SPRM1lc, a heterodimeric amino acid permease light chain of the human parasitic platyhelminth, *Schistosoma mansoni*

P. J. SKELLY[1]*, R. PFEIFFER[2], F. VERREY[2] *and* C. B. SHOEMAKER[1]

[1] *Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115*
[2] *Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland*

(*Received 25 May 1999; revised 12 July 1999; accepted 12 July 1999*)

SUMMARY

The *Schistosoma mansoni* protein, SPRM1lc, is a light chain member of a new family of heterodimeric amino acid permeases. These proteins require covalent association with a type II glycoprotein (like h4F2hc) for functional surface localization when expressed in *Xenopus* oocytes. We previously reported that, when co-expressed with h4F2hc, the transport properties of SPRM1lc resemble system y and y^+ while its human homologue, E16, functions as an L-type permease. Here we extend the functional characterization of SPRM1lc in oocytes and show by competitor studies that its amino acid transport capacity is similar to that of whole adult schistosomes. We demonstrate by Northern and Western analysis that SPRM1lc is expressed within both larval and adult schistosomes. In all stages, SPRM1lc is associated into a high molecular weight complex that can be disrupted by reducing agents, consistent with the hypothesis that a significant fraction of the endogenous SPRM1lc is linked by a disulphide bond to an uncharacterized schistosome amino acid transporter heavy chain. Immunofluorescence localization detects SPRM1lc in miracidia, daughter sporocysts and adult worms. Confocal microscopy demonstrates that SPRM1lc is found in the apical membrane of the syncytial, double-lipid bilayer tegument which surrounds adult worms. Aqueous biotinylation studies on living worms show that SPRM1lc is exposed on the host-interactive surface of this tegumental membrane. Host exposed, functionally important surface proteins such as SPRM1lc could form the basis of an effective schistosomiasis vaccine. These studies are the first to describe a helminth amino acid transporter, and the first to characterize an invertebrate heterodimeric amino acid transporter.

Key words: schistosome, amino acid permease/transporter, nutrient uptake, tegument.

INTRODUCTION

Schistosomes are parasitic platyhelminths that cause the debilitating disease schistosomiasis affecting several hundred million people globally. The parasites exhibit a complex life-history in which they undertake asexual multiplication in an intermediate freshwater snail host and attain sexual maturity in their final vertebrate hosts. Adult schistosomes live in the vertebrate bloodstream where they import nutrients across the syncytial, double-lipid bilayer membrane, called the tegument, which surrounds the entire worm. This suggests that nutrient transporter proteins (or permeases) must be located in the outer, apical membrane of the tegument and must be exposed to the nutrients in the serum. Such host-exposed membrane proteins might make excellent targets for immunological or chemotherapeutic intervention.

Several glucose transporters have been characterized in schistosomes (Skelly *et al*. 1994) and one of these, SGTP4, is detected only at the host interactive apical membrane of the tegument in mammalian stage *Schistosoma mansoni* (Skelly & Shoemaker, 1996). SGTP4 expression is rapidly induced upon transformation of larval cercariae into schistosomula at the time of infection. The protein is then deposited onto the surface of the worms as they shed their cercarial glycocalyx and synthesize the new tegument. Presumably this transporter is needed to replenish hexose reserves utilized during the movement of the cercariae from the snail to its final host, and to accommodate the shift from oxidative glucose metabolism to largely anaerobic lactate fermentation. SGTP4 remains present in the apical tegumental membrane through adult development. A second glucose transporter, SGTP1, is localized within the basal membrane of the tegument and internal membranes, and may distribute the glucose from the tegument to the rest of the worm (Skelly, Tielens & Shoemaker, 1998). Little is known as to how schistosomes facilitate the uptake of other critical nutrients during their complicated life-cycle.

Recently we reported that cDNA encoding an *S. mansoni* amino acid permease, SPRM1lc, functioned

* Corresponding author: Department of Immunology and Infectious Diseases, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA. Tel: +617 432 1339. Fax: +617 738 4914. E-mail: psk@hsph.harvard.edu

https://doi.org/10.1017/S0031182099005181 Published online by Cambridge University Press

within *Xenopus laevis* oocytes to promote amino acid uptake when it was co-expressed with a human protein, h4F2hc (Mastroberardino *et al*. 1998). The h4F2hc was required for SPRM1lc, as well as for several mammalian homologues, to reach the oocyte plasma membrane and function as permeases. A disulphide linkage forms between the h4F2hc and the permease light chains, but the resulting amino acid transport specificity is determined by the light chain. Thus SPRM1lc represents a member of a new family of amino acid permease light chains which function as heterodimers in association with a heavy chain 'chaperone'. In this paper, we characterize the expression and localization of *S. mansoni* SPRM1lc in the parasite, and relate its functional characterization to the amino acid transport of mammalian-stage schistosomes.

MATERIALS AND METHODS

Parasites

A Puerto Rican strain of *Schistosoma mansoni* was maintained by passage through *Biomphalaria glabrata* snails and CBA/J mice. Parasite life-stages were obtained as previously described (Hackett, 1993).

Cloning of an S. mansoni *amino acid permease (SPRM1lc) cDNA*

A 1 kb fragment of the amino terminal coding region of a previously characterized schistosome glucose transporter cDNA, SGTP1, was radio-isotope labelled by random primer synthesis and used as a probe to screen approximately 60000 plaques of a size-selected adult cDNA library at low stringency (50 °C in 6× SSC). Hybridization with this SGTP1 fragment resulted in the purification of a new glucose transporter (SGTP4) and the identification of an additional weakly hybridizing species, later designated SPRM1lc. Two independent isolates of the SPRM1lc cDNA were sequenced. Synthetic oligonucleotides were used to obtain the complete overlapping sequence of both strands of all coding region DNA by the method of Sanger, Nicklen & Coulson (1977).

Functional expression of SPRM1lc in Xenopus *oocytes*

The complete coding region of SPRM1lc was amplified in a polymerase chain reaction with oligonucleotides homologous to their amino- and carboxyl-terminal coding DNA which also contained *Bam*HI recognition sites, using conditions as described (Skelly *et al*. 1994). Following amplification, the coding DNA fragment was purified, digested with *Bam*HI and ligated into the compatible *Bgl*II site of the *Xenopus* expression vector, pSP64T (Melton *et al*. 1984). A recombinant plasmid in which the SPRM1lc coding sequence was properly orientated relative to the SP6 promoter was identified and used to synthesize SPRM1lc cRNA *in vitro* (Skelly *et al*. 1994). At least 5 *Xenopus* oocytes, isolated as earlier (Mastroberardino *et al*. 1998), were injected with 33 nl of H_2O containing 5 ng of either SPRM1lc cRNA or cRNA derived from the human cDNA clone, h4F2hc or both and were incubated in ND96 medium at 16 °C for 24 h. Tritiated L-arginine or L-leucine was used in amino acid uptake experiments as detailed (Mastroberardino *et al*. 1998). Assays examining the ability of selected amino acids to inhibit arginine uptake were carried out at a final arginine concentration of 50 μM and either a 10-fold or a 100-fold excess of potentially inhibitory amino acids.

Expression of SPRM1lc in Sf9 cells

SPRM1lc was expressed in Sf9 insect cells by first ligating the entire coding DNA (obtained by PCR as outlined above) into the baculovirus transfer vector pVL941 at the unique *Bam*HI site. The correctly oriented SPRM1lc transfer plasmid was co-transfected with the baculovirus DNA, Baculogold. Recombinant SPRM1lc production in Sf9 membrane fractions was assessed by Western analysis as outlined earlier (Zhong *et al*. 1995).

Membrane preparation and gel electrophoresis

Membrane preparations of different life-stages were prepared, proteins were resolved by SDS–PAGE and Western blot analysis was performed as outlined (Skelly & Shoemaker, 1996).

Generation of anti-SPRM1lc antibodies

A peptide (NH2-CSDIIELDGHLKPVDSRLDIS-COOH) was synthesized (by Dr C. Dahl, Harvard Medical School) based on the sequence at the carboxyl-terminus of SPRM1lc corresponding to amino acids 483–503. The peptide was coupled to the carrier proteins BSA and ovalbumin and the peptide–BSA conjugate was used to immunize a rabbit; anti-SPRM1lc antibodies were purified by affinity to the synthetic peptide coupled to ovalbumin as described (Skelly & Shoemaker, 1996).

Humoral immunity to recombinant SPRM1lc

Serum was obtained from 12 naturally infected Brazilians whose mean faecal egg count was 708 per gram faeces (range: 12–2150). Serum was also obtained from 5 C57/Bl6 mice and 5 Balb/c mice experimentally infected with 150 cercariae 7 weeks previously. Serum samples were obtained from 2 chronically infected C57/Bl6 mice and 2 mice that had been exposed to irradiated cercariae on 4 separate occasions as described (Reynolds & Harn, 1992). All of these sera were tested by Western analysis for the

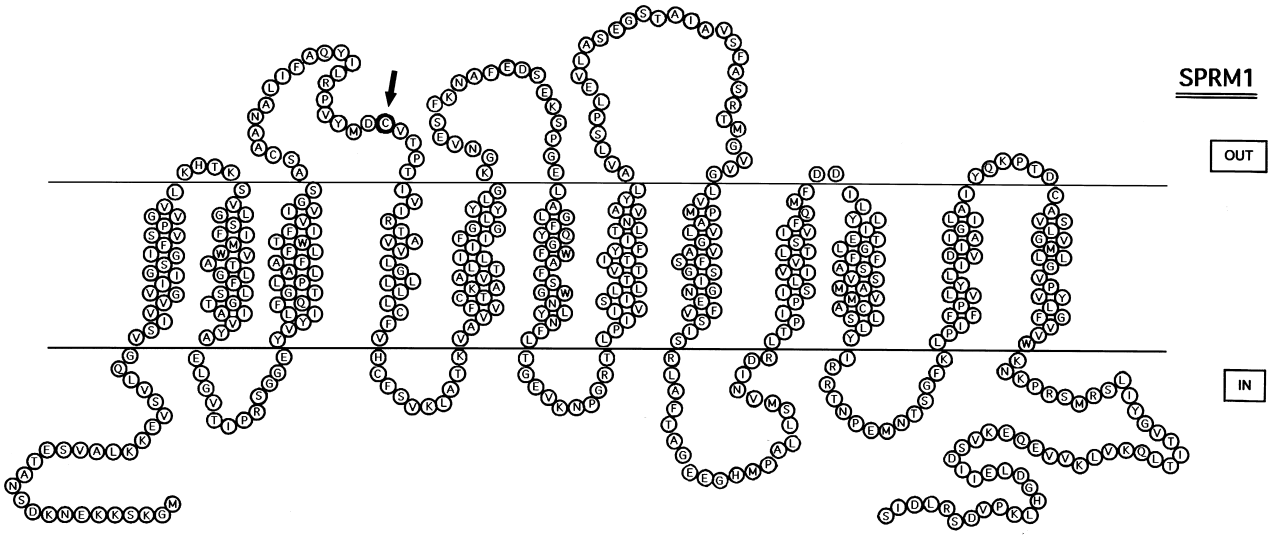

Fig. 1. Representation of the complete SPRM1 amino acid sequence and predicted membrane topology. 'OUT', exterior of the cell, 'IN', interior. The arrow indicates a conserved cysteine, (C), residue.

presence of antibody recognition of recombinant SPRM1lc (expressed in Sf9 insect cells) at a dilution of 1:100.

Immunocytochemistry

Immunofluorescent detection of SPRM1lc in whole miracidia, sporocysts or adult worm sections (all fixed in cold acetone for 5 min) was essentially carried out as described earlier for adult sections (Zhong *et al*. 1995; Skelly & Shoemaker, 1996).

Biotinylation of living adult parasites

Living adult parasites were labelled with sulphosuccinimidobiotin, following which a surface membrane-enriched preparation was isolated as described (Skelly & Shoemaker, 1996). Biotinylated surface proteins were recovered by affinity to streptavidin-agarose beads and were subjected to SDS–PAGE and Western analysis (Skelly & Shoemaker, 1996).

RESULTS

Characterization of cDNA encoding SPRM1lc

A novel cDNA was identified by a low stringency hybridization screen of an adult *Schistosoma mansoni* library using an *S. mansoni* glucose transporter cDNA as probe (see Materials and Methods section). The cDNA is 2305 bp and contains an open reading frame potentially encoding a 503 amino acid protein, later designated SPRM1lc, of 54842 Da with a pI of 8·04. The SPRM1lc cDNA predicts a 3′-untranslated region of 654 bp ending in a poly(A) tract preceded by a typical consensus poly(A) addition site (AATAAA) 16 bases upstream. The GenBank accession number for SPRM1lc is L25068.

As illustrated schematically in Fig. 1, SPRM1lc is predicted to have the 12 hydrophobic, transmembrane domains typical of glucose transporters and amino acid permeases. There are 3 sizeable predicted extracellular loops (20–30 amino acids in length) spanning transmembrane domains 3–4, 5–6 and 7–8. The protein has a similar size and substantial sequence homology with a group of proteins that serve as light chains for a newly described family of heterodimeric amino acid transporter proteins (Mastroberardino *et al*. 1998). For instance, SPRM1lc exhibits 37% identity (and 58% similarity) with the 507 amino acid human amino acid permease light chain, E16.

Functional expression of SPRM1lc in Xenopus *oocytes*

Because of its substantial homology with characterized amino acid permeases, many unsuccessful attempts were made to demonstrate enhanced amino acid uptake within *Xenopus* oocytes injected with cRNA encoding SPRM1lc. However, SPRM1lc function was not detected until we co-injected oocytes with both SPRM1lc cRNA and cRNA encoding the human 4F2 heavy chain, h4F2hc (Mastroberardino *et al*. 1998). These studies revealed that h4F2hc was required for the oocyte surface localization of SPRM1lc and that the amino acid specificity was due to SPRM1lc.

To compare the function of SPRM1lc with that observed in living schistosomes, we performed experiments in *Xenopus* oocytes in which we characterized the inhibitory effect of various unlabelled amino acids on the transport of radio-isotope labelled arginine. Incubation conditions were comparable to those reported by Asch & Read (1975*a*) using adult male schistosomes; uptake conditions included either a 10- or 100-fold excess of the unlabelled amino acids. In Table 1, the results of both studies are compared. There is a broad concurrence between the

https://doi.org/10.1017/S0031182099005181 Published online by Cambridge University Press

Table 1. Inhibition of arginine uptake by selected amino acids into *Xenopus* oocytes expressing SPRM1lc and h4F2hc

	Inhibition of arginine uptake (%)			
	10-Fold inhibitor excess		100-Fold inhibitor excess	
Inhibitor	Oocytes	*S. mansoni* males*	Oocytes	*S. mansoni* males*
PRO	25±11	9	7±1·5	6
GLU	20±7	27	7±1·5	13
ALA	30±16	20	47±14	43
PHE	35±13	42	83±22	62
LYS	65±16	43	94±12	74
ARG	85±43	61	97±40	84
TRP	<0	35	<0	70

* Data from Asch & Read (1975*a*).

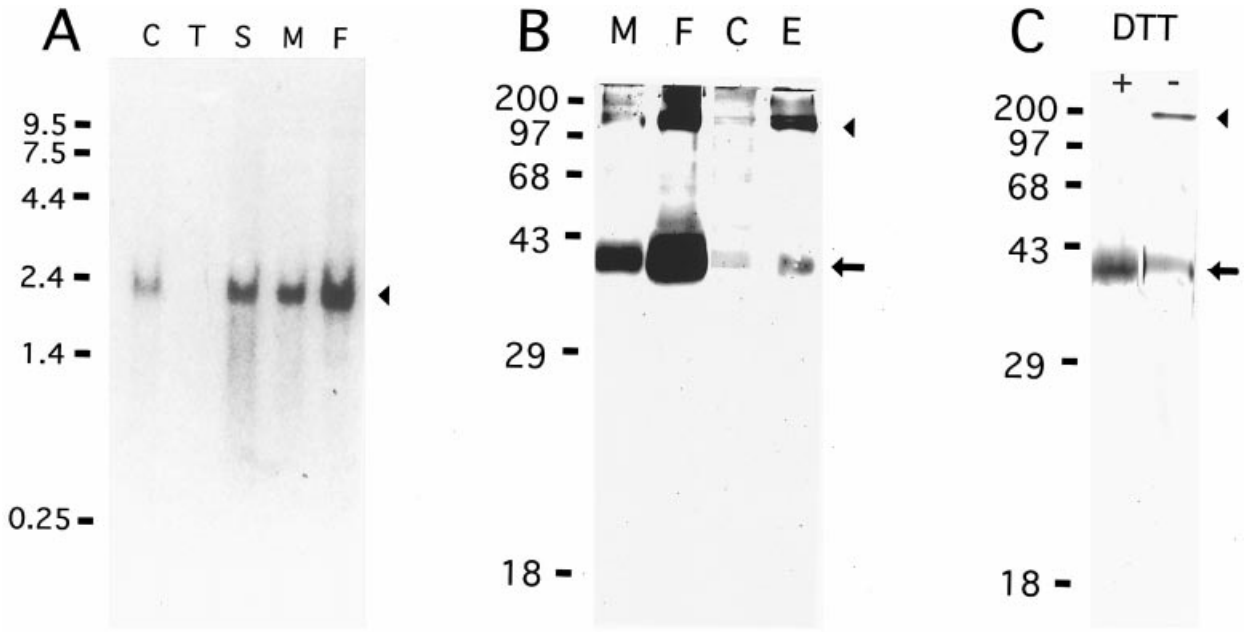

Fig. 2. Developmental expression of SPRM1lc. (A) Northern analysis. mRNAs from whole cercariae (C), isolated cercarial tails (T), schistosomula (S) and adult males (M) and females (F) were resolved. The position of the 2·3 kb SPRM1 transcript is indicated (arrowhead). The positions of molecular mass markers are indicated at left (kb). (B) Western analysis. Schistosome membrane preparations from adult males (M), adult females (F), cercariae (C) and eggs (E) were resolved by SDS/15% PAGE. The position of SPRM1lc is indicated (arrow). A high molecular weight SPRM1 complex is also detected (arrowhead). The positions of molecular mass markers are indicated at left (kDa). (C) Schistosome adult membrane preparation resolved by SDS/15% PAGE in the presence (+) or absence (−) of the reducing agent DTT. The position of SPRM1lc is indicated (arrow). The high molecular weight SPRM1 complex dissociates and is not detected in the presence of DTT (arrowhead). The positions of molecular mass markers are indicated at left (kDa).

properties of SPRM1lc and the amino acid transporting ability of adult male parasites. For both SPRM1lc/h4F2hc in oocytes and whole worms, unlabelled proline or glutamine exert little effect, alanine and phenylalanine exert a modest effect while lysine and arginine exert the greatest inhibitory effect on labelled arginine uptake (Table 1). Of the tested amino acids, only tryptophan does not follow this trend. Tryptophan inhibits arginine uptake in *S. mansoni* males but not in oocytes expressing SPRM1lc and h4F2hc.

Developmental expression of SPRM1lc

Poly(A) RNA from whole cercariae (approximately 1 μg), isolated cercarial tails (0·3 μg), schistosomula (2·5 μg) and adult males and females (1 μg each) was separated on a formamide–agarose gel, blotted onto a nylon membrane and hybridized with a radioisotope labelled SPRM1lc probe (Fig. 2A). The major SPRM1lc gene product is a 2·3 kb transcript (arrowhead, Fig. 2A) that is detected in all preparations with the exception of isolated cercarial tails.

https://doi.org/10.1017/S0031182099005181 Published online by Cambridge University Press

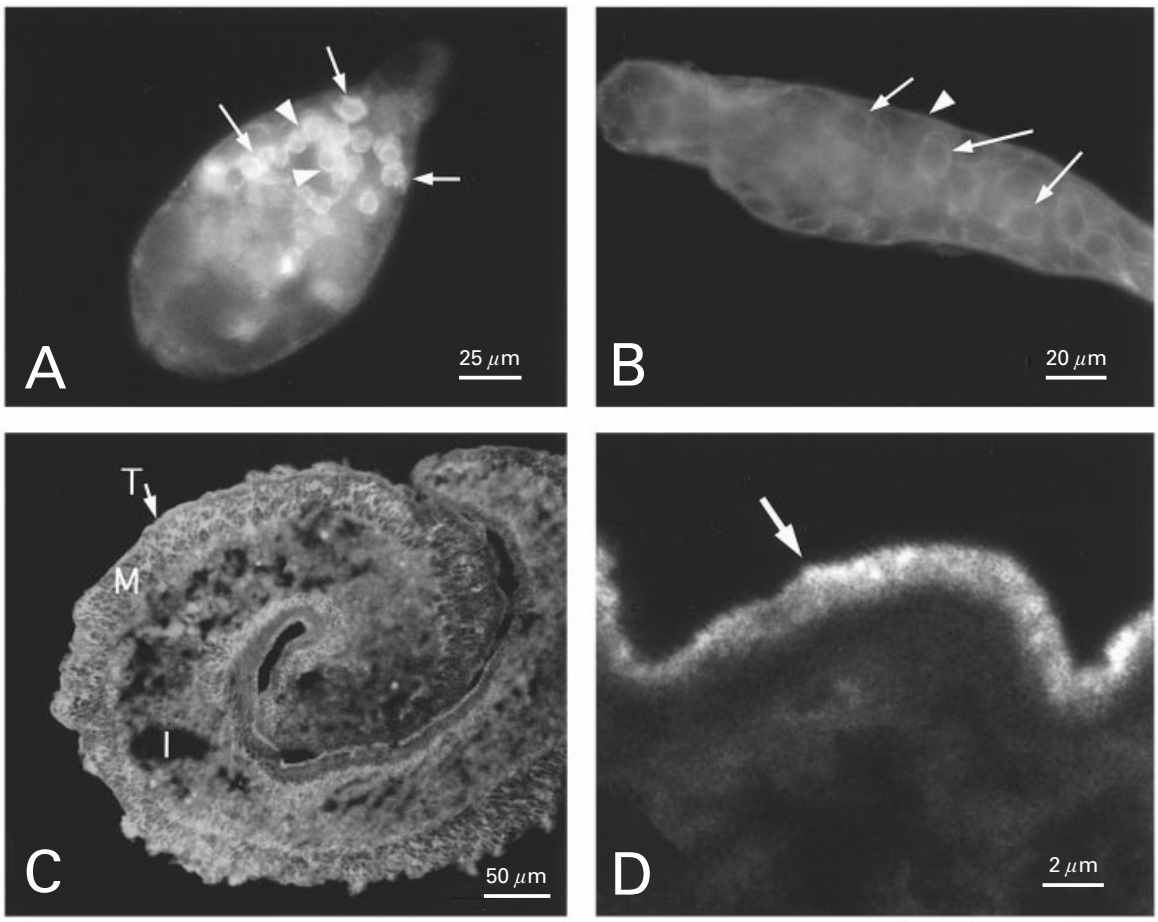

Fig. 3. Immunolocalization of SPRM1lc. In miracidia (A) the peripheral ganglia of the neural mass stain with anti-SPRMlc antibodies as do the cell bodies of neurons lying outside the neural mass (arrows). In daughter sporocysts (B) the outer sporocyst tegument (arrowhead) contains SPRM1lc as do the germ cell outer membranes within the sporocyst (arrows). In adult sections (C) the tegument (T, arrow), muscle area (M) and interior parenchyma contain SPRM1lc whereas the intestine (I) does not. (D) High magnification laser scanning confocal image of the adult tegument showing the presence of SPRM1lc in the outer tegument (arrow).

Antigen affinity-purified anti-SPRM1lc antibodies directed against a peptide based on the carboxyl terminal sequence were used to examine the developmental expression of SPRM1lc in the following life-stages: eggs, cercariae, adult male and female parasites (Fig. 2B). A protein of about 40 kDa, as estimated by SDS–PAGE mobility, is detected in membrane extracts of all life-stages tested under non-reducing conditions. A second band of about 150 kDa is also seen in all life-stages (arrowhead). This high molecular weight species disappears and the intensity of the low molecular weight species increases when the gel is run under reducing conditions (Fig. 2C, arrowhead).

In situ *localization of SPRM1lc*

The distribution of SPRM1lc within miracidia is confined to a collection of cells in the median anterior of the parasite (Fig. 3A). The staining equates with the position of the peripheral ganglia of the neural mass (arrowheads). The cell bodies of neurons that lie outside the neural mass and that connect with lateral papillae (arrows) also stain for SPRM1lc (Fig. 3A). Daughter sporocysts exhibit extensive staining for SPRM1lc (Fig. 3B). The protein is found in the sporocyst tegument (arrowhead) and within the sporocyst on the surface membranes of the germ cells (arrows, Fig. 3B). In sections of adult parasites anti-SPRM1lc antibodies stain a number of tissues (Fig. 3C). The tegument (T, arrow, Fig. 3C) and the tegumental cytons (beneath the peripheral muscle layers (M)), as well as the connections between the two, stain for SPRM1lc. Because the resolving power of the light microscope is insufficient to permit distinction between staining of muscle fibre membranes and staining in the connections of the cytons it is not clear whether the muscle also stains for the protein. The parenchyma within the body of the parasite stains broadly for SPRM1lc. The intestine (I) does not stain for this protein. Using laser scanning confocal microscopy it is clear that SPRM1lc is present within the outer tegument of adult parasites (Fig. 3D, arrow).

To test whether the SPRM1lc located in the outer tegument of adult worms is surface exposed, living

https://doi.org/10.1017/S0031182099005181 Published online by Cambridge University Press

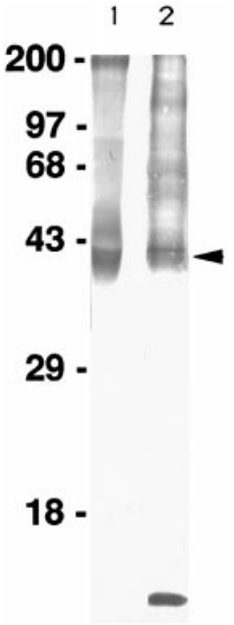

Fig. 4. SPRM1lc is surface exposed and available for surface biotinylation. Western blots contain tegumental extracts of biotinylated worms resolved by SDS/10% PAGE. Lane 1 contains the total extract and lane 2 contains the streptavidin-bound (surface-exposed) portion of the extract. SPRM1lc is detected in both the total as well as the surface-exposed extract (arrowhead). The positions of molecular mass markers are indicated at left (kDa).

parasites were labelled with sulphosuccinimidobiotin then washed, and a tegumental preparation was extracted by a short treatment with the detergent, digitonin. Western blots contain tegumental extracts of biotinylated worms and lanes contain either the total worm extract (Fig. 4, lane 1) or the streptavidin-bound (biotinylated) portion of the extract (lane 2). SPRM1lc is detected in both extracts (lanes 1 and 2, arrowhead) indicating that a portion of this protein is exposed at the host–parasite interface. As previously reported, the control non-surface protein paramyosin is detected with anti-paramyosin antibodies in the total extract but not in the biotinylated fraction, showing that biotinylation of internal proteins did not occur (Skelly & Shoemaker, 1996). The control internal protein paramyosin is readily biotinylated when labelling is carried out using permeabilized parasites (data not shown).

SPRM1lc is not immunogenic during infection

Recombinant SPRM1lc, expressed in insect cells, can be readily detected by Western analysis in membrane extracts using our anti-SPRM1lc antibodies (data not shown). When these same extracts were individually analysed for recognition by serum from naturally infected humans, or mice that were either chronically infected by schistosomes or multiply challenged by irradiated cercariae, we found no evidence of immune recognition of the recombinant SPRM1lc (data not shown).

DISCUSSION

Adult *S. mansoni* are parasitic platyhelminths that live in the venous blood of their vertebrate hosts. While the parasites have a mouth and a gut, they can take in nutrients such as glucose and amino acids from the blood directly across their body surface or tegument (Fripp, 1967; Asch & Read, 1975*b*). Nutrient uptake across the tegument is presumably accommodated through specific tegumental membrane transporter proteins. It is not understood how the parasite can interact with its host for nutrient uptake without simultaneously provoking a debilitating immune response directed against the nutrient transporter proteins. Should a damaging immune response against these transporter proteins become possible, this might form the basis of an effective schistosomiasis vaccine.

In this report, we describe the cloning and characterization of a cDNA encoding SPRM1lc, a 55 kDa light chain for a schistosome amino acid transporting heterodimer. This protein is a member of a newly described family of light chains that associate with a second protein (the heavy chain) which appears to serve as a chaperone to permit a functional surface localization for the amino acid transporter (Mastroberardino *et al.* 1998). It is not yet known whether the heavy chain is required for function once the light chain has reached the plasma membrane, although the 2 chains appear to remain associated. The substrate specificity of the heterodimer appears to be determined by the light chain since h4F2hc in association with different light chains results in different amino acid transport specificities (Mastroberardino *et al.* 1998). Neither the schistosome heavy chain, nor any non-mammalian heavy chain, has yet been identified. However, it is noteworthy that when schistosome membrane proteins are resolved under non-reducing conditions and examined by Western analysis using anti-SPRM1lc antibodies, in addition to detecting SPRM1lc (running at 40 kDa), a higher molecular weight species, running at about 150 kDa, is also seen. The 150 kDa band presumably represents SPRM1lc cross-linked with an endogenous heavy chain. Since the higher molecular weight species is not detected when the proteins are resolved under reducing conditions, this suggests that a cystine cross-link plays at least some role in stabilizing the heterodimer. The cysteine at position 136 in SPRM1lc is conserved among members of this new protein family and has been implicated in the disulphide bond formation. Indeed, mutagenesis of this cysteine residue prevents SPRM1 from forming a disulphide cross-linkage with 4F2hc in oocytes (Pfeiffer *et al.* 1998).

https://doi.org/10.1017/S0031182099005181 Published online by Cambridge University Press

Schistosomes as members of the phylum Platyhelminthes are among the earliest multicellular organisms to evolve. Database searches have identified additional close homologues of SPRM1lc in the nematode, *Caenorhabditis elegans*, but no clear homologues were found in any single-celled organisms, including several for which the entire genome has been sequenced. Thus, heterodimeric amino acid transporters appear to be an adaptation which developed very early in the evolution of multicellular organisms. These data provide the first direct evidence for the existence of such heterodimeric amino acid transporter heavy chains in invertebrate species.

In oocytes expressing both SPRM1lc and h4F2hc, the basic amino acids, histidine, arginine and lysine are all transported in significantly greater amounts than controls (Mastroberardino *et al*. 1998). In addition, transport of leucine, phenylalanine, methionine and glutamine is enhanced. Interestingly, SPRM1lc exhibits a partial sodium dependence for the transport of neutral amino acids. These transport characteristics differ from those of the human light chain (E16) when co-expressed with h4F2hc in oocytes and are unlike any single mammalian amino acid transport system previously described. The mixed properties of the transport mediated by SPRM1lc resembles both mammalian system y^+L and system L and has not been previously observed in one transport system. It remains to be determined whether this type of transport is a normal component of other invertebrates.

The 2·3 kb transcript detected by Northern analysis is the size expected if the cloned cDNA contains the complete coding region and 3′ untranslated sequences. The transcript levels seen in different developmental stages suggest that the protein is expressed to a higher degree in the various intramammalian-stage parasites as compared to larval cercariae. The detection of SPRM1lc by Western analysis in all life-stages examined implies that it acts as a housekeeping protein. Note that in all cases where the 40 kDa form of SPRM1lc is detected, we also detect the apparently identical 150 kDa species. This suggests that SPRM1lc heterodimerizes with the same or similar heavy chain in each life-stage.

Although SPRM1lc is detected in several life-stages, the localization of the protein changes significantly. In miracidia the protein seems largely confined to the ganglia that enclose the neural mass as well as to other associated ganglia. As mentioned earlier, some of the transport characteristics of SPRM1lc resemble those of mammalian system L. While this system is expressed widely in mammalian tissues, it is notably a major amino acid transport system in the brain (Castagna *et al*. 1997). Perhaps the brain system L of mammals and SPRM1lc in schistosome miracidia fulfil similar functions in the neural tissues of these organisms.

Within the intermediate snail host, miracidia undertake a complex metamorphosis to form mother sporocysts. Mother sporocysts reproduce germ cells asexually which then differentiate to develop into daughter sporocysts which migrate throughout the snail, settling mostly in the hepatopancreas. The germ cells continue to multiply asexually and later develop into infectious cercariae which are released from snails into fresh water where they seek their vertebrate hosts. We detect SPRM1lc throughout the migratory daughter sporocysts in which the protein is found on the sporocyst tegument and on the outer surfaces of the germ cells. It would thus appear that SPRM1lc is responsible for amino acid uptake both into the sporocysts and into the dividing germ cells.

In adult parasites, SPRM1lc is also widely distributed in several tissues. The protein is seen throughout the parenchyma as well as in the tegument, the tegumental cytons and the connections between the two. Laser scanning confocal microscopy has revealed that, within the tegument, SPRM1lc is detected in the apical membranes. The localization of SPRM1lc in the tegument resembles that of glucose transporter protein, SGTP4, which is almost certainly responsible for uptake of glucose from the serum of vertebrate hosts (Skelly & Shoemaker, 1996). The exposure of SPRM1lc to host serum is confirmed by the availability of the protein for surface biotinylation and suggests that SPRM1lc functions both in uptake of amino acids from the host and their distribution throughout the body of the worm. It is noteworthy that SPRM1lc is not clearly detected in the intestine and thus may not be available to transport amino acids across the gut. Whether the schistosome gut functions to take up nutrients such as amino acids or glucose has not been rigorously demonstrated.

Cells typically employ multiple different amino acid transporter systems, some having a specialized role and others general (Castagna *et al*. 1997). In early studies on amino acid transport into adult male schistosomes at least 5 amino acids transport systems were implicated (Asch & Read, 1975*a*). SPRM1lc may represent the molecular basis of one of these systems. Basic amino acids (such as arginine) are reported to be taken up by adult schistosomes via complex transport systems, at least one of which also carries some neutral amino acids (Asch & Read, 1975*a*). Import of arginine into adult male schistosomes can be almost totally inhibited by the other basic amino acids histidine and lysine as well as by phenylalanine and to a lesser extent by alanine, but not by proline or glutamate (Asch & Read, 1975*a*). This is the profile of transport expected if these amino acids were all competing for entry into schistosomes via SPRM1lc since a similar pattern of inhibition of arginine uptake was seen for SPRM1lc/h4F2hc co-expression in oocytes. However, the

uptake of arginine by the schistosome transport system is also greatly inhibited by tryptophan, an amino acid that does not utilize SPRM1lc in oocytes. Despite this difference it remains likely that the schistosome transport system described by Asch & Read (1975*a*) and the tegumental transporter protein SPRM1lc, characterized in this report, are related.

Despite the fact that SPRM1lc is a surface-exposed tegumental membrane protein that must interact intimately with the host in the acquisition of amino acids, it does not provoke a detectable humoral immune response. The serum from infected mice or humans does not contain antibody that convincingly detects recombinant SPRM1lc by Western analysis. Perhaps the extracellular epitopes of SPRM1lc are concealed, or masked by host-acquired antigens. It has been suggested that schistosomes divert the immune system by exposing only epitopes that evoke non-protective immunity. If so it should be possible to present SPRM1lc (and/or its endogenous heavy chain partner) in an immunogenic form to the host to act as an effective vaccine and thereby potentially prevent infection.

REFERENCES

ASCH, H. L. & READ, C. P. (1975*a*). Membrane transport in *Schistosoma mansoni*: transport of amino acids by adult males. *Experimental Parasitology* **38**, 123–135.

ASCH, H. L. & READ, C. P. (1975*b*). Transtegumental absorption of amino acids by male *Schistosoma mansoni*. *Journal of Parasitology* **61**, 378–379.

CASTAGNA, M., SHAYAKUL, C., TROTTI, D., SACCHI, V. F., HARVEY, W. R. & HEDIGER, M. A. (1997). Molecular characteristics of mammalian and insect amino acid transporters: implications for amino acid homeostasis. *Journal of Experimental Biology* **200**, 269–286.

FRIPP, P. J. (1967). The sites of (1–14C) glucose assimilation in *Schistosoma haematobium*. *Comparative Biochemistry and Physiology* **23**, 893–898.

HACKETT, F. (1993). The culture of *Schistosoma mansoni* and production of life cycle stages. In *Protocols in Molecular Parasitology*, Vol. 21 (ed. Hyde, J. E.), pp. 89–99. Humana Press, Totowa, New Jersey.

MASTROBERARDINO, L., SPINDLER, B., PFEIFFER, R., SKELLY, P. J., LOFFING, J., SHOEMAKER, C. B. & VERREY, F. (1998). Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. *Nature, London* **395**, 288–291.

MELTON, D. A., KRIEG, P. A., REBAGLIATI, M. R., MANIATIS, T., ZINN, K. & GREEN, M. R. (1984). Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Research* **12**, 7035–7056.

PFEIFFER, R., SPINDLER, B., LOFFING, J., SKELLY, P. J., SHOEMAKER, C. B. & VERREY, F. (1998). Functional heterodimeric amino acid transporters lacking cysteine residues involved in disulfide bond formation. *FEBS Letters* **439**, 157–162.

REYNOLDS, S. R. & HARN, D. A. (1992). Comparison of irradiated-cercaria schistosome vaccine models that use 15- and 50-kilorad doses: the 15-kilorad dose gives greater protection, smaller liver sizes, and higher gamma interferon levels after challenge. *Infection and Immunity* **60**, 90–94.

SANGER, F., NICKLEN, S. & COULSON, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* **74**, 5463–5467.

SKELLY, P. J., KIM, J. W., CUNNINGHAM, J. & SHOEMAKER, C. B. (1994). Cloning, characterization, and functional expression of cDNAs encoding glucose transporter proteins from the human parasite *Schistosoma mansoni*. *Journal of Biological Chemistry* **269**, 4247–4253.

SKELLY, P. J. & SHOEMAKER, C. B. (1996). Rapid appearance and asymmetric distribution of glucose transporter SGTP4 at the apical surface of intramammalian-stage *Schistosoma mansoni*. *Proceedings of the National Academy of Sciences, USA* **93**, 3642–3646.

SKELLY, P. J., TIELENS, A. G. M. & SHOEMAKER, C. B. (1998). Glucose transport and metabolism in mammalian-stage schistosomes. *Parasitology Today* **14**, 402–406.

ZHONG, C., SKELLY, P. J., LEAFFER, D., COHN, R. G., CAULFIELD, J. P. & SHOEMAKER, C. B. (1995). Immunolocalization of a *Schistosoma mansoni* facilitated diffusion glucose transporter to the basal, but not the apical, membranes of the surface syncytium. *Parasitology* **110**, 383–394.