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Research article

The riboflavin (vitamin B2) transporter protein (SmaRT) of the human intravascular parasitic trematode Schistosoma mansoni

Akram A. Da'dara, Roshni Gondane, Patrick J. Skelly *

Molecular Helminthology Laboratory, Department of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

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ABSTRACT

Schistosomes are intravascular parasitic worms infecting >200 million people globally. Here we examine how the worms acquire an essential nutrient - vitamin B2 (riboflavin). We demonstrate that all intravascular life stages (schistosomula, adult males and females) take up radiolabeled riboflavin. This process is impeded in the presence of excess unlabeled riboflavin and at 4 °C. We have identified a transporter homolog in worms designated SmaRT (*Schistosoma mansoni* riboflavin transporter) that localizes to the tegument and internal tissues of adults. CHO–S cells transfected with plasmid encoding SmaRT import significantly more radiolabeled riboflavin compared to controls. Uptake of radiolabel is impeded when SmaRT-expressing cells are incubated in an excess of unlabeled riboflavin but not by an excess of an irrelevant metabolite. Uptake is mediated in a sodium-independent manner and over a wide range of pH values (pH 5.5–9). This is the first identification of a *bone fide* riboflavin transporter in any platyhelminth.

1. Introduction

Schistosomiasis (bilharzia) is an infectious disease caused by parasitic platyhelminths belonging to the genus *Schistosoma*. These parasites infect over 200 million people, most commonly in Africa but also in parts of the Middle East, the Caribbean, South America, and Asia [1]. Infective larvae (cercariae) emerge from intermediate fresh-water snail hosts before penetrating the skin of a definitive host (e.g., a human or rodent). Within this final host the cercariae transform into juvenile forms called schistosomula. These travel through the vasculature to the liver and grow into adult male or females. Adult female parasites produce hundreds of eggs daily, many of which are secreted with feces or urine. However, some eggs can become trapped in host tissues, such as in the liver and bladder, and these induce inflammatory immune responses that can result in hepato-splenic, intestinal or urogenital disease [2].

Schistosome parasites can survive for several years in the blood stream of their mammalian hosts where they have easy access to all metabolites required for growth. The worms have a mouth and an intestine, and they ingest host blood as food. In addition, many metabolites can be transported into the worms directly through their outer tegumental membranes (skin) [3]. In the Molecular Helminthology Laboratory, we investigate the cellular and molecular biology of the worm tegument [4–10]. We have cloned and characterized surface proteins of *Schistosoma mansoni* that are required for the import of glucose, amino acids, and water [11–17]. How the parasites acquire other essential nutrients such as vitamins is not well understood and is the subject of the research presented here.

In this work we focus on parasite acquisition of vitamin B2, also known as riboflavin. Riboflavin is a water-soluble vitamin that is

* Corresponding author.
E-mail address: patrick.skelly@tufts.edu (P.J. Skelly).

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required for the formation of the essential coenzymes, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) [18]. These coenzymes are needed for core energy metabolism and cellular respiration. They also play central roles in the metabolism of other vitamins such as vitamin B3 (niacin), vitamin B6 (pyridoxine), and vitamin B9 (folate). Animals have lost the ability to synthesize riboflavin and must obtain it largely from food. Schistosomes too are auxotrophic for riboflavin and must acquire it from their hosts in order to ensure robust growth and development. How the worms acquire riboflavin is the subject of this work.

In humans, uptake of riboflavin is mediated by one of three plasma membrane riboflavin transporters (RFVT1-3) [19]. These transporters have different subcellular locations and tissue specificities. Riboflavin is imported into enterocytes via carrier-mediated uptake by RFVT3 [20]. To look for schistosome riboflavin transporters we first queried the *Schistosoma mansoni* genome database for RFVT homologs. In this manner, we identified a homologous sequence that we designated SmaRT for **S**. **ma**nsoni **R**iboflavin **T**ransporter. The cloning and functional characterization of SmaRT is presented here.

2. Material and methods

2.1. Parasites and mice

Adult male and female *Schistosoma mansoni* parasites (Puerto Rican strain, NMRI) were recovered by vascular perfusion from Swiss Webster mice that had been infected 7 weeks previously with approximately 100 cercariae [21]. Schistosomula were generated from cercariae recovered from infected snails, as previously [22]. All parasites were maintained in DMEM/F12 medium to which was added 10% heat-inactivated fetal bovine serum, 1 μM serotonin, 0.2 μM Triiodo-*l*-thyronine, 200 U/ml penicillin, 200 μg/ml streptomycin, 8 μg/ml human insulin. Parasites were cultured at 37 °C, in an atmosphere of 5% CO_2. All protocols involving animals were sanctioned by the Institutional Animal Care and Use Committees (IACUC) at Tufts University (Protocol: G2021-51).

2.2. Cloning cDNA encoding the schistosome riboflavin transporter homolog SmaRT

To look for homologs in schistosomes of known riboflavin transporters, we used the human RFVT2 sequence (GenBank accession number NP_001350047.1) to blast against all available *Schistosoma mansoni* sequences at NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins). A partial homolog was identified, designated XP_018646404.1 *Schistosoma mansoni* hypothetical protein Smp_194010. With this sequence as a guide, analysis of available *S. mansoni* genome data at WormBase ParaSite Assembly: ASM23792v2, allowed us to identify a potential full-length riboflavin transporter homolog as well as the SmaRT gene. Several primers were designed, including some upstream of the predicted start site and downstream of the predicted stop and these were used in PCRs using adult cDNA as template. In this manner the full-length SmaRT cDNA sequence was identified. In the most recent (March 2023) WormBase ParaSite Version (WBPS18 (WS285)), the gene is now identified as Smp_333240.1.

The predicted SmaRT sequence was analyzed for the presence of potential transmembrane domains at https://dtu.biolib.com/DeepTMHMM. Potential N-Glycosylation sites were queried with the NetNGlyc 1.0 Server (https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0). Multiple alignment analysis was conducted using CLUSTAL O (1.2.4) at http://www.ebi.ac.uk/Tools/msa/clustalo/. Phylogenetic analysis was performed at phylogeny.fr (http://www.phylogeny.fr/).

2.3. Anti-SmaRT antibody production

The SmaRT-specific peptide, NH_2-^{175}STLALQGVGSEPEC^{189}-COOH, was synthesized commercially and used to immunize New Zealand rabbits. Anti-SmaRT antibodies were affinity-purified from the immune rabbit serum using the same peptide (GenScript, Piscataway, NJ, USA).

2.4. Western blotting

Approximately 5 μg of an adult schistosome worm homogenate was resolved by SDS-PAGE and then blotted to PVDF, as previously [23]. The blot was incubated with anti-SmaRT antibody at 1:100 dilution for 1h and then washed three times with PBS containing 0.05% Tween-20 (PBST). Horseradish peroxidase-labeled anti-rabbit IgG (1:5000, GE Healthcare) was then added to samples for 1h. The blot was washed again as above and developed using ECL Western Blotting Detection Reagents (GE Healthcare). Western blot images were obtained using a ChemiDoc Touch Imaging System (Bio-Rad).

2.5. Immunolocalization of SmaRT

Worms were embedded in OCT compound and then frozen in liquid nitrogen. A cryostat was used to generate adult parasite frozen sections (7 μm thick) and these were fixed in ice-cold acetone for 30 min at −20 °C. CHO cells transfected with plasmid encoding SmaRT were fixed in 4% paraformaldehyde for 20 min, 48h after transfection. For immunolocalization, parasite sections and CHO cells were first washed ×3 in PBS. They were then incubated in PBS containing 1% BSA (blocking buffer) for 1h. Primary, purified anti-SmaRT, antibody at 1:100 dilution was next applied to the samples for 1h. Following this, samples were washed ×3 in PBST before being incubated for 1h with Alexa Fluor-488-*anti*-rabbit IgG (H + L, Invitrogen) at 1:100 dilution in blocking buffer, as in previous immunolocalization work [24]. Samples were again washed in PBS before being mounted in Fluoromount and examined by inverted fluorescent microscopy using a TH4–100, Olympus microscope. To stain nuclei, sections were incubated with DAPI (0.3 μM) for 5 min.

To stain actin, some sections were incubated with phalloidin (1 unit mixed with the secondary antibody, Invitrogen) for 1h.

2.6. SmaRT gene expression analysis

The level of expression of the SmaRT gene in three intravascular life stages of the parasite (schistosomula, adult males and adult females) was measured by RT-qPCR (reverse transcription quantitative PCR), by employing a custom TaqMan gene expression system (Applied Biosystems). To do this, RNA was first recovered from the various parasite stages with TRIzol reagent and cDNA was generated as reported previously [23]. The levels of SmaRT gene expression in the three life stages was assessed using triose phosphate isomerase as endogenous control [22]. The following reporter probe labeled with 6-carboxyfluorescein (FAM) and primer set were used (ThermoFisher Scientific): SmaRT-F: 5′- CTAGTTCAAGTCCCAATCCACCATA -3′, SmaRT-R: 5′-CAAATAAACAGCGTATCGCAATCCA-3′, SmaRT probe: 5′-FAM-CTGCCAGTGCTATCCTG-3′-NFQ. The final RT-qPCR reaction mixture contained cDNA, primer and probe and a TaqMan PCR mix in a final volume of 20 μL. PCR was performed using a Step One Plus Real Time PCR System Instrument and all samples were analyzed in triplicate. The ΔΔCt method was used for relative quantification, [22].

2.7. Riboflavin uptake experiments in parasites and SmaRT-transfected CHO cells

The ability of schistosomes to take up riboflavin was measured using [^{3}H]-labeled riboflavin (American Radiolabeled Chemicals, Inc., MO, USA). Uptake experiments were performed in Hanks Balanced salt solution (HBSS, pH7.4) containing 1.26 mM calcium and 1.0 mM magnesium (ThermoFisher Scientific) at 37 °C or at 4 °C (on ice). First, a codon-optimized SmaRT DNA sequence was synthesized and cloned in pcDNA3.1(+) at GeneScript. Next, CHO–S cells (Invitrogen) were transfected with this SmaRT plasmid using FreeStyle transfecting reagent, as described by the manufacturer (Invitrogen). About 72h later, cells were harvested, washed 3X with HBSS, counted using a hemocytometer, and distributed at 1×10^6 cells per sample. [^{3}H]-labeled riboflavin (0.5 μCi, equivalent to 0.165 μM; Specific Activity 30 Ci/mmol) was added to each sample that was then incubated at 37 °C. At the end of the incubation period (as noted in the figure legends), cells were washed several times with ice-cold HBSS and then solubilized in 100 μl lysis/solubilization buffer (0.5 N NaOH/1% SDS) at 37 °C for 2h. Lysates were transferred to scintillation fluid (5 ml, ULTIMA GOLD XR LSC COCKTAIL, Sigma) and any radioactivity associated with cells was counted using a Triathler Liquid Scintillation Counter (Hidex) and expressed as counts per min (CPM). Control cells included those transfected with an irrelevant plasmid and untransfected cells.

In some experiments, uptake of [^{3}H]-Riboflavin was competed with an >500-fold excess of unlabeled riboflavin or ribose (100 μM each). The effect of pH on riboflavin uptake by SmaRT was studied by adjusting the pH of the buffer to 5.5, 6.0, 7.4, 8.0 or 9.0. To study the effect of sodium ions on uptake, NaCl and Na_2HPO_4 in Dulbecco's phosphate-buffered saline (DPBS) were replaced with equimolar quantities of choline chloride and KH_2PO_4, respectively.

Uptake experiments using parasites were carried out essentially as just described for CHO–S cells, here using replicate parasite samples, each comprising 10^3 3-day old cultured schistosomula.

2.8. Statistical analysis

Data are presented as Mean ± SEM. For comparison of two groups, means were compared by *t*-test (two-tailed, unpaired). For

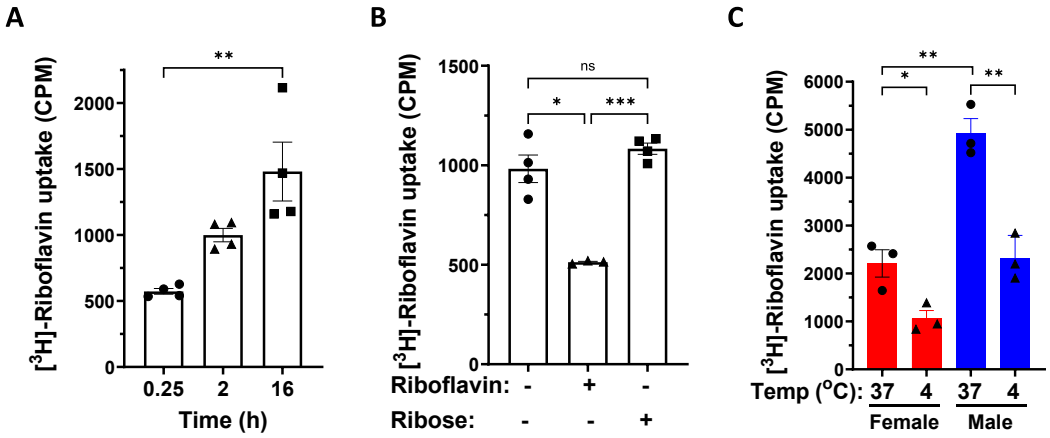

Fig. 1. Schistosomes import riboflavin. Radiolabeled (^{3}H) riboflavin uptake (mean counts per minute (CPM) ± SEM) by groups of 1,000, 3-day old schistosomula **(A)** at the indicated time points and **(B)** in the presence of either an excess of unlabeled riboflavin (center bar) or unlabeled control metabolite (ribose, right bar). Significantly less radiolabel is imported by worms in the presence of unlabeled riboflavin v unlabeled ribose. **(C)** Radiolabeled riboflavin uptake (mean CPM ± SEM) by groups of adult male (blue bars) or female (red bars) worms at 4 °C versus at 37 °C, as indicated. Data shown in panels B and C were obtained at the 2h incubation time point. Uptake is significantly greater by males versus females and is significantly greater at 37 °C for both males and females. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

comparison of more than two groups, one-way ANOVA with Tukey's post hoc analysis was employed. Analysis was performed using GraphPad Prism 10.0 (GraphPad Software). A probability value (p) of less than 0.05 was considered significant.

3. Results

3.1. Schistosomes import riboflavin

Fig. 1A shows that when 3-day old cultured schistosomula are incubated in buffer containing ^{3}H-riboflavin, radiolabel is taken up by the worms, with more being imported by the parasites the longer they are in culture. Fig. 1B shows that radiolabeled riboflavin uptake by schistosomula (left bar) is significantly impeded in the presence of excess unlabeled riboflavin (center bar; *, $p < 0.05$) but not in the presence of an equivalent amount of an irrelevant metabolite (ribose, right bar; ns, not significant). Radiolabel uptake in the presence of excess riboflavin is significantly lower than that in the presence of excess ribose (***, $p < 0.001$). Fig. 1C shows that significantly more radiolabeled riboflavin is taken in by individual male (blue bars) or individual female (red bars) adult schistosomes at 37 °C versus at 4 °C. In addition, individual males take in significantly more radiolabel than individual females. *, $p < 0.05$; **, $p < 0.01$.

3.2. Cloning the schistosome riboflavin transporter SmaRT

As described in Methods, a sequence encoding a partial schistosome homolog of human riboflavin transporter 2 (RFVT2) was identified by BLAST analysis. Using this to interrogate the available *S. mansoni* genome, we identified a full-length schistosome homolog which we designated SmaRT (GenBank accession number OR495727). This 531-amino-acid protein is predicted to have a molecular weight of 58,777 Da and a pI of 6.79. Fig. 2A shows the SmaRT sequence aligned with human homolog, RFVT3 (GeneBank accession number: NP_001357014.1). Note that, of the three human riboflavin transporters (RFVT 1–3), BLAST analysis reveals that

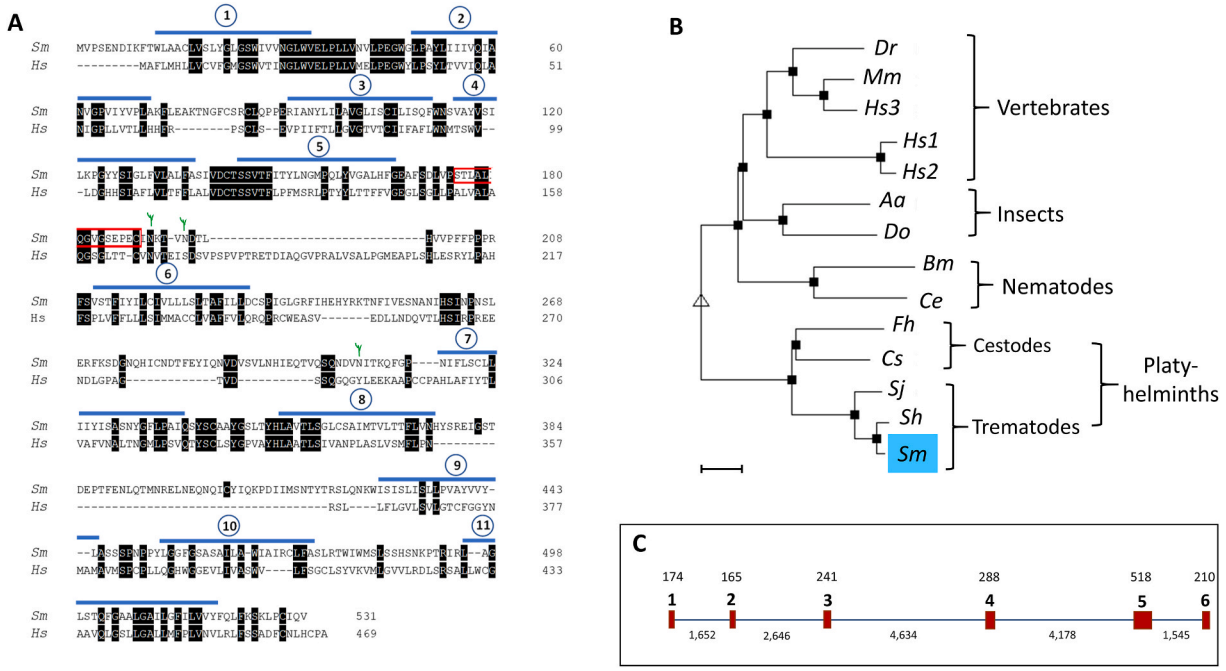

Fig. 2. Analysis of the schistosome riboflavin transporter SmaRT protein and gene. (A) Amino acid sequence alignment of the schistosome riboflavin transporter SmaRT (***Sm***) v human (***Hs***) riboflavin transporter 3 (RFVT3) generated using Clustal Omega software. Residues identical between the two transporter proteins are indicated by white text in a black background. The predicted presence of 11 transmembrane domains (numbered 1–11) is indicated by blue lines above the Sm sequence. Potential N-linked glycosylation residues are indicated by the symbol Y and the peptide used to generate anti-SmaRT antibodies is indicated by a red box. **(B)** A phylogenetic tree showing diverse riboflavin transporter homologs from different animal groups generated by multiple sequence alignment with hierarchical clustering. The *S. mansoni* sequence is indicated by a blue box. Accession numbers: Sm, *Schistosoma mansoni*, WMM48041.1; Sh, *Schistosoma haematobium*, XP_051072327.1; Sj, *Schistosoma japonicum*, KAH8867350.1; Cs, *Clonorchis sinensis*, KAG5444576.1; Fh, *Fasciola hepatica*, THD24419.1; Ce, *Caenorhabditis elegans*, NP_001033515.2; Bm, *Brugia malayi*, XP_001896372.2; Do, *Drosophila obscura*, XP_022210981.2; Aa, *Aedes albopictus*, XP_019552209.2; Hs1, Human RFVT1, NP_060456.3; Hs2, Human RFVT2, NP_001350047.1; Hs3, Human RFVT3, NP_001357014.1; Dr, *Danio rerio*, NP_001035447.1; Mm, *Mus musculus*, NP_001158291.1. **(C)** Depiction of the SmaRT gene. Six exons (numbered 1–6) are represented by red rectangles whose sizes (in bp) are given above each. Numbers below the line represent the sizes (in bp) of each intron.

SmaRT is most similar to RFVT3 (i.e., using default parameters this alignment returns the highest BitScore 167 bits(422) and lowest E-value – 3e-50). In Fig. 2A identical residues are indicated by white text in a black box. SmaRT is predicted to possess 11 transmembrane domains (numbered 1–11 and indicated by blue lines over each encoding sequence). The 3 potential N-linked glycosylation sites at residues ^{191}N, ^{195}N and ^{309}N are indicated by green symbols. The peptide ^{175}STLALQGVGSEPEC^{189}, against which anti-SmaRT antibodies were generated, is indicated by a red box. A phylogenetic tree of riboflavin transporter homologs generated by multiple sequence alignment with hierarchical clustering is shown in Fig. 2B. Accession numbers for all sequences are listed in Fig. 2 legend. The *S. mansoni* sequence is indicated by a blue box. Homologs from all members of the phylum Platyhelminthes (including other trematodes and cestodes) form a clade distinct from those in nematodes, insects, and vertebrates. The *S. mansoni* sequence is 91% identical to its homolog from *S. haematobium* and 74% identical to its homolog from *S. japonicum*. Fig. 2C represents the SmaRT gene, which comprises 6 exons (red boxes numbered 1–6) and 5 introns. The size of each exon (in base pairs) is indicated above it and the size of each intron is given below the line. The SmaRT gene spans a 16,537 bp region on the reverse strand of the Z chromosome at SM_V10_Z:67,496,290–67,512,826 (https://parasite.wormbase.org/Schistosoma_mansoni_prjea36577/Gene/Summary?g=Smp_333240;r=SM_V10_Z:67496290-67512826;t=Smp_333240.1).

3.3. Localization of SmaRT

Fig. 3A shows that anti-SmaRT antibodies (generated against the peptide indicated by the red box in Fig. 2A) recognize a single prominent protein band running at about the expected sized of SmaRT in a lysate of adult worms, as assessed by Western blot analysis (arrowhead). This antibody was used to identify the tissue distribution of the SmaRT protein (green staining) in adult worms by immunofluorescence microscopy. Blue shows DAPI-stained nuclei (in Fig. 3B and C) and red is phalloidin-stained actin (muscle tissue, in Fig. 3C). Fig. 3B shows an adult worm cross section illustrating very widespread staining for SmaRT and Fig. 3C confirms this in longitudinal section. The inset in Fig. 3C shows clear SmaRT localization (indicated by the white arrows) exterior to the muscle tissue (red) - showing that the protein is expressed in the peripheral adult worm tegument.

3.4. Developmental expression of SmaRT

As shown in Fig. 4, in the three intravascular life stages examined, (schistosomula, adult male and adult female worms), highest relative expression of SmaRT is seen in schistosomula. The figure also shows that, in relative terms, males express significantly more SmaRT than females, with schistosomula expression being significantly greater than either males or females (****, $p < 0.0001$).

3.5. Functional expression of SmaRT in CHO–S cells

Forty-eight hours after plasmid encoding SmaRT was used to transfect CHO cells, the cells were fixed in 4% paraformaldehyde and stained with anti-SmaRT antibody. Fig. 5A (top panel) shows clear staining, indicating robust protein expression in the transfected cells. Staining the cells with secondary antibody alone reveals no staining (Fig. 5A, bottom panel). Fig. 5B shows that the cells transfected with SmaRT (left bar) import significantly more radiolabeled riboflavin compared to cells transfected with a control plasmid (middle bar) or untransfected cells (None, right bar), **, $p < 0.01$; ***, $P < 0.001$; ns, not significant. Fig. 5C shows that uptake

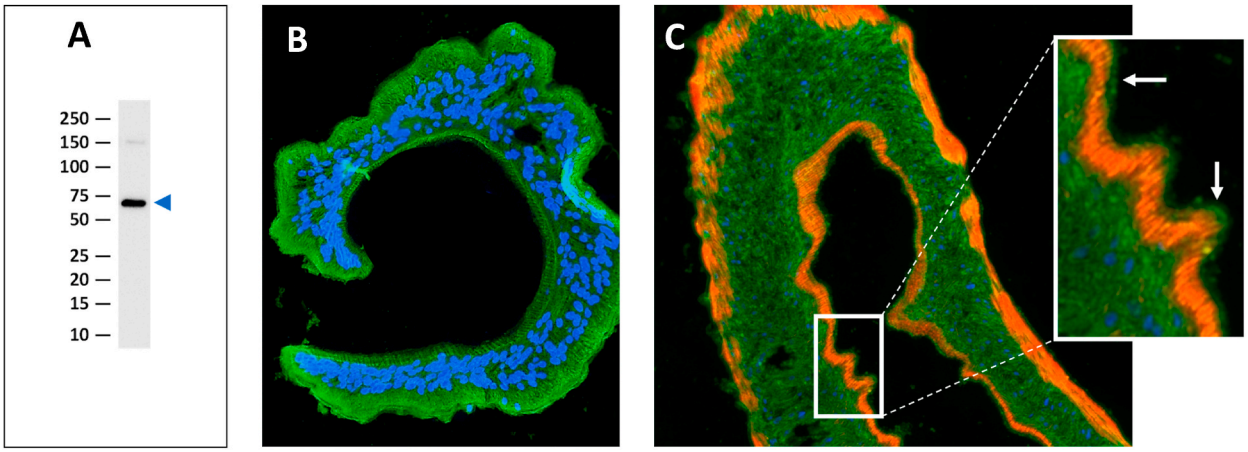

Fig. 3. Immunolocalization of SmaRT. (A) Western blot: a single major protein of the expected size of SmaRT is detected (arrowhead) using anti-SmaRT antibodies in an adult worm lysate. The positions of migration of molecular mass markers are indicated (left) where numbers represent kilobase pairs. **(B)** Cross section of an adult schistosome showing the wide distribution of SmaRT (green). DAPI-stained nuclei are visible (blue). **(C)** Longitudinal section of an adult schistosome confirming the wide distribution of SmaRT throughout the worm's body (green). DAPI-staining shows nuclei (blue) and phalloidin staining shows muscle (red). The inset highlights detection of SmaRT external to the peripheral muscle, i.e., in the tegument (white arrows).

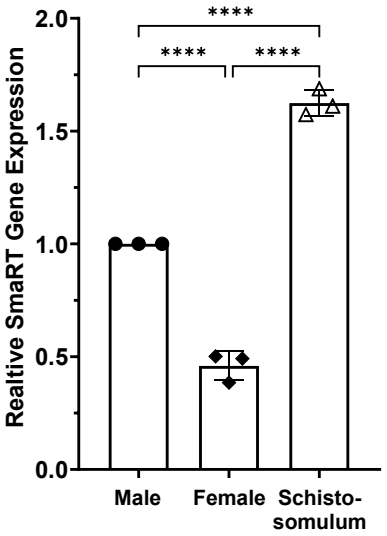

Fig. 4. Developmental Expression of SmaRT. Relative expression of the SmaRT gene in schistosome intravascular life stages, schistosomula and adult male and female worms, as indicated, determined by RT-qPCR. Relative expression in males (set at 1) is significantly higher than in females and schistosomula expression is significantly higher than both males or females (****, $p < 0.0001$).

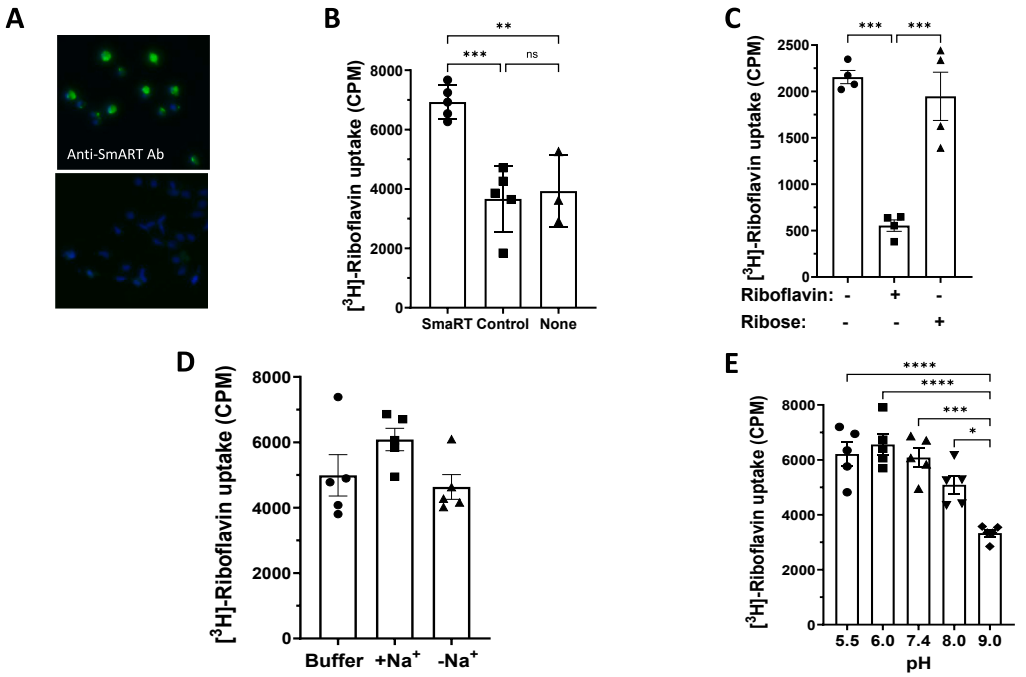

Fig. 5. Functional characterization of SmaRT following expression in CHO–S cells. (A) CHO cells transfected with plasmid encoding SmaRT are stained with affinity purified anti-SmaRT antibody, 72h post transfection (green, top panel) but not by secondary antibody alone (bottom panel). DAPI staining shows nuclei (blue). **(B).** Radiolabeled riboflavin uptake (mean CPM ± SEM) by ~10^6 CHO–S cells, in replicate, transfected with plasmid encoding either SmaRT (left) or an irrelevant protein (Control, center) or not transfected (None, right), after 6h incubation. Significantly more radiolabel is imported by SmaRT-transfected cells versus either control (**, $p < 0.01$; ***, $p < 0.001$; ns, not significant). **(C)** Radiolabeled riboflavin uptake (mean CPM ± SEM) by ~10^6 SmaRT-expressing CHO–S cells, in replicate, in the presence of either an excess of unlabeled riboflavin (center bar) or unlabeled control metabolite (ribose, right bar) after 2h incubation. Significantly less radiolabel is imported by the cells in the presence of unlabeled riboflavin (***, $p < 0.001$). **(D)** Radiolabeled riboflavin uptake (mean CPM ± SEM) by ~10^6 SmaRT-expressing CHO–S cells, in replicate, in HBSS (left bar) or in buffer either containing (center bar) or lacking (right bar) sodium (Na^+). Data shown are from the 6h incubation time point. There is no significant difference in uptake under any condition ($p > 0.05$). **(E)** Radiolabeled riboflavin uptake (mean CPM ± SEM) by ~10^6 SmaRT-expressing CHO–S cells, in replicate, in buffer at the different pH values. Data shown are from the 6h incubation time point. Uptake is significantly lower only at pH9.0, (*, $p < 0.05$; ***, $p < 0.001$; ****$p < 0.0001$).

of radiolabeled riboflavin (left bar) is significantly lessened in the presence of excess unlabeled riboflavin (middle bar, ***, $p < 0.001$) but not in the presence of an equivalent amount of an irrelevant metabolite (ribose, right bar). Fig. 5D shows that SmaRT acts in a sodium independent manner; uptake is not significantly different in SmaRT-transfected cells incubated in HBSS (buffer, left bar) or in buffer with (center bar) or without (right bar) Na^+ ($p > 0.05$). Fig. 5E shows that SmaRT functions under a wide range of pH values, as indicated, and is only significantly impaired at the most alkaline condition tested (pH 9, rightmost bar; *, $p < 0.05$; ***, $p < 0.001$; ****$p < 0.0001$).

4. Discussion

Our laboratory focuses on understanding the molecular capabilities of the tegument of intravascular schistosomes. We have found that the blood stage worms express several ectoenzymes that are capable of cleaving host metabolites in a manner that could promote parasite survival. For instance, the ectoenzyme SmATPDase1 can cleave the pro-inflammatory damage associated molecular pattern (DAMP) extracellular adenosine triphosphate (ATP), as well as the related pro-thrombotic metabolite adenosine diphosphate (ADP) [25,26]. Further, the GPI-linked alkaline phosphatase ectoenzyme SmAP can dephosphorylate adenosine monophosphate (AMP) to generate the anti-inflammatory molecule adenosine [27,28]. SmAP can also cleave the immune mediator sphingosine-1-phosphate as well as the pro-thrombotic metabolite polyP [28,29]. These capabilities are predicted to make the local vascular environment around the worms more immunologically benign and less prone to clotting – outcomes predicted to benefit the worms *in vivo* [30].

The schistosome host-interactive plasma membrane additionally expresses transporter proteins and permeases that permit the worms to import nutrients directly from the host's bloodstream across the parasite's skin and into the tegument proper [3,31]. From here, such metabolites can be shuttled to the interior tissues. For instance, SGTP4 is a sugar transporter protein that is highly expressed in the tegumental plasma membrane of intravascular life stages that can transport glucose and other hexoses into the body of the worms [11,12,32]. A second glucose transporter SGTP1, expressed on the tegumental basal membrane, as well as widely on internal tissues, can then direct some of the sugars from the tegument deeper into the body of the worms [33]. SPRM1 is a heterodimeric amino acid permease that is expressed both at the tegument surface and in the internal tissues of schistosomes [13,15]. This protein can transport selected amino acids from the external environment across the tegument and from here to the internal tissues of the blood stage worms. The parasites also express an abundant tegumental aquaporin protein - SmAQP - that regulates water flux across the parasite surface [16,17]. How the worms acquire other essential metabolites such as vitamins (like vitamin B2) is not understood.

Recent work in our lab has focused on *S. mansoni* metabolism of another B vitamin (pyridoxal, vitamin B6). The active form of vitamin B6 is pyridoxal phosphate (PLP) [34]. We showed that live intravascular stage parasites can cleave exogenous PLP to liberate pyridoxal and that this reaction is mediated by one of the ectoenzymes mentioned above – SmAP [34]. Heat-inactivated recombinant SmAP cannot cleave PLP. Furthermore, the ability of parasites whose SmAP gene has been knocked down to cleave PLP is significantly reduced in comparison to controls [34]. We hypothesized that SmAP-mediated dephosphorylation of PLP creates an excess of free pyridoxal around the worms and some of this pyridoxal can be imported by the parasites where it engages in key, vitamin B6-driven biochemistry. However, precisely how that vitamin B6 might be imported by the worms is unknown. Solute Carrier 19A2 (SLC19A2) and SLC19A3 proteins have both been shown to be capable of transporting vitamin B6 in animal cells *in vitro* [35]. However, no close homologs of these proteins can be found in schistosomes using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

In this paper we investigated vitamin B2 (riboflavin) uptake by schistosomes. We first show that intravascular life stages incubated in the presence of radiolabeled riboflavin import this essential metabolite and this is not surprising. Uptake of radiolabeled riboflavin by schistosomula *in vitro* is diminished in the presence of excess unlabeled riboflavin but not in the presence of an excess of the control metabolite ribose. This evidence, coupled with the fact that uptake of radiolabeled riboflavin by live adult male and female worms is significantly higher at 37 °C compared to 4 °C suggests that the process in schistosomes is active and carrier-mediated. In other systems, riboflavin uptake is indeed facilitated by plasma membrane riboflavin transporter proteins [19,20]. Here we used the sequence of one such transporter protein to look for homologs in schistosomes. In this manner we identified the 531-amino-acid *S. mansoni* riboflavin transporter homolog that we designated SmaRT. This multi-membrane spanning protein shares several sequence motifs that are found in previously characterized riboflavin transporters. Using the SmaRT sequence, we were able to identify homologs in the other major schistosome species parasitizing humans – *S. japonicum* and *S. haematobium* and in other platyhelminths.

We generated anti-SmaRT antibodies that detect a single major band of the expected size in parasite lysates by western blot analysis. Using these antibodies, we showed that the protein is widely expressed throughout the tissues of the adult worms, as assessed by immunofluorescence microscopy. Among those tissues, SmaRT is clearly expressed in the adult worm tegument, and we hypothesize that, at the host-interactive plasma membrane, it functions to take up riboflavin from the external environment. Given the importance of the vitamin for core cellular metabolism, it is not surprising that SmaRT is widely expressed throughout the body of the parasites. This likely permits the rapid dissemination of riboflavin throughout the body of the worm.

To better characterize SmaRT, we first expressed the protein in CHO–S cells and confirmed expression by showing that our anti-SmaRT antibodies clearly stain these cells. Next, we compared the ability of the SmaRT-expressing cells to import radiolabeled riboflavin compared to control cells expressing a second (irrelevant) protein or cells expressing no new protein (as an additional control). In this experiment, cells transfected with SmaRT take up significantly more radiolabeled riboflavin compared to either control, providing direct evidence that SmaRT is a *bone fide* riboflavin transporter. This finding is supported by the observation that uptake of radiolabel by SmaRT-expressing CHO cells is significantly lower in the presence of an excess of unlabeled riboflavin but not in the presence of an equivalent excess of an irrelevant metabolite. In agreement with the known characteristics of some other riboflavin transporting proteins, SmaRT too functions in a sodium-independent manner and works well over a relatively wide range of

pH values [36–38]. While we do not know the conditions in the microenvironment that contains SmaRT, our characterization shows that the protein could maintain its vital function over a wide pH range. This is the first report of the identification and characterization of a vitamin transporter in any platyhelminth. Blocking SmaRT function in schistosomes would minimize access by the worms to the vital metabolite riboflavin. While identifying inhibitory compounds that block only the schistosome transporter and not those of the host is a challenge, this approach, if successful, would debilitate the worms and could form the basis of a new anti-schistosomiasis treatment.

Ethical approval statement

All protocols involving animals were approved by the Institutional Animal Care and Use Committees (IACUC) of Tufts University (Protocol: G2021-51).

Data availability statement

Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Akram A. Da'dara: Writing – review & editing, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Roshni Gondane:** Writing – review & editing, Investigation, Formal analysis. **Patrick J. Skelly:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Patrick Skelly reports financial support was provided by ational Institutes of Health, NIAID, United States. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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