

Schistosoma mansoni: Comparison of Cloned Tropomyosin Antigens Shared between Adult Parasites and *Biomphalaria glabrata*¹

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WESTON, D. S., AND KEMP, W. M. 1993. *Schistosoma mansoni*: Comparison of cloned tropomyosin antigens shared between adult parasites and *Biomphalaria glabrata*. *Experimental Parasitology* 76, 358–370. Rabbit antisera, raised against whole, homogenized hepatopancreas from uninfected *Biomphalaria glabrata*, were used to screen a cDNA library prepared from adult *Schistosoma mansoni*. Of 1.8×10^5 recombinants screened, 34 clones were specifically immunoreactive with the antisera. Twenty of the clones were subcloned for further analysis, and 13 of the 20 were demonstrated to hybridize to a cDNA probe encoding schistosome tropomyosin I (pSMTM) at high stringency (Xu *et al.* *Experimental Parasitology* 69, 373–392, 1989). One partial cDNA clone (pSM4), which failed to hybridize to pSMTM, was used to rescreen the adult worm cDNA library and a 1381-bp cDNA clone, SmTMII, was identified and characterized. SmTMII contained a single 284-amino acid open reading frame and was shown to be 65.8% homologous to SMTM. Computer-assisted analysis of the predicted protein structure depicted a hydrophilic molecule with the extensive alpha helical secondary structure characteristic of tropomyosins. SmTMII was expressed in *Escherichia coli* and the resulting 45-kDa fusion protein was recognized by both a polyclonal antiserum specific for SMTM and the antiserum of mice chronically infected with *S. mansoni*. Northern analysis showed the SmTMII mRNA in the adult stage to be about 1.6 kb in size. Analysis of the SmTMII gene by Southern blot revealed a complex hybridization pattern suggesting the presence of introns or multiple gene copies. The 650-bp *Eco*RI fragment of SmTMII was used to screen a cDNA library prepared from uninfected *Biomphalaria glabrata* and two clones, each encoding a novel snail tropomyosin, BgTMII, were isolated and characterized. The two clones, consisting of 2816 and 2314 bp, respectively, differed from each other only in the polyadenylation signal used and each contained a single and identical 284-amino acid open reading frame having 94.7% homology with the previously reported snail tropomyosin, BG39 (Dissois *et al.* *Molecular and Biochemical Parasitology* 43, 245–256, 1990). BgTMII was shown to have a higher amino acid homology with SMTM (68.3%) than with BG39 (65.1%) and a higher amino acid homology with SMTM than the two schistosome tropomyosins (SMTM and SmTMII) have with each other (65.8%). Implications of these observations in relation to the concept of host–parasite antigen mimicry are discussed. © 1993 Academic Press, Inc.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Schistosoma mansoni*; Trematode; *Biomphalaria glabrata*; Mollusc; Antigen sharing; Antigen mimicry; Tropomyosin; cDNA cloning; Polymerase chain reaction (PCR); Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE); Phosphate-buffered saline (PBS); Ribonucleic acid (RNA); Deoxyribonucleic acid (DNA); Complementary DNA (cDNA); Kilobase pairs (kbp); Base pairs (bp); Kilodaltons (kDa).

INTRODUCTION

The sharing of antigens between *Schistosoma mansoni* and *Biomphalaria glabrata*

¹ Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. M97554 and M97555.

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has been demonstrated by several different immunological techniques. These shared antigens have been suggested to play a possible role in immune evasion in the immunocompetent host and to contribute to the maintenance of host antigen polymorphisms (Damian 1987). Damian (1964) further proposed that at least some of these

shared antigens reflected a genetic accommodation between the host and parasite as a result of selection pressure and termed this hypothesis "molecular mimicry."

Fairley (1919–1920) first demonstrated antigen sharing while attempting to develop a method for immunodiagnosing schistosomiasis in infected humans. Since this initial observation, evidence for antigen sharing between schistosomes and biomphalarid snails has expanded to include the demonstration of circulating anti-snail antibodies in schistosome-infected humans (Capron *et al.* 1969; Jackson and Demoor 1976; Chieffi *et al.* 1982; Rivera-Marrero and Hillyer 1985; and Mansour *et al.* 1989), experimental animal infections and/or experimental immunizations (Kemp *et al.* 1974; Hamburger *et al.* 1989), and the detection of circulating anti-schistosome antibodies in the serum of animals immunized against normal snail tissue (Capron *et al.* 1965, 1968, 1969; Tsuji and Yokogawa 1972; Deelder 1973; Deelder *et al.* 1975a,b; Jackson 1976; Henning *et al.* 1977; Yoshino and Bayne 1983; Devine and Kemp 1984; Bayne *et al.* 1984; Rasmussen *et al.* 1985; Dissous *et al.* 1986; Dissous and Capron 1989).

The genetic basis for certain shared antigens was first demonstrated by the molecular cloning of Bg39, a snail antigen which immunologically cross-reacted with Sm39 and Sm43 from *S. mansoni* (Dissous *et al.* 1990). The Bg39 cDNA was shown to have a 51–65% homology with tropomyosins of other species, the highest homology (65.5%) being with schistosome tropomyosin, SMTM (Xu *et al.* 1989). The demonstration of Bg39 cross-reactive epitopes associated with vesicles of the epidermal ridges of miracidia suggest a possible role of the cross-reactive tropomyosin in miracidia and/or sporocyst/snail interactions.

In the present report we confirm and extend these previous observations by identifying and characterizing a novel schistosome tropomyosin isoform (SmTMII) and a novel tropomyosin isoform from *B. gla-*

brata (BgTMII). BgTMII differs from BG39 in a region of the protein defined by amino acids 189–213, giving this isoform higher homology to both parasite tropomyosins than does BG39, and higher homology with SMTM than the two schistosome tropomyosins have for each other.

MATERIALS AND METHODS

Parasite materials. All parasite material used was derived from a life cycle of the LE strain of *S. mansoni*, derived from a patient from Belo Horizonte, Brazil, passaged through a Brazilian strain of *B. glabrata* and C57BL/6 mice.

Antigens and antisera. *B. glabrata* hepatopancreas homogenate (SNAG) was prepared by separating six snail bodies from their shell fragments and washing them twice in ice-cold PBS containing 0.1% sodium azide and 2 mM EDTA (Sigma, St. Louis, MO). The washed hepatopancreas of each snail was homogenized in PBS containing 0.8 mM phenylmethylsulfonyl fluoride (Sigma) and the supernatant saved after a 30-min centrifugation at 30,000g. Polyclonal antiserum was raised in New Zealand rabbits by injecting 0.5 mg SNAG with Freund's complete adjuvant subcutaneously. Two subsequent injections of SNAG with Freund's incomplete adjuvant were given at 4-week intervals with blood being collected 10 days postinjection. Immune and preimmune sera were absorbed against a Lambda ZAP/*Escherichia coli* lysate to minimize reactivity to procaryotic antigens.

Monospecific polyclonal antibodies from rabbit anti-SNAG directed toward SmTMII were prepared by a method described by Smith and Fisher (1984). Briefly, the recombinant SmTMII fusion peptide (described below) was fractionated by SDS-PAGE and transferred to nitrocellulose. The appropriate band was identified by Ponceau S staining, excised, incubated in blocking solution (20% fetal calf serum in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20), washed, and incubated in rabbit anti-SNAG (1:500) overnight at 4°C. Following a final wash step the bound antibodies were eluted by three consecutive washes in 270 µl glycine elution buffer [5 mM glycine (pH 2.3); 500 mM NaCl, 0.5% Tween 20, and 100 µg/ml bovine serum albumin (BSA)] for 30 sec and quickly neutralized with 30 µl 500 mM sodium phosphate (pH 7.0). The three elutions were pooled and immediately diluted 1:10 in blocking solution. Also used in this report was a polyclonal mouse serum raised against SMTM (Xu *et al.* 1989; kindly provided by Dr. Philip T. LoVerde, State University of New York, Buffalo, NY).

Procaryotic expression and purification of SmTMII. The open reading frame of pSmTMII was amplified by

the PCR using specific oligonucleotides containing appropriate restriction endonuclease sites to facilitate cloning into the procaryotic expression vector, pFLAG (Hopp *et al.* 1988). Proteins expressed in pFLAG are fused to a small (1 kDa) hydrophilic peptide encoding an epitope recognized by a commercially available, calcium-dependent, monoclonal antibody (M1). Protein expressions were carried out at 30°C, induced with isopropyl- β -D-thiogalactopyranoside (IPTG) (Boehringer-Mannheim, Indianapolis, IN) added to 2 mM final concentration, and incubated at 30°C for 4 hr or overnight. Soluble proteins from the cells were prepared by standard procedures (Sambrook *et al.* 1989). After adding CaCl_2 to 1 mM, recombinant SmTMII was purified by affinity chromatography using the M1 monoclonal antibody covalently attached to agarose (International Biotechnologies, Inc., New Haven, CT). Eluted fractions were concentrated with Centrprep-10 concentrators (Amicon, Beverly, MA), and the protein concentration was determined by the Bio-Rad (Richmond, CA) protein assay.

Preparation and screening of cDNA expression libraries. Total RNA was isolated from adult *S. mansoni* worm pairs or uninfected *B. glabrata* using the modified guanidinium thiocyanate method of Chomczynski and Sacchi (1987). Polyadenylated RNA was subsequently purified from total RNA on oligo(dT)-cellulose spun columns (Clontech, Palo Alto, CA). Parasite or snail poly(A⁺) RNA (5 μ g) was used as substrate for complementary DNA synthesis, using the Uni-ZAP cDNA Synthesis kit (Stratagene). Fractionated cDNA was ligated into the *EcoRI/XhoI* sites of the lambda ZAP XR vector and packaged *in vitro* with commercial packaging extract (Stratagene). The libraries were amplified on host strain PLK-F' to titers exceeding 2.0×10^{10} pfu/ml of lysate. Antibody screening of the schistosome library was performed by standard methodology (Sambrook *et al.* 1989). Recombinants were plated on host strain XL1-Blue at a density of 50,000 recombinants per 137-mm NZYM agar plate. Plaque lifts were performed in duplicate using 10 mM IPTG saturated nitrocellulose filters (Schleicher & Schuell, Keene, NH) and incubated in blocking buffer (20% fetal calf serum in 1xTBST [10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20]). Filters were probed with rabbit anti-SNAG (1:1000) for 1 hr, or overnight at 4°C, followed by a 1-hr incubation in the appropriate alkaline phosphatase-conjugated secondary antibody (1:7500; Promega, Madison, WI). Color development was performed using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega), according to manufacturer's instructions. Normal rabbit serum, 1:1000, was used as a negative control on plaque-purified rabbit anti-SNAG reactive clones.

Screening of the library by hybridization was per-

formed as described by Sambrook *et al.* (1989). Low stringency hybridizations were carried out at 65°C with two 20-min washes in $5 \times \text{SSC}$ ($20 \times \text{SSC} = 3.0 \text{ M NaCl}$; 0.3 M sodium citrate, pH 7) at 65°C. Restriction fragments used as probes were fractionated by agarose gel electrophoresis and purified using Gene-Clean II (Bio 101, La Jolla, CA). Radioactive DNA probes were synthesized to high specific activity by the random primed labeling method (Feinberg and Volgelstein 1983). Hybond-N nylon membranes (Amersham, Arlington Heights, IL) were used for plaque lifts and all prehybridizations, hybridizations, and washes were performed under conditions suggested by the manufacturer. Membranes were exposed to XAR5 film (Eastman Kodak Co., Rochester, NY) with a HyperScreen intensifying screen (Amersham) at -70°C .

DNA cloning and sequencing. Restriction enzymes and other DNA-modifying enzymes were purchased from Boehringer-Mannheim Corp. Plasmid pSMTM (Xu *et al.* 1989) was donated by Dr. Philip T. LoVerde (State University of New York, Buffalo, NY). Unless otherwise indicated all subcloning was performed using the pBluescript phagemid vector (Stratagene). Generation of nested deletions for sequencing was performed on linearized plasmid DNA with exonuclease III/S1 nuclease treatments (Heinikoff 1984). Double-stranded sequencing was performed by the dideoxynucleotide chain termination method (Sanger *et al.* 1977) using Sequenase Version 2.0 (United States Biochemical, Cleveland, OH).

SDS-PAGE and Western blotting. Antigen preparations were separated by SDS-PAGE according to standard procedures (Sambrook *et al.* 1989). Proteins separated by SDS-PAGE were transferred to nitrocellulose membranes (Bio-Rad Laboratories) using a Bio Trans semidry transfer apparatus (Gelman Sciences, Ann Arbor, MI) for 1–2 hr at 200 mA. Membranes were blocked against nonspecific binding and treated as described above for immunological screening of the cDNA libraries.

Northern blot and Southern blot procedures. For Northern analysis adult *S. mansoni* poly(A⁺) RNA was affinity purified by oligo (dT) cellulose adsorption (Invitrogen Corp., San Diego, CA). This mRNA was fractionated on a 1.2% agarose gel in the presence of 2.2 M formaldehyde and transferred to a Hybond-N (Amersham) nylon membrane (Thomas 1980). Hybridization was carried out at 65°C in $6 \times \text{SSPE}$ [$20 \times \text{SSPE}$ is 3.6 M NaCl, 0.2 M sodium phosphate, and 0.02 M EDTA (pH 8.0)], $2 \times$ Denhardt's solution (Sambrook *et al.* 1989), 0.1% SDS, 100 μ g/ml herring sperm DNA, and 1.8×10^6 CPM/ml radioactively labeled DNA probe. Membranes were washed under conditions suggested by the manufacturer.

Parasite genomic DNA was purified by standard procedures (Sambrook *et al.* 1989). Genomic DNA (20

µg) was digested with an appropriate restriction enzyme, fractionated on a 0.7% agarose gel, and transferred to Hybond-N nylon membranes (Southern, 1975). Hybridization was carried out at 68°C in 6 × SSC, 5 × Denhardt's solution, 0.5% SDS, 100 µg/ml herring sperm DNA, and 2.1 × 10⁶ CPM/ml radioactively labeled DNA probe. Membranes were washed under conditions suggested by the manufacturer. All autoradiography was performed as described above.

Computer sequence analysis. The Genetics Computer Group (GCG) sequence analysis software package (Devereux *et al.* 1984) was used to analyze the nucleotide sequence and the deduced amino acid sequence. To identify homologies of nucleotide sequence with other known sequences, the WordSearch and FastA programs were used to scan the Genbank and EMBL databases.

RESULTS

Cross-reactive cDNA clones. To isolate schistosome genes which encoded epitopes shared with its snail intermediate host, an adult *S. mansoni* Lambda ZAP expression library was prepared and screened with hyperimmune rabbit serum directed against normal snail hepatopancreas antigens (rabbit anti-SNAG). Of 1.8 × 10⁵ recombinants screened with this antiserum, 34 clones were demonstrated to be immunoreactive. Twenty of these clones were subcloned by *in vivo* excision of the pBluescript phagemid. Thirteen of the 20 purified clones specifically hybridized to a cDNA probe encoding schistosome tropomyosin (pSMTM) at high stringency (Xu *et al.* 1989). The 650-bp *Eco*RI insert from one of the nonhybridizing clones, pSM4, was used to probe the clone pool and hybridized to 6 of the remaining 20 clones. One remaining clone, pSM20, hybridized to neither pSMTM or pSM4. The inserts of pSM4 and pSM20, 1.1 and 2.1 kbp, respectively, were partially sequenced by the dideoxynucleotide chain termination method using vector-specific primers. Computer-assisted analysis of the DNA sequence information identified pSM20 as schistosome myosin heavy chain, previously described and partially cloned (Newport *et al.* 1988; Grossman *et al.* 1990) and recently fully cloned

(Weston *et al.*, 1993). Clone pSM4 was very similar to schistosome tropomyosin but different enough to suggest it encoded a novel tropomyosin isoform. The 700-bp *Eco*RI fragment of pSM4 was labeled by random priming and used to rescreen the same Lambda ZAP cDNA library. Out of 60,000 recombinants screened 77 positive clones were observed, 12 of which were purified for further analysis. The PCR was performed directly on the purified phage particles using one insert-specific minus strand primer and one vector-specific plus strand primer to test the new clones for additional 5' sequence information. Of the 12 clones, 3 appeared to have additional 5' information (data not shown). The clone with the largest insert (about 1.4 kbp), named pSmTMII, was further analyzed. Both strands of the insert were sequenced by the dideoxynucleotide chain termination method using a primer walking strategy. The pSmTMII cDNA sequence, shown in Fig. 1, contained 1381 bp, including a 19 adenosine nucleotide tail. Computer-assisted analysis revealed a single open reading frame beginning with the start methionine at position 106 and ending at position 957. The insert also contains 420 bp of 3' untranslated region with the consensus polyadenylation signal AATAAA beginning 29 bp upstream of the poly(A⁺) tail.

pSmTMII encodes a novel schistosome tropomyosin. Study of the deduced amino acid sequence of pSmTMII revealed a protein containing 284 amino acids with a calculated molecular weight of about 33 kDa. Analysis of the amino acid content showed 45% of all the residues to be charged, 63% of which were acidic, giving the protein a predicted pI value of 4.32. In addition, a Kyte-Doolittle plot (data not shown) of predicted hydrophobicity showed extensive hydrophilicity throughout the protein. A Chou-Fasman plot of secondary structure predicted alpha helices throughout the molecule (data not shown). A comparison of the pSmTMII open reading frame with

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1  aggaacctcattttaatcatttcttatttctttaaacttcttcttgattttaattattatccttattatcaattcataagattaaagtataattca 100
101 ttatcatggaacatattaaaaagaaaattgttggcaatgaaattggcaataagagaaatgctgtagatgaagctgatcaatttgaagcaaaattacgtgaaaa 200
      M E H I K K K M L A M K L D K E N A V D E A D Q L E A K L R E K
201  AGAATTAGAAATGCAAACTAAAGATGAAGAAGTTGCAGAAGTACTTAAAAAATTCACCAAGTTGACACGGACAAAGAACGGCACAACCTCAATTAGCT 300
      E L E M Q T K D E E V A E V L K K I Q Q V D T D K E T A Q T Q L A
301  GAACTAATACTAAATTAGAAGAAGACAGATAAACGAGCAACTGAGGCCGAAGCTGAAGTCGCCCTCACTACAGAAGCGAATTCGACAGCTTGAAGATGAAT 400
      E T N T K L E E T D K R A T E A E A E V A S L Q K R I R Q L E D E
401  TGAATCTACTGAGACTCGTCTTCAAGAAGCTACTGTTAAGCTTGAAGAAGCAAGTAAAGCTGCTGATGAAAGTGATCGTGGCTGTAAGTATTGGAGAA 500
      L E S T E T R L Q E A T V K L E E A S K A A D E S D R G R K V L E N
501  TCGTACTTTCGAGATGAAGAAGCTATCAATCAGTTAGAAGAACAATTAAGAATCTACATTTATGGCTGAAGATGCTGATCGAAAAATACGATGAGCGG 600
      R T F A D E E R I N Q L E E Q L K E S T F M A E D A D R K Y D E A
601  GCTCGTAAGCTGGCTATCACGGAAGTGAAGCTGAAGCGGCTGAGTCTCGCCTGGAAGCTGCCGAAAGTAAATACAGAAGTGAAGAAGAGTTACGCA 700
      A R K L A I T E V E I E R A E S R L E A A E S K I T E L E E E L R
701  TTGTTGTAATAATGTAATATCTTTGAGATATCTGAACAAGAGCCAGCCAGCGTGAAGAGGCATATGAAGAGATATACGTTATTTAACTGAGCGCTT 800
      I V G N N V K S L E I S E Q E A A Q R E E A Y E E N I R D L T E R L
801  AAAAGCTGCTGAGGATCGAGCTCAAGAATCTGAACGACTTGCTAATCTTTACAAGCTGATGCTGATCGCTTGAAGATGAATTTGGTCACCGAGAAAG 900
      K A E S E R A Q E S E R L V N T L Q A D A D R L E D E R K E
901  AAATATAAGCACTCAGTGAAGAATTGGATTCAACTTTTCAGAACTTACTGGAACtaattataataataacttttactcttcttactaagcattttctatc 1000
      K Y K A L S E E L D S T F A E L T G N *
1001 cttacctgatcagctccttaaatgaattcaatatatatatactgcttatatgatgaatgcttggcaactatttctctcattcattttctctcc 1100
1101 tttttatagttgatcaatatcctatgtatgtttatgattatccttaaaattattgatcgaaatgaaatgttaacgggtccaaattcttttcttttact 1200
1201 tatatcaatgatcatgatgatggtttattccttgaatttcaccctgttctctagatttctcttatgtttagaaccttaaatttgttgggtgggggta 1300
1301 atttttgagcaagtacacataatgcaaatgaatgaatatatatattcaacgttaactagtaaaaaaaaaaaaaaaaaaaaaa 1381

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FIG. 1. Nucleotide sequence of the cDNA and the deduced amino acid sequence for *S. mansoni* tropomyosin II. The amino acids are shown using single-letter code below their codons beginning with the underlined ATG and ending with the termination codon taa. Untranslated nucleotide sequences are shown in small letters while translated sequences are shown in capital letters. A predicted polyadenylation signal AATAAA is underlined twice.

other known protein sequences showed 65.8% homology with schistosome tropomyosin (Xu *et al.* 1989). Tropomyosins from *B. glabrata* (Dissous *et al.* 1990) and the nematode, *Trichostrongylus colubrifomis* (Frenkel *et al.* 1989), had 65.1 and 58.1% homology scores, respectively, followed by *Drosophila melanogaster* with 53.5% homology (Fig. 2). In addition, the amino acid sequence of this schistosome tropomyosin follows the canonical heptad repeat pattern found in all tropomyosins described to date (Smillie 1979; data not shown), strongly suggesting that clone pSmTMII encodes a schistosome tropomyosin.

Expression and immunological characterization of SmTMII. The 284 amino acid open reading frame of SmTMII was cloned into the pFLAG vector and expressed at high levels in *E. coli*. The recombinant protein was purified on an affinity column containing sepharose coupled to the M1 mAb specific for the FLAG peptide fused to the

recombinant SmTMII. SDS-PAGE analysis showed the purified peptide to migrate at about 44 kDa (Fig. 3a, lane 1). On the other hand, in previous attempts to express SmTMII in *E. coli* the SmTMII protein was observed to migrate as a 39-kDa unfused peptide (data not shown). The observed migration of unfused SmTMII (39 kDa) is higher than the 33-kDa predicted molecular weight. However, it has previously been reported that tropomyosin proteins migrate anomalous to their predicted molecular weights during SDS-PAGE analysis (Lau *et al.* 1985; Mak *et al.* 1980). These workers suggest that this phenomenon is attributable to differential binding of detergent or other factors, rather than resulting from significant variation in molecular size. Recombinant SmTMII was specifically recognized by rabbit anti-SNAG, confirming that the initial immunoreactivity of pSM4 is consistent for the recombinant protein (Fig. 3a, lane 2). In addition, SmTMII was recognized by antisera directed against schisto-

	20	40	60	80	100	
SmTMII	MEHIKKMLAMKLDKENAVDEADQLEAKLREKELEMOQKDEEVAEVLKKIQQVDTDKETAQTQLAETNTKLEETDKRATEAAEAVASLQKRIRQLEDELE					
SMTM	.DG....I....E....MER.V.Y.EL.KK..E.REKRES.I..LNN.MK.AQI.CDE..ET.Q.QMN.....N.....AMTR...L..ED..					
BgTMII	.DA.....ME.....I.R.E.MEQ.V.DV.ETKNKLE..FNNLQ..FSNLQN.FD..NEG.T.AQ.....ASE.HVA.L.SDT.G.N..QL..ED..					
BG39	.DA.....ME.....I.R.E.M.Q...DV.ETKNKLE..FNNLQ..FSNLQN.FD..NEG.T.AQ.....ASE.HVA.L.SDT.G.NR..QL..ED..					
TCI	.DA.....Q...IE.D..L.R..AA.E.V.QITEKLERVE..LRDTQ..MM.TEN.LDK..ED..AATSQ...KE.KVQ.....A.NR.MTL..E..					
DMII	.DA.....Q...V..DG.LER.LVC.QEA.DANTRAEKAE..ARQLQ...T.ENELDOT.EA.TLVTG....KN.ALQN...S...A.NR..QL..ED..					
	120	140	160	180	200	
SmTMII	STETRLQEAATVKLEEASKAADESGRKVLNRTFADEERINQLEEQLEKSTFMAEDADRKYEAAARKLAITEVELERAESRLEAAESKITELEELRIV					
SMTM	VSSS..T.TLT.....T.E.E....D..I.SI..D..L...D.Q..AKYI.....A..DF....A.....V.....V..					
BgTMII	RS.E...S...E.....E.....S.SL..D..LDG..A...AKYI.....E.....D.....A.....A..I.....KV..					
BG39	RS.E...S...E.....E.....S.SL..D..LDG..A...AKYI.....E.....D.....A.....A.VW..D...H..					
TCI	RA.E..KI..E...THNV..E.V...M..GS.Q...A.TI..A...AQML..E...V...MV..AD...E.A..G.N..V...V..					
DMII	RS.E..GS..A..S...Q...E..A..I...AL...MDA..N...AR..L..E..K...V...MV..AD...E.A..G.N..V...V..					
	220	240	260	280		
SmTMII	GNNVSLSEIQEAAQREEAYEENIRDLTERLKAADRAQESERLVNTLQADADRLDELVTEKEKYKALSEELSTFAELTGN					
SMTM	...M.A.....S.....S.....T.....Q..T.A..Q.SK..NEV.H...D.LA...R...G...Q.....Y				65.8%	--
BgTMII	...M.....DS.....T.....Q...D..N..T.A..T.SK..KEV.....LA...R..SI..D.....A.Y				63.3%	68.3%
BG39	...I.T.S.QNDQ.S...DS.Q.T...Q...D..N..T.A..T.SQ..KEV.....LA...R..SI..D.....A.Y				60.6%	65.1%
TCI	...L...V..EK.L...Q...TISS...E..T..EFA..S.QK..KEV.....H...R..I...Q...S.Y				58.1%	58.1%
DMII	...L...V..EKS...E.KNQ.KT.NT...E..A..EFA..S.QK..KEV.....D..L...R..DIGDD..TA.V..ILKE				53.5%	56.0%

FIG. 2. Alignment of tropomyosin amino acid sequences from other invertebrate species for homology comparison with SmTMII. Amino acid single-letter code is used with dots representing positions having identity with SmTMII. Sequences of *S. mansoni*, SMTM (Xu *et al.* 1989); *B. glabrata*, BgTMII (this report), BG39 (Dissous *et al.* 1990); *T. colubriformis*, TCI (Frenkel *et al.* 1989); *D. melanogaster*, DMII (Hanke and Storti 1988) are compared. The homology scores with respect to SmTMII are listed at the end of each sequence in boldface print.

some tropomyosin isoform, SMTM (Fig. 3a, lane 3), and pooled sera from mice chronically (12 weeks) infected with *S. mansoni* (Fig. 3a, lane 4).

To determine the native molecular weight of SmTMII, adult worm antigens were blotted onto nitrocellulose and probed with rabbit anti-SNAG antibody rendered monospecific for SmTMII by affinity purification. This antibody preparation recognized two protein bands on Western blots of whole parasite antigens (Fig. 3b, lane 1). The two immunoreactive bands corresponded to molecular weights of approximately 36 and 39 kDa. When this antigen preparation was probed with antiserum directed against SMTM the same two bands were reactive, with the 36-kDa band having more reactivity than the 39-kDa band (Fig. 3b., lane 2). The 36- and 39-kDa proteins appear to be highly abundant in the adult worm antigen preparation and present at approximately a 2:1 ratio as determined by Ponceau-S staining of the blotted membrane (data not shown).

SmTMII gene characterization. Northern analysis was performed to investigate if

the 1.4-kbp insert of pSmTMII represented the size of the transcript in the adult worm. The entire insert of pSmTMII was radiolabeled and used to probe fractionated and blotted adult mRNA. Autoradiography of the blot revealed a single hybridizing band of about 1.6 kb, indicating that the cDNA insert of pSmTMII was nearly full-length (Fig. 4).

The organization of the schistosome tropomyosin II gene was analyzed by Southern blot using the pSmTMII insert to probe adult *S. mansoni* genomic DNA digested with *EcoRI*, *HindIII*, and *EcoRV* (Southern 1975). Treatment with each restriction enzyme produced multiple bands having an array of signal intensities (Fig. 5). These data suggest the presence of intervening sequences and/or multiple gene copies. Furthermore, an *EcoRI* map of an SmTMII genomic clone isolated by our laboratory revealed the presence of at least three introns between the internal *EcoRI* sites of the cDNA (unpublished data).

Cloning of a novel snail tropomyosin. To initiate studies of intermediate host shared antigens the 650-bp *EcoRI* fragment of

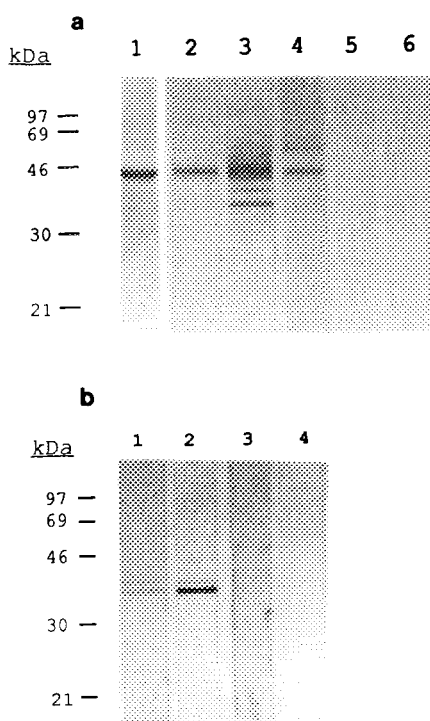


FIG. 3. (a) Coomassie stain and Western blot of recombinant SmTMII. SmTMII was expressed in *E. coli* and purified by affinity chromatography. Purified SmTMII, shown by Coomassie stain after 12% SDS-PAGE (lane 1), was transferred to nitrocellulose and probed with rabbit anti-SNAG, 1:1000 (lane 2); mouse anti-SMTM, 1:250 (lane 3); pooled infected mouse serum, 1:100 (lane 4); normal mouse serum, 1:100 (lane 5); and preimmune rabbit serum, 1:100 (lane 6). (b) Western blot of soluble whole-worm antigens probed with monospecific antibody directed against SmTMII, 1:20 (lane 1); mouse anti-SMTM, 1:250 (lane 2); preimmune rabbit and normal mouse serums, both 1:100 (lanes 3 and 4).

pSmTMII was used to screen a *B. glabrata* Lambda ZAP cDNA library by low stringency hybridization. Out of 120,000 recombinants screened 20 positive clones were obtained. Of these 10 were plaque purified, subcloned as described above, and analyzed by restriction digest and agarose gel electrophoresis. One clone (pBgTMII) containing an insert of about 2.8 kbp was chosen for further analysis. Both strands of the pBgTMII insert were entirely sequenced yielding a cDNA sequence 2816 bp in

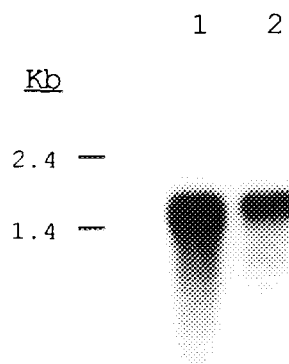


FIG. 4. Northern blot hybridization of mRNA from adult-stage *S. mansoni*. Poly(A⁺) RNA from adult parasites, 5 μ g (lane 1) and 0.75 μ g (lane 2), was fractionated on a 1.2% agarose gel in the presence of 2.2 M formaldehyde, transferred to a nylon membrane, and probed with ³²P-labeled SmTMII insert DNA. Film exposure was about 48 hr with an intensifying screen at -70°C. RNA size standards are indicated in kilobases (kb).

length, including a 31 adenosine nucleotide tail. Computer-assisted analysis of the sequence revealed a single open reading frame of 284 amino acids beginning with a methionine codon at position 193 and closing at position 1044. The insert contained a long 3' untranslated region 1772 bp in length with the putative polyadenylation signal ATTAAA 18 bp upstream of the poly (A⁺) tail (Fig. 6). In addition, a second snail tropomyosin cDNA clone 2314 bp in length was entirely sequenced and found to be identical to pBgTMII, but lacked approximately 500 bp of 3' untranslated region. The two alternative transcripts appeared to employ one of two polyadenylation signals found at positions 2296-2301 (AATAAA) and 2762-2767 (ATTAAA) of the BgTMII cDNA (Fig. 6).

Comparison of the 284 amino acid-encoded protein of pBgTMII revealed it to have 94.7% identity with BG39. The differences in the two proteins were due to a region defined by residues 189 to 213 in which the two snail tropomyosins share only 52% homology. In addition, there were three single base substitutions within the coding

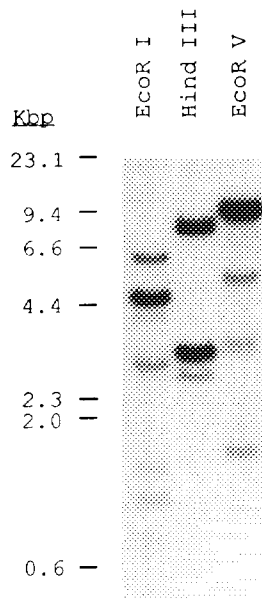


FIG. 5. Southern blot hybridization of genomic DNA isolated from *S. mansoni*. Genomic DNA (20 μ g) was digested with *EcoRI* (lane 1), *HindIII* (lane 2), and *EcoRV* (lane 3), fractionated on a 0.7% agarose gel, transferred to a nylon membrane, and probed with ^{32} P-labeled SmTMII insert DNA. Film exposure was about 96 hr with an intensifying screen at -70°C . DNA size standards are indicated in kilobase pairs (kbp).

region of pBgTMII with respect to BG39, all of which resulted in an amino acid change (Fig. 7).

DISCUSSION

The immunological cross-reactivity between antigens of *S. mansoni* and *B. glabrata* has been demonstrated by numerous independent groups over the past three decades, but molecular cloning techniques have only recently been applied to the study of this phenomenon. In this report an adult schistosome cDNA expression library was screened to identify clones expressing epitopes recognized by anti-snail tissue antibodies. From this screening three parasite recombinant antigens were found to contain epitopes that cross-reacted with antibodies against snail host antigens: tropomy-

osin I (SMTM) (Xu *et al.* 1989), tropomyosin II (SmTMII), and myosin heavy chain (Weston *et al.*, 1993).

Tropomyosin belongs to a conserved class of regulatory proteins associated with muscle and nonmuscle cells. In striated muscle tropomyosin interacts with the troponin complex to regulate calcium-mediated muscle contraction via interaction with actin and myosin (Smillie 1979). Tropomyosin can also be found in nonmuscle cells and is believed to take part in cytoskeletal activities such as cytoplasmic streaming, phagocytosis, and cell division (Cote 1983). Previous immunolocalization studies have identified SMTM epitopes strongly associated with the tegument and muscle tissue of adult schistosomes (Xu *et al.* 1989). These localization data most likely include SmTMII epitopes and SMTM epitopes since both isoforms share immunological cross-reactivity. Recombinant SmTMII was also recognized by pooled sera from mice with chronic *S. mansoni* infections. These data, however, do not establish if SmTMII is an immunogen during infection or is simply cross-reacting with antibodies directed toward SMTM, a known immunogen, during *S. mansoni* infections (Xu *et al.* 1989).

The native molecular weight of SmTMII was determined by probing SDS-PAGE-fractionated adult parasite antigens with monospecific antibody to SmTMII. Two bands of approximately equal intensity were detected on Western blots having molecular weights of 36 and 39 kDa. Anti-SMTM recognized the same two bands, with the 36-kDa band reacting more strongly than the 39-kDa band. Furthermore, SmTMII expressed in *E. coli* as an unfused protein migrates at 39 kDa. These data suggest that the 36-kDa band is SMTM and the 39-kDa band is SmTMII, although in the initial report characterizing SMTM (Xu *et al.* 1989) the authors report the native molecular weight of SMTM to be about 40 kDa.

FIG. 6. Nucleotide sequence of the cDNA and deduced amino acid sequence for *B. glabrata* tropomyosin II. The amino acids are shown in single-letter code below their codons beginning with ATG (in bold letters) and ending at the termination codon tga. Untranslated nucleotide sequences are shown in small lettering and the coding region is shown in capital lettering. The putative polyadenylation signals AATAAA, for the 2314-bp cDNA, and ATTAAG, for the 2816-bp cDNA are underlined.

In order to more thoroughly investigate the cross-reactivity between host and parasite antigens it was necessary for us to clone tropomyosin isoforms from *B. glabrata*. The first described snail tropomyosin, BG39, has recently been cloned and described as an antigen sharing cross-reactivity with Sm39 and Sm43 of *S. mansoni* (Dissous and Capron 1989; Dissous *et al.* 1990). In light of the discussion above, it is likely that Sm39 and Sm43 represent SMTM and SmTMII, respectively, but

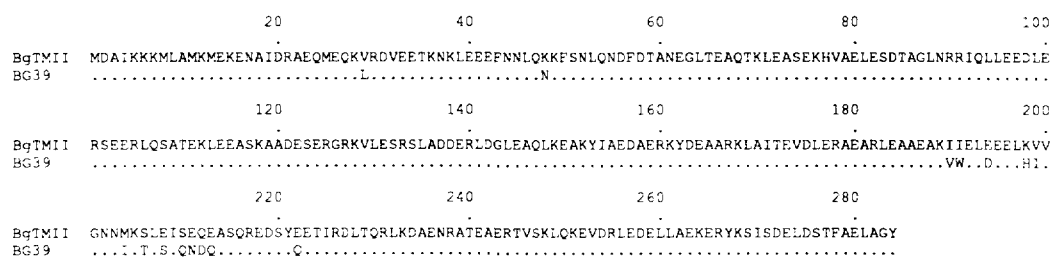


FIG. 7. *B. glabrata* tropomyosin peptide alignment. Comparison of amino acid homology between BgTMII and BG39. Amino acids are shown in single-letter code with dots representing positions of identity.

more experimental data are required to confirm this. In the present study we have described the cloning of BgTMII from a *B. glabrata* cDNA library and identified it as a novel tropomyosin isoform. BgTMII and BG39 shared 94.7% identity with one another, the difference being due primarily to amino acids 189–213 in BgTMII which shared only 52% identity with BG39, but BgTMII shared 88 and 84% identity with SMTM and SmTMII, respectively. These data indicate that the domain defined by amino acids 189–213 of BgTMII may encode additional host-like epitopes not present in BG39. This region corresponds to exon 6 in the *D. melanogaster* TmII gene which is alternatively spliced to generate isoform diversity (Hanke and Storti 1988). Whether or not this mechanism is occurring in *B. glabrata* is currently under investigation.

Comparison of host and parasite tropomyosins show BgTMII and BG39 to have 68.3 and 65.5% identity with SMTM (Xu *et al.* 1989), respectively, as well as 63.4 and 60.6% identity with SmTMII, respectively. Thus, BgTMII has higher homology with SMTM than the two schistosome tropomyosin isoforms have with each other (65.8%). These data and the homology scores from other invertebrate species suggest that the *B. glabrata* tropomyosin II (BgTMII) protein is more closely related to both *S. mansoni* tropomyosin peptides than to the *T. colubriformis* tropomyosin protein which has 58.1% identity with BgTMII. Interest-

ingly, these data appear to contradict the classical phylogenic order which places the nematodes (pseudocoelomates) between the platyhelminths (acoelomates) and the molluscs (protostome coelomates). However, while the organisms as a whole may conform to the phylogenic order, certain genes and their products may not. It has been shown that *E. coli* has obtained one of its glyceraldehyde-3-phosphate dehydrogenase genes from some ancient eukaryotic host by a naturally occurring horizontal gene transfer (Doolittle *et al.* 1990). With the close proximity and ancient evolutionary history of the host–parasite relationship between schistosomes and biomphalarid snails it is entirely feasible that this phenomenon has occurred between the two organisms. This possibility is consistent with the hypothesis of molecular mimicry, as formulated by Damian (1964, 1987). Damian hypothesized that in host–parasite relationships a strong selective pressure exists to drive parasites toward antigenic convergence with immunologically competent hosts. Data from molecular analysis of similar genes in the host and the parasite could be interpreted as lending significant support to this concept, especially in light of the preceding discussion of phylogenetic relationships. Furthermore, immunolocalization studies have located BG39 cross-reactive epitopes in vesicles in the epidermal ridges and subtegumental cytons of miracidia (Dissous *et al.* 1990), suggesting that schistosome tropomyosin epitopes are

associated with the protective tegument surrounding the sporocyst and a spatial and temporal context for tropomyosin involvement at the host-parasite interface. Such an anatomical location would be necessary and predicted if these antigens are indeed contributing to immune evasion, as originally suggested by Damian (1964).

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