peptides were injected subcutaneously into rabbits as previously described (Zhong *et al*. 1995). Immune sera were collected, and

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Table 1. Amino acid sequence comparisons of TGTP1 and TGTP2 from *Taenia solium* with several glucose transporters from other organisms

	TGTP1 (%)		TGTP2 (%)	
Protein*	Identity	Similarity	Identity	Similarity
TGTP2	28·0	47·2	—	—
GLUT1	31·9	49·2	37·2	53·0
GLUT3	30·8	48·8	37·3	53·6
GLUT4	32·2	49·9	36·0	52·3
SGTP1	56·2	67·6	26·9	43·8
SGTP2	25·2	42·1	41·9	56·0
SGTP4	47·9	64·6	27·7	47·3

* TGTP1 and 2 are *T. solium* glucose transporters; GLUT1, 3 and 4 are human glucose transporters; SGTP1, 2 and 4 are *Schistosoma mansoni* glucose transporters.

the anti-peptide antibodies were purified by affinity chromatography to the ovalbumin-coupled peptides conjugated to NHS-activated HiTrap columns (Pharmacia Inc.). Retained antibodies were eluted in 0·1 M glycine, pH 2·5, neutralized with Tris, pH 8, and dialysed exhaustively against PBS. Purified antibodies against the C-terminal peptides are referred to as anti-TGTP1 or anti-TGTP2.

Immunofluorescence microscopy was performed on frozen sections of the parasite tissues. Sections 6–8 μm thick were air dried on poly-L-lysine coated slides, rehydrated with PBS, pre-incubated for 30 min with 20% normal goat serum in PBS as a blocking reagent, washed with PBS–0·15% Tween 20, and incubated overnight at 4 °C with the anti-TGTP1 or anti-TGTP2 antibody fractions (100 μg/ml in PBS–0·15% Tween 20, 3% BSA). Slides were extensively washed and incubated in the dark for 1 h at room temperature with a fluorescein-conjugated $F(ab)_2$ goat anti-rabbit IgG (Boehringer Mannheim, Biochemicals) at 2 μg/ml. The slides were washed again and mounted with cover-slips using 1:9 glycerol–PBS. Control sections were incubated with the second antibody only. All sections were photographed in a Nikon Optiphot Epi-fluorescence microscope.

Expression of TGTPs in insect cells

For the recombinant expression of the TGTP1 and TGTP2 in insect cells, the complete coding sequences of TGTP1 and TGTP2 were amplified by PCR as described earlier and subcloned into the baculovirus vector pVL1393. Recombinant plasmids containing TGTP1 and TGTP2 coding sequences in the correct orientation and baculovirus DNA were used to co-transfect Sf9 cells as described previously (Zhong *et al*. 1995). Detection of TGTP1 and TGTP2 in membrane fractions of transformed insect cells after 48 h of infection, and of *T. solium* and *T. crassiceps* cysts, was carried out by Western blot (Towbin, Staehelin, & Gordon, 1979). Membrane fractions from Sf9 cells and cysts were prepared as described by Zhong *et al*. (1995), except that frozen cysts were homogenized using a Polytron homogenizer (Brinkmann Instruments) at maximum speed for 1–2 min at 4 °C before sonication. A goat anti-rabbit IgG conjugated to peroxidase (Sigma) 1:1000 dilution was used as second antibody and detected by reaction with 0·05% 4-chloro-1-naphthol.

RESULTS

Characterization of 2 T. solium *glucose transporter cDNAs*

Two full-length cDNA clones were obtained from a *T. solium* cysticerci cDNA library with probes based on homologies to conserved amino acid sequence motifs of human and *Schistosoma mansoni* glucose transporters as described in the Materials and Methods section. One 1·8 kb clone showed strong coding homology to the *S. mansoni* glucose transporter, SGTP1, and was designated TGTP1. A second 1·7 kb clone was designated TGTP2 because of its greater coding homology with SGTP2, a *S. mansoni* glucose transporter homologue (Table 1). Extensive efforts to amplify additional TGTP cDNAs with other conserved sequence motif primer combinations, or low stringency hybridization with *S. mansoni* glucose transporter cDNA clones were unsuccessful. TGTP1 and TGTP2 have 3′-untranslated regions of 235-bp and 136-bp, respectively, ending in poly-A tails, whereas only TGTP2 has the typical consensus poly(A) addition site (AATAAA), 20-bases upstream of the poly-A tract.

The predicted translation products of TGTP1 and TGTP2 encoded proteins with characteristics typical of facilitated diffusion glucose transporters (Mueckler *et al*. 1985). They were about 55 kDa each with 12 predicted transmembrane α-helices, relatively large hydrophilic loops between transmembrane helices 1–2 and 6–7, and hydrophilic sequences at the amino- and carboxyl- ends (Fig. 1). A single putative *N*-linked glycosylation site on the first external loop, typical of eukaryotic transporters, was present only in TGTP1. Absence of this site has been reported in glucose transporters of plants and protozoa (Stack, Stein & Landfear, 1990; Langford *et al*. 1994; Sauer & Tanner, 1989; Stadler *et al*. 1995). In addition to their structural similarities, both proteins have substantial levels of protein sequence identity and similarity to all known members of the superfamily of facilitated diffusion glucose transporters. TGTP1 shows 56% identity and 67·6% similarity with SGTP1, whereas TGTP2 is most closely related with SGTP2 (42% identity and 56% similarity). Identity between TGTP1 and TGTP2 is only 28% (see Table 1).

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TGTP1                    MKGISGPLVLAIFTTCFGSSFLLGYNLGVANLPGDNIKKFLVNY  44

TGTP2                      MVNFHY.F.TVVIV.....QF.FQT..I.S.LPL.E.YILSI  42
GLUT1                 MEPSS.KLT.R.M..VGGAVL..-LQF...T..I.A.QKV.EE.YNQT  47
GLUT3                   MGTQKVTPA.IF..TVATI..-.QF...T..I.A.EKI..I.INKT  45
GLUT4 MPSGFQQIGSEDGEPPQQRVT.T....V.SAVL..-LQF...I..I.A.QKV.EQSYNET  59
SGTP1               MGVASNN..T.K...TVLI..V.....I......L...RR..EIYFNET  49
SGTP2                    MRQLKFF.PYCIITL....PF..HT..I.A.A.L..S.INTT  42
SGTP4                  MGSG.KFTKS.S.SVLLA.L....TI......L....E...E..SRT  47

TGTP1 YKPDN-------SSALNANFLYGQVTSVLVICAAIAAFTCGWVADGLGRKRSLMVNNGIG  97
TGTP2 CEDR-----GSSPSPEFVQAMSSL.VAGFP.GGIFG.LFG.S.SNKM...L..FIF.IPM  97
GLUT1 WVHRY----GESILPTTLTT.WSLSVAIFSVGGM.GS.SV.LFVNRF..RN.MLMM.LLA 103
GLUT3 LTDK----GNAPPSEVLLTS.WSLSVAIFSVGGM.GS.SV.LFVNRF..RN.MLIV.LLA 101
GLUT4 WLGRQGPEGPSSIPPGTLTT.WALSVAIFSVGGM.SS.LI.IISQW.....AML...VLA 119
SGTP1 VV.NT--------PE.DSS.F.TH.STIF.VA...G..S.........RNG.IL..V.. 101
SGTP2 LAARSVTCDE-----RFIDL.WSLCVTSFLLGGFFGGLIG.VL.NK....N..FLLSIPT  97
SGTP4 MLGK.ASEAENTANLVTPS...A..STAF.VAG..G..S..AI..C...RNG.I..SLLA 107

TGTP1 IVGSVISSVCVVANQPALLYVGRAISGLNSGLSIGIAAMFLTEIAPRHLRGMIGACNQLA 157
TGTP2 A...LLMMA.QA.VSFEMII...VLV.FAC.AFT...PVY.A....VRI...S.IMH... 157
GLUT1 F.SA.LMGFSKLGKSFEM.IL..F.I.VYC..TT.FVP.YVG.VS.TAF..AL.TLH..G 163
GLUT3 VT.GCFMGL.K..KSVEM.IL..LVI..FC..CT.FVP.YIG..S.TA...AF.TL...G 161
GLUT4 VL.GSLMGLANA.ASYEM.IL..FLI.AY...TS.LVP.YVG....T....AL.TL.... 179
SGTP1 I.G..VGP..LVK.........FVI.I...IT....SLY...V...D...G....H...V 161
SGTP2 VI..LLMMFSKM.QSFEMIII..FTI.IAC.AHTVVGP...S....VNF..AA.TF..FV 157
SGTP4 .I.GILVGP..AYS.....F...VFN.F.F.I.M...P.Y......LS...G..SLH... 167

TGTP1 ITIGIVISYVLTLSHLLNTPTLWPVAMGVGAIPAVIALIISPFTVESPRWLYLKKKDEKA 217
TGTP2 .VCA.L..QI.G.KE.MGSAK...YLL.LTI..S.VL.FLFWICPD...YIL.NSQ.LES 217
GLUT1 .VV..L.AQ.FG.DSIMGNKD...LLLSIIF...LLQC.VL..CP....F.LINRNE.NR 223
GLUT3 .VV..LVAQIFG.EFI.GSEE...LLL.FTIL..ILQSAAL..CP....F.LINR.E.EN 221
GLUT4 .V...L.AQ..G.ES..G.AE...LLL.FTI...ILQSAAL..CP....F.LINR.E.EN 239
SGTP1 .V..AF..FI.FTF....L.N...L.VAL..V..A.S.VTL..CP....F..M..HK.AE 221
SGTP2 .VSA.L..Q..S.PEVMG.TE...YLLALCTVSS..HILLLFTCP...TY..II.G.RRR 217
SGTP4 L....LV..LM..TYT........ISVA..SV..L....LL.YCP....F.FI..GK.AK 227

TGTP1 AREAFARINGSENVD-MFIAEMR-EELEVAQNQPEFKFTELFRRRDLRMPVIIAVLIQVM 275
TGTP2 .KS.LFWLR.DTE.VEEE.G.LLA.QENESE.HTK.PLKD...VKA..LALFV..VAHLA 277
GLUT1 .KSVLKKLR.TAD.T-HDLQ..K-..SRQMMREKKVTIL....SPAY.Q.IL...VL.LS 281
GLUT3 .KQILQ.LW.TQD.S-QD.Q..K-D.SARMSQEKQVTVL....VSSY.Q.I..SIVL.LS 279
GLUT4 .KQILQ.LW.TQD.S-QVL..LK-D.KRKLERERPLSLLQ.LGS.TH.Q.L....VL.LS 297
SGTP1 ..K..LQL.VK....-T..G.L.-..I...K...V....Q..TQ.......L..C....L 279
SGTP2 SEN.LVYLR.QDCDV-HAEL.LL-KLETEQSSTHKSNVCD.L.IPY..WGL.V.LVPHIG 275
SGTP4 ..K..Q.L.CIDDIN-ETFN..K-R.MHE.EKR.K...FR..TQ.......L..CI...F 285

TGTP1 QQLSGINAVVANSSEMLKSAKVSPDMLEYFVVGLGLLNVICTIVALPLLEKAGRRTLLLW 335
TGTP2 ..F.....ALFY.TSLFE.IGLT3Q-AV.ATL.V.SMI.VI.VASIF.I.RV...I..IG 336
GLUT1 .........FYY.TSIFEK.G.--QQPV.ATI.S.IV.TAF.V.S.FVV.R......H.I 339
GLUT3 .........FYY.TGIF.D.G.--QEPI.ATI.A.VV.T.F.V.S.F.V.R......HMI 337
GLUT4 .........FYY.TSIFET.G.--GQPA.ATI.A.VV.TVF.L.SVL.V.R......H.L 355
SGTP1 .........ITY..L..EL.GIPDVY.Q.C.FAI.V....V.V.S...I.R......... 339
SGTP2 ..F....GILYYFVSLFI.NGLTKQVAS.ANL.T.VTIL.GAFASIFVIDRK...P..MF 335
SGTP4 .........ITY..T...T.GIPLVYIQFC..AVPAI..LM.VLSVY.I.R......... 345

TGTP1 PSLVVAIILLLLVIFVNIANYGGVVNKT-PFVLVSAVLVFIYVAAFAMGLGPMPALIVAE 395
TGTP2 GLS.MLFSAVIIT.GLALRSHASGLV------YLAITF.Y.F.GG..I.P.SI.WFV... 390
GLUT1 GLAGM.GCAI.MT.ALALLEQLPWMS------YL.I.AI.GF..F.EV.P..I.WF.... 393
GLUT3 GLGGM.FCST.MTVSLLLKDNYNGMS------F.CIGAILVF..F.EI.P..I.WF.... 391
GLUT4 GLAGMCCCAI.MYVALLLLERVPAMS------Y..I.AI.GF..F.EI.P..I.WF.... 409
SGTP1 .TVSL.LS....T...NL.DS.PQST.N-AMGII.II.IL..ICS..L....V.....S. 398
SGTP2 GTS.CLFS...FTLTLI.KQVTEINKL.ILSIVLTYTFL.GFSVS-------I.WFL.S. 388
SGTP4 .TVLL.FS..C.T.S.D..SSTKDPTTARTAGII....IILTICG..L....I.GV.... 405

TGTP1 IFRQGPRAAAYSLSQSIQWACNLIVVASFPSLNELLKGYVYLPYLVVVAVCWVVFFLFMP 454
TGTP2 M.V.ET.DP.IVITVIVN.LAQIVISLGY.P.LKY..D.SFM.FIGLLVIFIALLYF.L. 450
GLUT1 L.S....P..IAVAGFSN.TS.F.G.MC.QYVEQ.CGP..FIIFT.LLVLFFIFTYFKV. 453
GLUT3 L.S....P..MAVAGCSN.TS.FL.GLL...AAHY.GA..FIIFTGFLITFLAFT.FKV. 451
GLUT4 L.S....P..MAVAGFSN.TS.F.IGMG.QYVA.AMGP..F.LFA.LLLCFFIFT..RV. 469
SGTP1 ..................LS....LC.Y.VIQKNIG..SF..F....VI..IF...... 458
SGTP2 L.T.EN.D..V.IAAATN.L..A..ALI..Q.VIYIGI.AFI.FICALL.VLIFVG.YL. 448
SGTP4 ....E..........GVNLL...L.LF.Y..I.DAIHH.SF..F..I.II..IF...Y.I 465

TGTP1 ETKNRTFDEVARDLAFGSIVVGKRTAALQAPVFTKEDEEAATALRRSDEEDAKVDA      510
TGTP2 ...G.APCD.QDEFVRMTGGAEDDVTLGSYTRSLPSENGENMTKSDRVKF          500
GLUT1 ...G.....I.SGFRQ.GASQSDK.PEELFHPLGADSQV                     492
GLUT3 ..RG...EDIT.AFEGQAHGADRSGKDGVMEMNSI.PAKETTTNV              496
GLUT4 ..RG....QISAAFHRTPSLLEQEVKPSTELEYLGP..ND                   509
SGTP1 ..................N.......T..EDRNL.VFTKQGNNEGPA.ESLLYPRSDNDKG 518
SGTP2 ...GK.PASIEDYEMRVCGFR.TAEHENPTFTDIID.TTQY                  489
SGTP4 ......C.SN.....TAKV.ACQ.PSR.TYKNEEPFYSDE                  505

SGTP1 MYA                                                        521
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Fig. 1. Alignment of the deduced amino acid sequences of *Taenia solium* TGTP1, TGTP2 and other glucose transporters. GLUT1, GLUT3 and GLUT4 are human glucose transporters (Kayano *et al.* 1990); SGTP1, SGTP2 and SGTP4 are *Schistosoma mansoni* glucose transporters (Skelly *et al.* 1994). Positions of amino acid identity with TGTP1 are shown as a dots. Hyphens indicate gaps introduced to maximize alignment. Underlining shows the putative membrane-spanning segments determined using the algorithm of Eisenberg *et al.* (1984).

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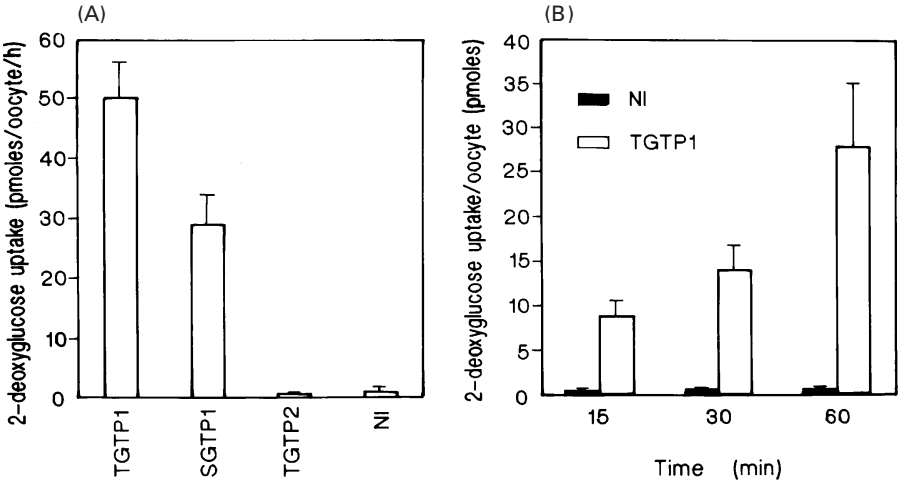

Fig. 2. Functional expression of the TGTP1 in *Xenopus* oocytes. (A) Transport of 2-deoxyglucose by oocytes injected with TGTP1, TGTP2 and SGTP1 cRNA. The basal transport is represented by uninjected oocytes (see Materials and Methods section). (B) The 2-deoxyglucose transport versus time by oocytes injected with TGTP1 cRNA compared with uninjected oocytes (NI). Data are represented as the mean ± standard deviation of uptake by 4–5 individual oocytes of a representative experiment.

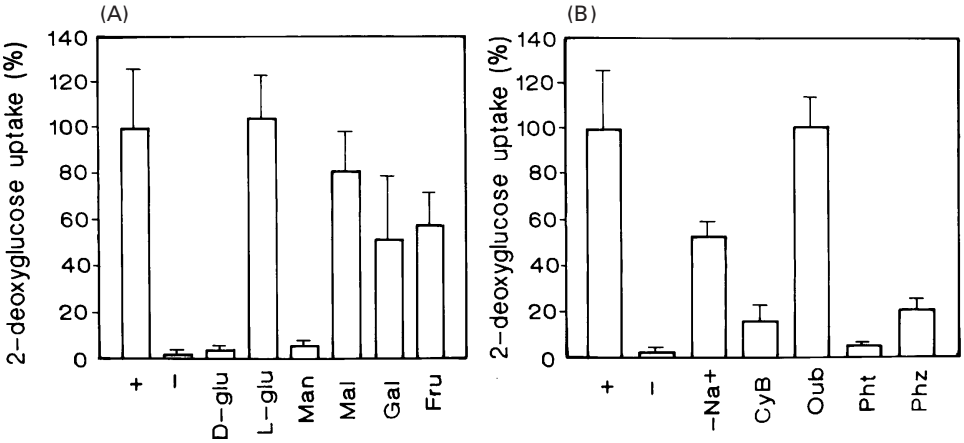

Fig. 3. Sugar specificity and sensitivity to inhibitors of TGTP1 cRNA injected oocytes. (A) Effect of competing 10 mM sugars on the transport of 2-deoxyglucose by oocytes injected with TGTP1 cRNA. The values for uninjected (−) and positive control (+) oocytes, were obtained in the absence of competing sugar. D-GLU, D-glucose; L-GLU, L-glucose; MAN, D-mannose; MAL, D-maltose; GAL, D-galactose; FRU, D-fructose. (B) Inhibition of 2-deoxyglucose transport by several potential transport inhibitors at 1 mM. Uninjected (−) and positive (+) control oocytes, were obtained in the absence of inhibitors. −Na, oocytes injected with TGTP1 RNA incubated in buffer lacking sodium; CyB, cytochalasin B; Oub, ouabain; Pht, phloretin; Phz, phlorizin. The 2-deoxyglucose transport by the oocytes injected with TGTP1 cRNA in the absence of sugar competitors or inhibitors was set to 100%.

Functional expression of T. solium *glucose transporters in* Xenopus *oocytes*

To functionally characterize the *T. solium* glucose transporters, the coding DNA of both TGTP clones were expressed in *Xenopus* oocytes. Oocytes were injected with coding RNA that was produced *in vitro* and then measured for their ability to take up radioisotope labelled 2-deoxyglucose as compared with uninjected control oocytes. As shown in Fig. 2, oocytes injected with TGTP1 RNA, took up significantly more 2-deoxyglucose than controls, and uptake was linear over a 60 min incubation. Oocytes injected with TGTP2 RNA had no increased glucose uptake during a 60 min incubation, as was previously found for its *S. mansoni* homologue, SGTP2 (Skelly *et al.* 1994). The oocyte expression system was used to characterize the substrate specificity of TGTP1. Sugar stereospecificity was assessed by competition studies using either D-glucose or L-glucose (Fig. 3A). The natural stereoisomer D-glucose, but not L-glucose, competed effectively for transport of

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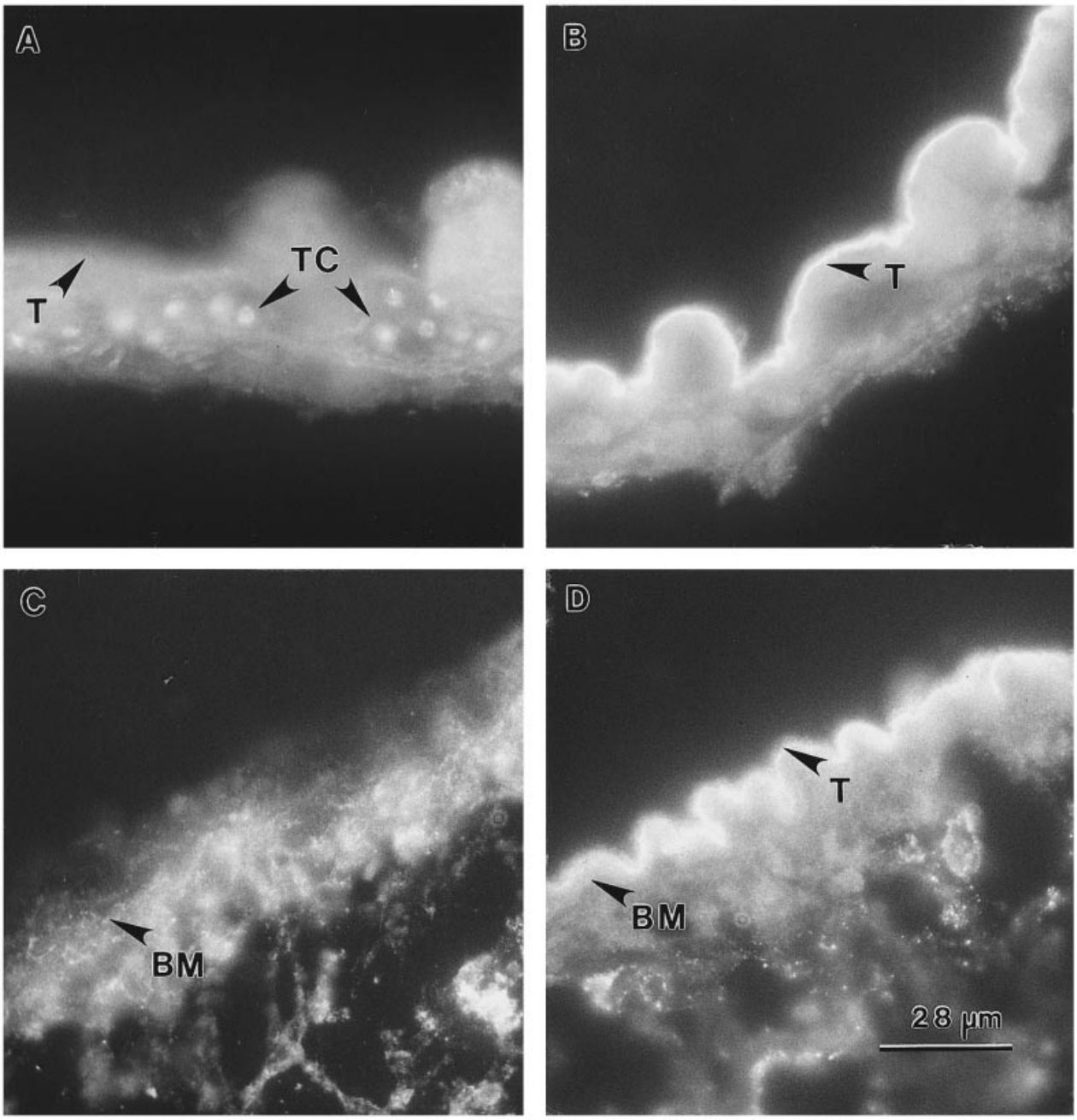

Fig. 4. Immunolocalization of TGTP1 and TGTP2 in tissue sections of *Taenia solium* (A and B) and *T. crassiceps* (C and D) larvae. Tissue sections were initially incubated with anti-TGTP1 (A and C) or anti-TGTP2 (B and D) affinity-purified antibodies and developed with a fluorescent anti-rabbit IgG. Control sections incubated in the absence of primary antibody did not show any fluorescence (not shown). BM, basal membrane; TC, tegumental cytons; T, tegument.

2-deoxyglucose. Transport inhibition studies were also carried out using the D-isomers of the following sugars at 10 mM; mannose, maltose, galactose and fructose (Fig. 3A). Mannose inhibited uptake to a level similar to that observed with D-glucose, whereas galactose and fructose were much less inhibitory. Maltose did not significantly inhibit glucose uptake by TGTP1 ($P > 0{\cdot}05$). The inhibitor of facilitated glucose transporters, phloretin, completely abolishes TGTP1-dependent glucose transport at 1 mM (Fig. 3B). Cytochalasin B, another inhibitor of facilitated glucose transporters, decreases glucose transport by TGTP1 by 80%. In contrast, no differences in the glucose uptake were seen when the oocytes were incubated in the presence of ouabain, which disrupts sodium movement by inhibiting the sodium/ATPase pump. These results demonstrate that the translation product of TGTP1 is a facilitated diffusion glucose transporter. Somewhat surprising was the moderate reduction in activity detected when sodium was not

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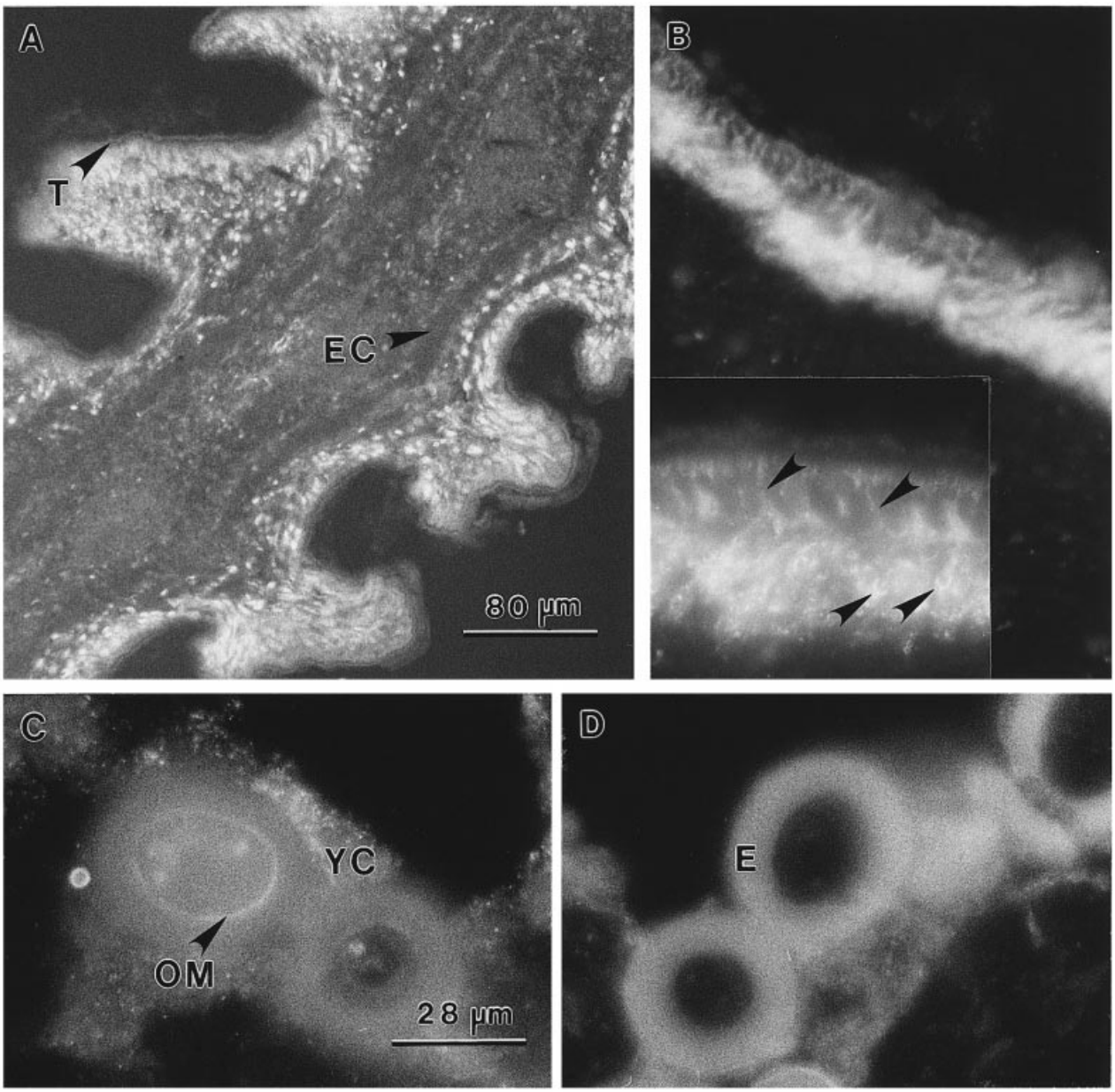

Fig. 5. Immunolocalization of TGTP1 in tissue sections of adult *Taenia solium* (A) or *T. saginata* (B and C). Sections were initially incubated with anti-TGTP1 affinity-purified antibodies and developed with fluorescent anti-rabbit IgG. Insert shows higher magnification (×60). (C) Staining of eggs within the uterus of *T. saginata*. (D) Control staining, in the absence of primary antibody. Equivalent tissue sections stained with anti-TGTP2 antibodies and in the absence of the primary antibody did not show any fluorescence (data not shown). EC, excretory canals; E, embriophore; OM, oncospheral membrane; YC, yolk cell; arrows downwards show the cytoplasmic canals and arrows upwards show the tegumental cytons.

included in the medium, and the clear sensitivity to phlorizin, another inhibitor of sodium-dependent sugar transport.

Immunolocalization of TGTP1 and TGTP2 in Taenia *parasites*

Specific rabbit antisera against TGTP1 and TGTP2 were elicited with peptides corresponding to the carboxyl terminus of the 2 glucose transporters. For localization studies, antibodies were purified by antigen affinity. The resulting antibody preparations recognized proteins of about 40–45 kDa in Western blots containing membrane fractions from *T. solium* cysts (data not shown). The specificity of each antibody was demonstrated on Western blots containing membrane fractions of recombinant insect cells expressing 1 of the TGTPs. In each case, a broad band of about 40–45 kDa in the relevant membrane fraction from recombinant insect cells was apparent and the antibodies did not recognize the other TGTP (data not shown).

Immunofluorescence was carried out on frozen sections from the bladder wall of *T. solium* larvae and

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showed that TGTP1 was localized in the tegument as well as in several structures underneath its basal membrane, including the tegumental cytons and other cellular types (Fig. 4A). Similar observations were made in localization studies of TGTP1 in *T. crassiceps* cysts, with a less intense fluorescence in the tegument (Fig. 4C). At higher magnification fluorescence appeared patchy, suggesting association of TGTP1 with the vesicular bodies within the tegument, the cytoplasmic canals and the cytons (not shown). Immunolocalization of TGTP1 on adult worm sections from *T. solium* and *T. saginata* also revealed localization in a number of structures underneath the tegument including cytons, muscle cells and deeper cellular bodies in the vicinity of the excretory canals which might correspond to the canal bodies and flame cells (Fig. 5A and B). The TGTP1 could also be visualized on sections of eggs inside the uterus of a gravid proglottid of *T. saginata*, within the yolk cell, and in the oncosphere membrane (Fig. 5C). These observations suggest that TGTP1 is involved in the uptake of glucose into a diversity of *T. solium* tissues in all developmental stages.

Immunolocalization of TGTP2 on frozen sections from the bladder wall of both *T. solium* and *T. crassiceps* cysts showed an intense fluorescent strip on the external surface of the tegument and a diffuse fluorescence underneath (Fig. 4B and D). In contrast to TGTP1, no TGTP2 could be localized on adult worms sections from *T. solium* and *T. saginata*, nor in eggs within a gravid proglottid of *T. saginata*, suggesting that its expression is developmentally regulated and restricted to the cyst stage.

DISCUSSION

The lack of an alimentary canal in cestodes defines the physiology of this group because the body-covering or tegument not only provides protection against the environment and the host immune response, but carries out both absorption of nutritive molecules and excretion/secretion of waste materials (Smyth, 1994). Adult *T. solium* are elongated worms that only live in the intestine of human beings, whereas the larval stage, or cysticercus, is a cystic organism that can survive for long periods in a diversity of tissues of its intermediate host, usually pigs. In this context, glucose transporters were expected to be associated with the tegument on the external surface of both adult and larval forms and may be candidates for intervention against the parasite. For example, glucose transporters exposed on the tegumentary surface could be potential vaccine targets for the induction of protective immune responses within hosts.

We have isolated and characterized cDNA clones encoding 2 distinct *T. solium* proteins showing substantial amino acid sequence identity with the members of the facilitated diffusion glucose transporter family. Functional expression of TGTP1 in *Xenopus* oocytes confirms its role as a facilitated glucose transporter. Results from immunolocalization studies indicate that both proteins are expressed in *T. solium* and other taeniid species (see below). These proteins, designated TGTP1 and TGTP2 according to their homology to *Schistosoma mansoni* transporters, are the first glucose transporters characterized for cestodes.

The high amino acid sequence identity (56%), and similarity (68%) of TGTP1 and its counterpart from the trematode *S. mansoni*, SGTP1, as well as their similar sugar specificity, support the concept that both proteins are orthologous transporters in cestodes and trematodes. The partial sensitivity to Na and to phlorizin in transport assays using whole cestode larvae (von Brand *et al.* 1964; Arme *et al.* 1973; Pappas *et al.* 1973), have been interpreted as indicative of the simultaneous operation of the 2 distinct types of glucose transport, i.e. active and facilitated, in larval cestodes (Pappas, 1983). In fact, removal of the cyst wall during excystment allowed the localization of an active transport in the scolex and a facilitated diffusion in the wall of cysticercoids of *Hymenolepis diminuta* (Rosen & Uglem, 1988). Our observations that TGTP1 is significantly sensitive to sodium and phlorizin, as well as its wide distribution in the tissues of adult and larval taeniids, suggests that TGTP1 provides the sensitivity to sodium and phlorizin in the whole larva.

Our attempts to demonstrate functional expression of TGTP2 in the *Xenopus* oocyte system have been unsuccessful, similar to the experience with the related *S. mansoni* homologue, SGTP2 (Skelly *et al.* 1994). However, the presence of TGTP2 on the external surface of the tegument of *T. solium* and *T. crassiceps* cysts in immunolocalization studies suggests its role in sugar uptake. It is likely that the lack of function of TGTP2 is due to improper translation within oocytes, and/or improper transport to the plasma membrane. Also possible is that some aspect of the assay conditions inhibited TGTP2 function or that the transporter did not recognize 2-deoxyglucose, used in the assay.

Glucose transporters can be grouped in 2 distinct types; energy-dependent transporters able to take up glucose against a concentration gradient relying on an electrochemical potential, and the facilitated transporters that are energy independent and simply facilitate the diffusion of glucose down a concentration gradient (Lienhard *et al.* 1992). Among parasites, glucose transporters have been studied in *Leishmania* (Langford *et al.* 1994, 1995; Piper *et al.* 1995), *Trypanosoma* (Bringaud & Baltz, 1992; Tetaud *et al.* 1994), and *S. mansoni* (Skelly *et al.* 1994). The 2 glucose transporters of *T. solium* described here add to the list of known glucose

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transporters in parasites. As expected, differences in the mechanism of glucose transport appear to emerge according to the life-style of the parasite developmental stage; *Trypanosoma* and *Leishmania* amastigotes, living within cardiac or reticuloendothelial cells of their hosts, require an active transport system to take up enough glucose from the internal milieu of those cells, whereas adult schistosomes residing in the bloodstream and taeniid larvae in the tissues of their hosts (e.g. central nervous system) live in glucose-rich environments where facilitated diffusion should suffice.

Immunolocalization studies show that TGTP1 is expressed in all developmental stages of *T. solium* and other taeniid species, whereas TGTP2 appears to be restricted to the larval stage or cysticercus. Moreover, TGTP1 appears to be localized in a variety of the cell types in the embryo, the larval and the adult stages, whereas TGTP2 appears to be predominantly localized on the surface of the cyst. This developmentally regulated expression and asymmetric location of the 2 glucose transporters is similar to observations from *S. mansoni* where SGTP1 is detected in membrane extracts of all life-cycle stages, being confined to the basal membrane and its dilations in the adult worm, whereas another transporter protein SGTP4 only appears to be exposed on apical membranes of the adult worm's tegument (Zhong *et al*. 1995; Skelly & Shoemaker, 1996). Perhaps trematodes and cestodes have developed analogous arrays, based on 2 distinct transporters, to take up and distribute glucose through the syncytial tegument to the internal tissues. In this respect it will be interesting to look for a counterpart of SGTP4 in adult *T. solium* and for SGTP2 on the apical membrane of larval stages of schistosomes.

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