Acta Biochimica Polonica

Vol. 50 No. 1/2003

269–278

www.actabp.pl

Cloning and characterization of a *Schistosoma mansoni* 1H and 30S clones as two tegumental vaccine candidate antigens

Amr M. Shabaan[1], Magdy M. Mohamed[1]✉, Mohga S. Abdallah[2], Hayat M. Ibrahim[2] and Amr M. Karim[1]

[1]*Department of Biochemistry, Faculty of Science, Ain Shams University; Cairo, Egypt;*
[2]*Department of Chemistry, Faculty of Science, Helwan University, Cairo, Egypt*

Received: 10 April, 2002; revised: 29 October, 2002; accepted: 18 February, 2003

Key words: Tegumental antigen, Sm13, vaccine candidate antigen, anti-GST

Two *Schistosoma mansoni* cDNA clones 30S and 1H were identified by immunoscreening of sporocyst λgt11 library and by random sequencing of clones from λZap libraries, respectively. Clone 30S was one of 30 clones identified by an antibody raised against tegument of 3-h schistosomules. The clone was found to encode an 81 amino-acid protein fragment. It was expressed in *Escherichia coli* as a fusion protein of calculated molecular mass of about 35 kDa with C-terminus of *Schistosoma japonicum* glutathione-*S*-transferase (Sj26; about 26 kDa). The recombinant fusion protein was specifically recognized by serum of rabbits immunized with irradiated cercariae. Clone 1H is one of 76 expressed sequence tags derived from an adult worm library. It encodes the complete sequence of a tegumental membrane protein, Sm13. The 104 amino-acid open reading frame encodes a protein with a calculated molecular mass of about 11.9 kDa. Clone 1H was expressed in *E. coli* as an insoluble fusion protein with Sj26 of about 40 kDa. In Western blots, the fusion protein was recognized by serum from rabbits vaccinated with irradiated cercariae but not by preimmune rabbit sera. The cloning, characterization and expression of those proteins are therefore potentially usefull for vaccine development.

Schistosomiasis (Bilharzia) is a major debilitating and parasitic disease affecting humans with an estimate 200 million people in 75 countries currently inffected and a further 500–600 million exposed to infection (Waine *et al.,* 1994). It is caused by three major species of parasitic trematoda (Xiaochuan *et al.*, 1997). The most inportant schistosome species infecting humans is *Schistosoma mansoni,* which causes intestinal schistosomi-

✉Corresponding author: Dr. Magdy Mahmoud Mohamed, Department of Science, Chemistry Division, P.O. 1070, Taif Teacher's College, Taif, Saudi Arabia; e-mail: mutagen61@yahoo.com

Abbreviations: EST, expressed sequence tag; GST, glutathione-*S*-transferase; NRS, normal rabbit serum; various, vaccinated rabbit serum.

asis in Africa, Latin America, and the Caribbean. It is considered as the second most prevalent tropical disease and a leading cause of severe morbidity in several foci. There are many endemic areas where schistosomiasis is not yet recognized as an important public health problem (Bergquist, 1993). Egypt has one of the most heavily infected populations in the world, this is because the urinary and intestinal schistosomiasis are both endemic and occur with great frequency, for example in some endemic localities of the Nile valley, the infection rate exceeds 80% (Bogitsh & Cheng, 1990). A WHO report in 1993 recorded that the total number of infected individuals in Egypt in 1990 was estimated to be 5–6 millions (WHO, 1993).

In the search for a vaccine, the antigens associated with the tegumental membrane of the parasite are leading candidates. Smithers *et al.* (1989) have shown that protection levels induced by immunization with isolated adult worm tegumental sufrace membranes approach those obtained with irradiated cercaria in the same strain of mice. Analysis of antibody response reveals the principal tegumental antigens as proteins of 13, 15, and 25 kDa (Sm13, Sm15, and Sm25) (Smithers *et al.,* 1990).

The potential usefulness of tegumental membrane antigens for the development of an antischistosomiases vaccine has led to attempts to clone and characterize many tegumental proteins. By screening *S. mansoni* cDNA expression libraries with antibodies against tegumental membrane, clones encoding the 21.7 kDa (Knight *et al.,* 1989) 22.6 kDa (Stein & David, 1986) and 20.8 kDa (Mohamed *et al.,* 1998a) proteins have been previously identified.

MATERIALS

Biological materials. The λgt-11 and λZap cDNA libraries from different *S. mansoni* stages were kindly provided by Dr. P.T. LoVerde (SUNY, Buffalo, U.S.A), and Dr. M. Saber (TBRI, Cairo, Egypt).

Parasites. *Schistosoma mansoni* Egyptian strain (Frandson, 1979) and cercaria of *S. mansoni* were obtained from the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute. Molecular mass marker was from Sigma Chemical Co. Bulk dNTPs were from Promega Corp.

Serum. Serum from a rabbit vaccinated with irradiated *S. mansoni* cercariae (VRS) was kindly provided by Dr. P.T. LoVerde. This polyclonal antiserum was used to screen a *S. mansoni* sporocyst cDNA library at dilution 1:1000. Normal rabbit serum (NRS) was obtained from a preimmunized rabbit at dilution 1:1000. Anti-GST monoclonal antibody (1:10 000) was from Promega Corp. (U.S.A).

METHODS

DNA isolation and sequencing. The method of Young & Davis (1983) was used to immunoscreen a λgt11 adult *S. mansoni* cDNA library. The λ bacteriophage was amplified by PCR (Saiki *et al.,* 1988) using a pair of oligonucleotides complementary to sequences flanking the unique *Eco*RI site in the phage DNA. Plasmids were prepared by the alkaline lysis method (Sambrook *et al.,* 1989). Isolated inserts were sized on agarose gel following *Eco*RI and *Xho*I digestion. One kbp DNA ladder (GIBCO-BRL, Life Technologies Inc.; Grand Island, NY, U.S.A.) was used as a size marker. The inserts were sequenced from both ends using oligonucleotids primers complementary to the flanking T3 and T7 sequences. Sequecing was carried out using the dideoxynucleotide chain termination method (Sanger *et al.,* 1977). Sequence analysis and homology comparisons were counducted using DNASTAR software and Blast programs (Altschul *et al.,* 1990).

Preparation of *in vivo* excised λZap cDNA library and *in vivo* excision of the cDNA library were according to Tabor & Richardson (1989).

Sequenase V.2.0 (USB) sequencing kit was used to sequence the double-stranded DNA subcloned in a plasmid vector using the chain-termination DNA sequencing method (Sanger *et al.*, 1977).

Techniques for protein expression. Prokaryotic expression vectors (pGEX-4T-2 and pGEX-4T-3) were used to express the recombinant protein. The vectors have an IPTG-inducible *tac* promoter; a functional hybrid derived from the *trp* and *lac* promoters, which upon induction results in the synthesis of a fusion protein with the amino terminus of the *S. japonicum* glutathione *S*-transferase (Sj26) protein (Smith & Johnson, 1988). Proteins separated on SDS/PAGE were transferred onto a nitrocellulose membrane in a Trans-Blot SD semi-dry electrophoretic blotting cell (BioRad) according to the method described by Towbin *et al.* (1979).

RESULTS

Cloning and expression of clone 1H

λZap adult worm library

This library was *in vivo* excised and the resulting recombinant pBluescript SK plasmids were sequenced. Seventy six ESTs (expressed sequence tag) were generated from this library. One of the cDNA clones was isolated which had an insert size of calculated molecular mass of about 0.65 kbp as determined by *Eco*RI and *Xho*I digestion as shown in Fig. 1.

Full length sequencing of the *S. mansoni* clone 1H

Sequence analysis revealed an open reading frame corresponding to 104 amino acids and a calculated molecular mass of 11.9 kDa. Figure 2 shows the open reading frame starting with ATG at nucleotide 30 and ending with a TTA stop codon at nucleotide 379, a putative AATAAA polyadenylation signal is shown at

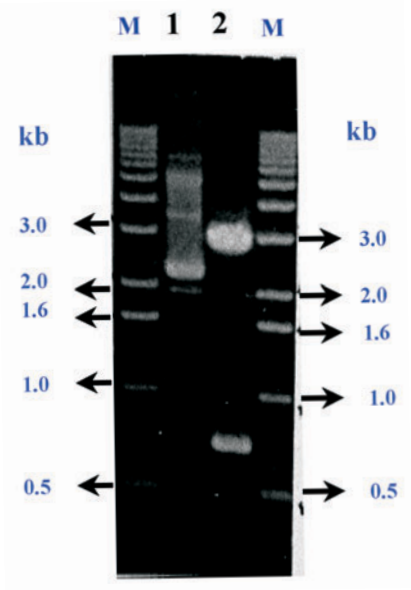

Figure 1. Agarose gel electrophoresis (1%) of plasmid pBlue-1H and insert 1H.

M: Marker (1 kbp DNA ladder). Lane 1: pBlue-1H undigested; lane 2: pBlue-1H digested with *Eco*R1 and *Xho*1.

position 411 and a poly (A) tail is located further downstream. It has an about 29 bp 5′ untranslated region and a 383 bp 3′ untranslated region. The nucleotide sequence used to search GenBank databases for homology has revealed the highest homology with sequence Af072886, the Sm13 gene. Clone 1H shows 100% identity with the genomic sequence from nucleotide 384 to nucleotide 878 of the genomic sequence. An interruption in the alignment between positions 536 and 581 reveals the presence of a single intron of 45 nucleotides. An identity of 100% is also revealed on alignment with the GenBank Sm13 mRNA sequence U67153 (Fig. 3). The putative AATAAA polyadenylation signal at position 411 is 12 nucleotides upstream of the poly (A) tail of the GenBank mRNA sequence.

Subcloning and expression of recombinant clone 1H

In order to study the biological characteristics of clone 1H, the coding sequence was subcloned in the expression vector pGEX-4T-3 and the protein was expressed in *E. coli* as a fusion protein with Sj26. The reactivity of the

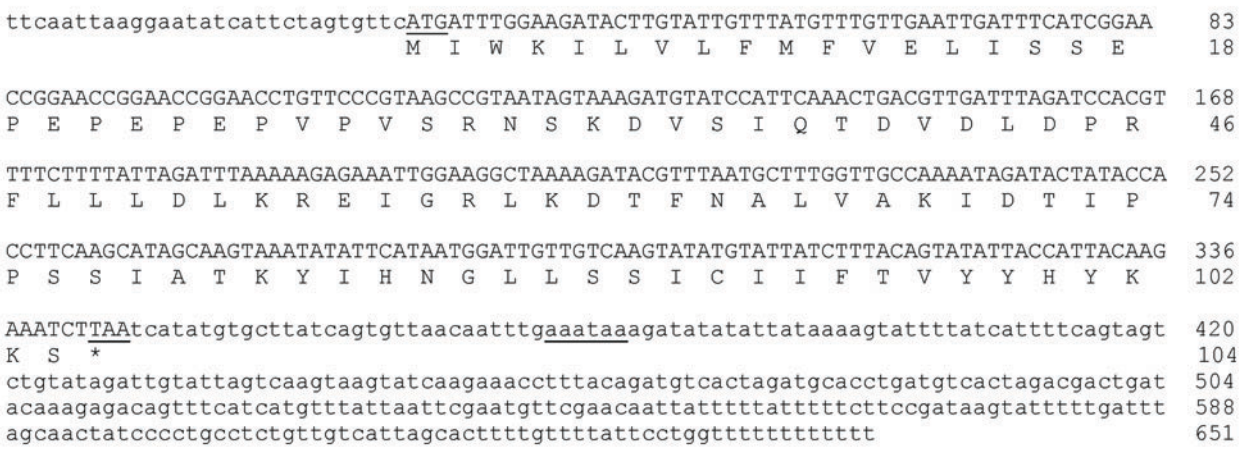

Figure 2. Nucleotide sequence and deduced amino-acid sequence for insert 1H.

The coding nucleic acid sequence is shown in upper case while the non-coding sequence is shown in lower case. The start codon (ATG), the stop codon (TAA) and the polyadenylation signal (AATAAA) are underlined.

fusion protein (Sj26-1H) with antibodies raised against native antigen was examined then transferred onto nitrocellulose membrane. Triplicate samples were reacted with

Figure 3. Blast N alignment of Sm13 insert (1H) sequence with GenBank sequences.

Top line: 1H insert sequence; U67153: Sm13 mRNA sequence; Af072886: Sm13 gene sequence.

by Western blot analysis. From zero to 5 h post-induction the transformed cells were harvested. The induced culture was pelleted and lysed. Aliquots from both the supernatant and the pellet were ran on 15% SDS/PAGE and VRS, NRS, and anti-GST. Figure 4 shows that both VRS and anti-GST recognized a band of 40 kDa. This is the expected size for a protein of calculated molecular mass of about 13 kDa fused with Sj26. NRS did not recognize the fu-

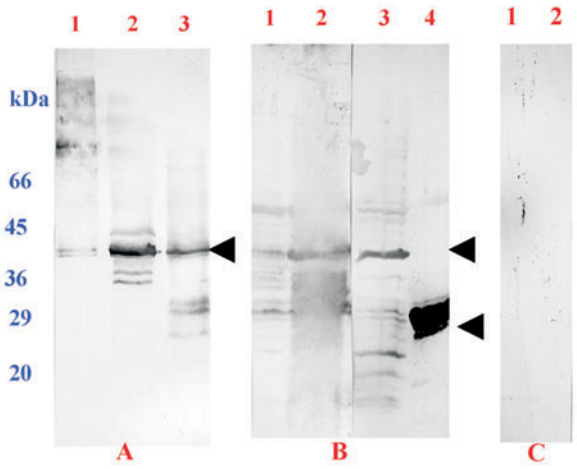

Figure 4. Western blot analysis of Sj26-1H fusion protein immunoreactivity.

Reaction with VRS (Panel A), anti-GST (Panel B) and NRS (Panel C). Lane 1: supernatant of bacterial cell lysate with induced pGEX-1H; lane 2: resuspended pellet of bacterial cell lysate with induced pGEX-1H; lane 3: unfractionated bacterial cell lysate with induced pGEX-1H; lane 4: bacterial cell lysate with induced native pGEX4T-3.

sion protein. The protein was present mostly in the pellet and to a much lesser extent in the supernanat fraction.

Cloning and expression of clone 30S

Immunoscreening and insert DNA preparation

S. mansoni sporocyst λgt11 cDNA library was immunoscreened using affinity purified VRS antibodies diluted 1:1000 in 1% BSA/1× PBST20. The diluted antibodies were initially adsorbed with lysate of *E. coli* infected with λgt11. This step was carried out in order to deplete the antibodies, which may cross react with *E. coli* or λgt11 antigens. Approximately 130 independent positive clones were isolated from 150 000 plaques screened in primary screening. These clones were further isolated and subjected to second and third rounds of immunoscreening to give finally 30 positive clones. One out of the thirty clones was amplified (clone 30S, which is a highly reactive clone that gives the darker plaque during immunoscreening of λgt11 sporocyst library), digested with *Eco*R1 and subcloned in the pGEM7ZF cloning vector. The insert size was about 0.62 kbp (Fig. 5). The entire insert 30S

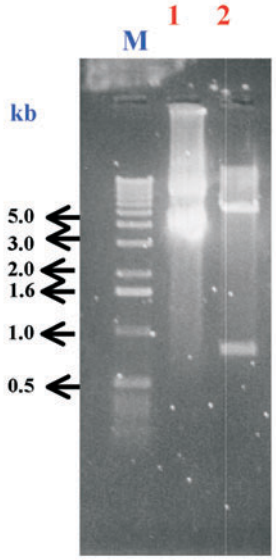

Figure 5. Agarose gel electrophoresis (1%) of recombinant plasmid with insert 30S.

Lane 1: pGEM-7Zf-30S undigested; lane 2: pGEM-7ZF-30S digested with *Eco*RI; lane M: molecular size marker (1 kbp ladder).

was sequenced from the Sp6 and T7 ends (Fig. 6). The nucleotide sequence of clone 30S was used to search databases for homology. BLASTN and BLASTX analysis revealed the highest homology to a hypothetical *Schizosaccharomyces* protein (Z99759). This suggested that we have identified a new schistosome vaccine candidate.

Subcloning and expression of recombinant clone 30S

The recombinant protein encoded by the pGEM 30S insert was expressed in the prokaryotic expression system pGEX-4T-2 under the control of the pGEX system. A single bacterial colony containing the pGEX 2T-30 plasmid was grown in culture and induced with 1 mM IPTG to produce the calculated molecular mass of about 40 kDa fusion protein

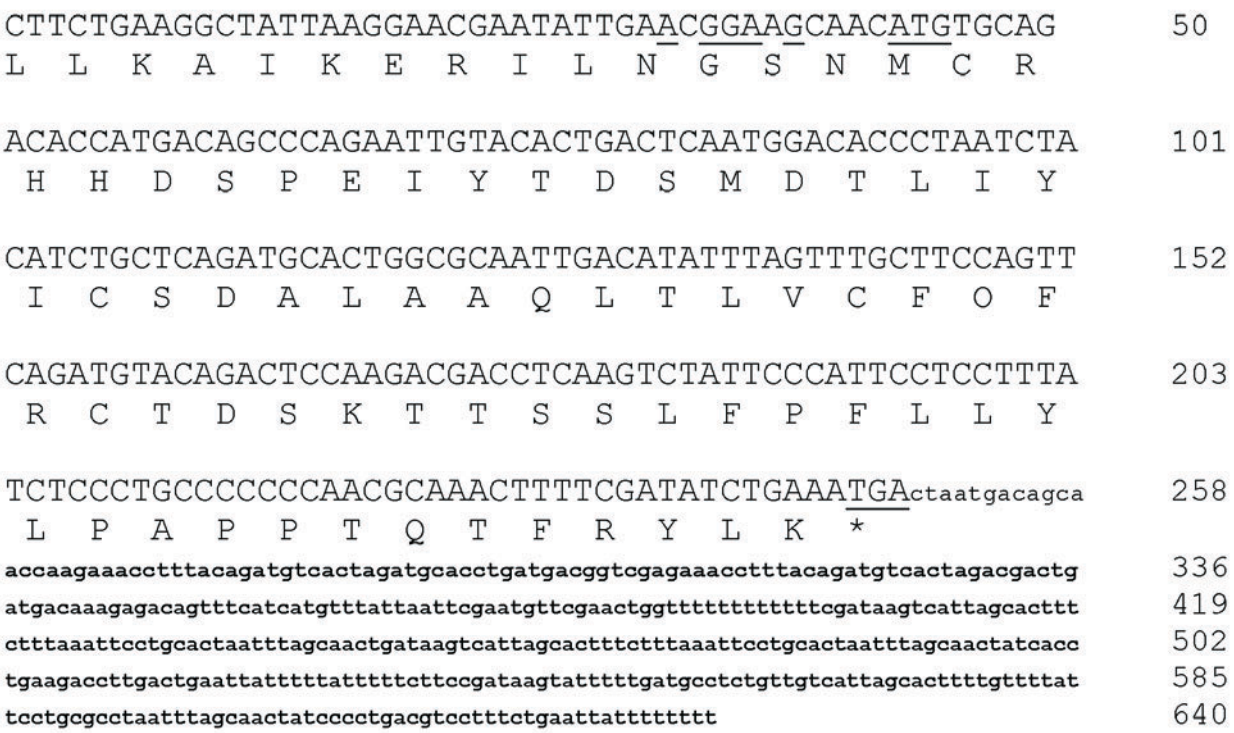

Figure 6. Nucleotide sequence and deduced amino-acid sequence for insert 30S.

The coding nucleic acid sequence is shown in upper case while the non-coding sequence is shown in lower case. The start codon (ATG), the stop codon (TGA) and the putative Shine–Dalgarano sequence are underlined.

with 26 kDa GST. Five hours post-induction the transformed cells were harvested. The induced culture was pelleted and the cell pellet lysed. The lysate was centrifuged, aliquots from both the supernatant and the pellet were ran on 15% SDS/PAGE. Figure 7 shows that the recombinant protein was found principly in the pellet.

Immunoreactivity of fusion protein

The reactivity of the fusion protein with antibodies against native antigen was examined by Western blot analysis. The nitrocellulose membrane was then cut into 3 stripes (Fig. 8). One stripe was reacted with VRS, the second with anti-GST, the third stripe was reacted with NRS. Both VRS and anti-GST were reactive as evidenced by a single band at about 36 kDa compared to NRS that showed no reaction. Therfore, the fusion protein of clone 30S an immunoresponse with VRS.

DISCUSSION

The present study was aimed at identifying schistosome cDNA clones which could be of importance for examining their immunogenicity and for a vaccine development against schistosomiasis. In order to achieve those goals two approaches were used.

The first approach was based on random sequencing of cDNA clones from excised λZAP cDNA libraries as a part of ESTs project. The availability of those clones will facilitate characterization of the schistosome counterparts of proteins identified in other organisms and should also enable studies to identify the function and expression profiles of as yet unidentified proteins.

Sm13 has been described recently as a tegumental antigen (Abath *et al.*, 2000) and Sm13 encoding EST clones were isolated in our laboratory. The authors used rabbit antiadult worm tegumental membrane antisera to im-

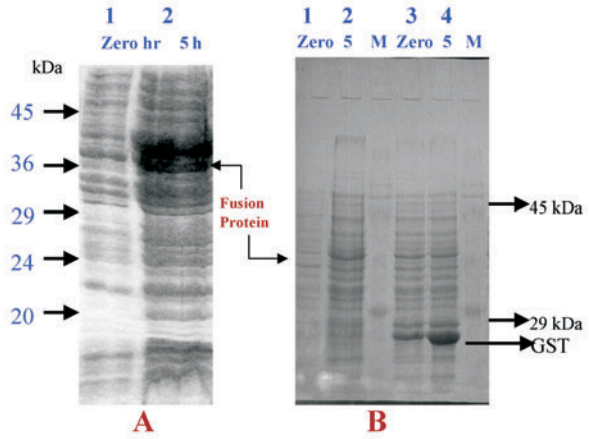

Figure 7. SDS/polyacrylamide gel electrophoresis.

Panels A and B are two different induction preparations for 30S fusion protein separated on 15% SDS/PAGE; lanes 1 and 2 are plasmid pGEX-2T-30S induction at time zero and 5 h, respectively; lanes 3 and 4 show induction of the parent pGEX-2T at time zero and 5 h; M: low molecular mass marker.

munoscreen a λgt11 adult *S. mansoni* cDNA library. A clone with a cDNA insert that encodes a protein of 104 amino acids was identified. The calculated molecular mass is 11.9 kDa. Antibodies against the λgt11 β-galactosidase fusion protein were used in Western blot analysis and indirect immunofluorescence to identify a 13-kDa protein (Sm13) in the tegument of adult worms. Searches of the nucleic acid data banks, protein databases and GenBank translations revealed no significant homology. However, EST database searches revealed three almost identical sequences (GenBank accession numbers AAS17940, N20684 and AA269243). The independent isolation of homologous cDNA confirmed the sequence of Sm13. The sequence (# AA269243) is that of the cDNA we describe here.

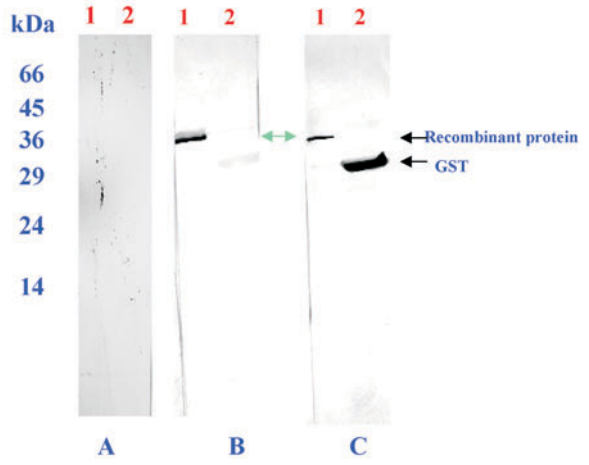

Figure 8. Western blot analysis: immunoreactivity reaction of pGEX-2T-30S fusion protein with NRS (panel A), VRS (panel B) and monoclonal anti-GST (panel C).

Lane 1: supernatant fraction of bacterial cell lysate with induced pGEX-2T-30S; lane 2: supernatant fraction of bacterial cell lysate with induced only pGEX-2T.

We sequenced the entire 0.65 kbp insert of clone 1H (EST GenBank accession # AA269243). Sequence analysis using DNASTAR software revealed a 104 amino-acid open reading frame (ORF) identical to that described by Abath *et al.* (2000). The ORF encodes the full length protein. It starts with an ATG at nucleotide 28 and ends with a TAA stop codon at nucleotide 343. A putative AATAAA polyadenylation signal is present at position 377. Our sequence also shows 100% identity with GenBank sequence AAS072886, the Sm13 gene, with the exception of an interruption in the alignment between positions 536 and 581 which reveals the presence of a single intron of 45 nucleotides. Comparison with the cDNA sequence described by Abath *et al.* (2000) (GenBank sequence SMU67153) shows that alternative splicing affects the length of the 3′ untranslated region which is about 325 nucleotides for our sequence and 50 nucleotides for SMU67153.

We have expressed Sm13 in *E. coli* as a fusion protein with the 26 kDa C-terminus of *S. japonicum* GST (Sj26). The fusion protein has a molecular mass of about 40 kDa. The Western blot (Fig. 4) shows the fusion protein present mainly in the pellet of bacterial lysates. This is expected for a hydrophobic membrane proteins. The immune relevance of Sm13 is demonstrated by its specific recognition by se-

rum from a rabbit vaccinated with irradiated cercariae.

Clone 1H (Sm13) appears to be yet another addition to likely membrane associated candidate antigens, which may be useful for the development of an anti-schistosomiasis vaccine. Along with Sm15 (Knight *et al.,* 1989) and Sm25 (Abath *et al.,* 1994) previously identified, Sm13 is one of the principle tegumental antigens recognized by mice protectively vaccinated with adult worm surface membranes (Simpson, 1990). They appear to be integral components of the membrane. The potential usefulness of those tegumental membrane antigens for the development of an antischistosomiasis vaccine has led to attempts to clone, identify and characterize those proteins.

The vaccine relevance of the antigens associated with the schistosome tegument is further emphasized by the cloning and characterization of a family of structurally related peripheral membrane proteins of about 22 kDa. Those proteins were identified by investigation immunoscreening of *S. mansoni* and *S. japonicum* cDNA expression libraries for vaccine candidates (King *et al.,* 1996; Mohamed *et al.,* 1998a). Well defined protective antisera were used for this purpose. Using this approach, Simpson (1990) identified a 22.6 kDa antigen by immunoscreening *S. mansoni* adult worm cDNA library with rabbit antimembrane serum.

The antigen which was previously described by Stein and David (1986), is recognized by mice protectively vaccinated with purified adult *S. mansoni* tegumental membranes. Sm22.6 is immunologically cross reactive with Sj22, an antigen identified by screening an adult worm library with hyperimmune rabbit serum raised against *S. japonicum* adult worm proteins. Sj22 shares 71% identity in the amino-acid sequence with Sm22.6. Another family member, Sm21.7 shares 47% amino-acid identity with Sm22.6. This antigen was identified as a vaccine dominant antigen after screening a sporocyst cDNA library with highly protective VRS and excluding clones which are also recognized by moderately protective mouse single sex infection serum.

The second approach we used to identify clones encoding *S. mansoni* vaccine candidates is based on immunoscreening. We have used an approach successfully used previously in this laboratory to identify and characterize a number of important vaccine candidates (Osman *et al.,* 1995; Keung *et al.,* 1995; Ghazalie *et al.,* 1996; Mohamed *et al.,* 1998b; 2000). This approach is based on studies which have shown that the early schistosomula stage is an important target for immune elimination (Taylor, 1991). Antigens on the surface of schistosomules are therefore important targets for a vaccine. In order to identify such antigens we solubilized proteins from the surface of 3-h schistosomules using the nonionic detergent NP-40. The NP-40 extracted antigens were used to prepare an affinity column which was subsequently used to fractionate sera from chronically infected humans or rabbits immunized with irradiated cercariae or immature female worms. In previous studies the affinity-purified antibodies were used to immunoscreen *S. mansoni* cercarial and adult worm cDNA libraries.

In this study, we selected to use a sporocyst stage library for immunoscreening. The sporocyst stage is an important stage for identifying antigens on the surface of 3-h schistosomules. Presumably the RNA messages directing the synthesis of those antigens are present in the sporocyst stage. Immunoscreening of this library using affinity purified antibodies derived from rabbits immunized with irradiated cercariae led to the identification of 30 immunoreactive clones. Of those, clone 30S was selected for further characterization. It has an insert of about 0.65 kb. Sequence analysis revealed an open reading frame corresponding to 81 amino acids ending with a TGA stop codon and a 452 nucleotide 3′ untranslated region with a putative ATTAAT polyadenylation signal 373 nucleotides upstream of a poly (A) tail. The reading frame, however, does not match that expected for a

λgt11 β-galactosidase fusion protein and it is likely that in clone 30S the immunoreactive peptide is 67 amino-acids long and is expressed from an internal ATG codon 51 nucleotides downstream of the *Eco*RI cloning site. This conclusion is supported by the presence of a 7 nucleotide purine rich sequence 11 nucleotides upstream of the start ATG. This sequence has 5 nucleotides complementary to the 16S rRNA 3′ end of *E. coli* and resembles the consensus Shine–Dalgarno sequence (ACGGAAGC) important for positioning the mRNA to start translation from the nearby ATG.

Database searches using the sequence of insert 30S and its translation in three reading frames did not reveal homology to any known nucleic acid or protein sequence. Insert 30S was cloned in the prokaryotic vector pGEX-2T for expression of the 81 amino acid ORF as a fusion protein with the 26-kDa C-terminus of *S. japonicum* GST. A protein of about 36 kDa was expressed, therefore the molecular mass of the peptide encoded by clone 30S was about 10 kDa. This is similar to the calculated molecular mass of 9.1 kDa. We have shown that the expressed fusion protein is recognized by serum from rabbits immunized with irradiated cercaria but not with normal rabbit serum, thereby verifying that the expressed protein is indeed a schistosome antigen of relevance to vaccine development. Further studies will be conducted to isolate clones encoding the N-terminal portion of the protein and to characterize the protein and study its effectiveness in protection experiments.

REFERENCES

Abath FCG, Hagan P, Jeffs SA, Simpson AJG. (1994) Partial characterization and kinetics of expression of Sm15, a *Schistosoma mansoni* tegumental antigen. *Parasitol Res.*; **80**: 64–9.

Abath FCG, Fredrerico EM, Raynond A, Yara MG, Norma L, Marcilio B, Andrew JG, Simpson AJG. (2000) Characterization of Sm13, a tegumental antigen of *Schistosoma mansoni. Parasitol Res.*; **86**: 745–52.

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ. (1990) Basic local alignment search tool. *J Mol Biol.*; **215**: 403–10.

Bergquist R. (1993) Schistosomiasis. In *Tropical disease research progress 1991–1992*. pp 29–36. 11th programme report of the UNDP & World Bank/ WHO.

Bogitsh BJ, Cheng TC. (1990) Blood flukes. In *Human parasitology*. Bogitsh BJ, Cheng TC. eds. pp 193–210. Saunders College Pub.

Frandson F. (1979) Studies of the relationship between *Schistosoma mansoni* and their intermediate hostsIII. The genus *Biomphalaira* and *Schistosoma mansoni* from Egypt, Kenya, Sudan, Uganda, West Indies (St. Lucia) and Zaire (two different strains: Katanga and Kinshasa*). J Helmin.*; **53**: 321–48.

Keung WL, Shalaby KF, Medhat AM, Hong SQY, Karim AM, LoVerde PT. (1995) *S. mansoni*: characterization of a gene encoding Sm23, an integral membrane protein. *Exp Parasitol.*; **80**: 155–8.

Kiang D, Ghazalie NE, Medhat AM, Abdel-Fatah M, Karim, AM, LoVerde PT. (1996) Identification and characterization of *Schistosoma mansoni* p17.7, a cyclophilin. *Mol Biochem Parasitol.*; **76**: 73–82.

King D, Karim AM, LoVerde PT. (1996) Cloning the gene encoding *Schistosoma mansoni* p50, an immunophilin. *Gene.*; **170**: 137–40.

Knight MC, Kelly V, Rodrigues X, Yi A, Smither W, Simpson AJG. (1989) A cDNA clone encoding part of major 5000-dalton surface membrane antigen of adult. *Schistosoma mansoni. Parasitol Res.*; **75**: 280–6.

Mohamed MM, Shalaby KF, LoVerde PT, Karim AM. (1998a) Characterization of Sm20.8, a member of schistosome tegumental antigens. *Mol Biochem Parasitol.*; **96**: 15–25.

Mohamed MR, Mohamed MM, Shalaby KA, Karim AM. (1998b) The primary structure of *Smansoni* ribosomal protein L37a. *Egyp J Biochem.*; **16**: 38–43.

Mohamed MR, Shalaby KA, Abdellah NM, LoVerde PT, Karim AM. (2000) *Schistosoma mansoni* myosin heavy chain: characterization of an immunoractive fragment with vaccine potential. *N Egypt J Med.*; **22**: 297–303.

Osman A, Kian D, LoVerde PT, Karim AM. (1995) *S. mansoni*: characterization of p50, an immunophilin. *Exp Parasitol.*; **80**: 550–9.

Saiki PK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science.*; **239**: 487–91.

Sambrook J, Fritsch EF, Maniatis T. (1989) Molecular cloning. *A laboratory manual.* 2nd edn. Cold Spring Harbor Laboratory Press, NY.

Sanger F, Nicklen S, Coulson AR. (1977) DNA sequencing with chain termination inhibitors. *Proc Natl Acad Sci U S A.*; **74**: 5463–6.

Simpson AJG. (1990) Schistosoma surface antigens: developmental expression and immunological function. *Parasitol Today.*; **6**: 40–5.

Smithers SR, Hachett F, Omer AP, Simpson AJG. (1989) Protective immunization of mice against *S. mansoni* with purified adult worm surface membranes. *Parasite Immunol.*; **11**: 301–18.

Smithers SR, Hackett F, Braga V, Simpson AJG. (1990) Immunoblotting identifies additional antigens recognized by mice protectively vaccinated with adult *Schistosoma mansoni* tegumental membranes. *Parasitol Res.*; **76**: 454–6.

Smith DB, Johnson KS. (1988) Single-step purification of polypeptides expressed in *E. coli* as fusions with glutathione *S*-transferase. *Gene.*; **67**: 31–40.

Stein LD, David JR. (1986) Cloning of a developmentally regulated tegument antigen of *schistosoma mansoni. Mol Biochem Parasitol.*; **20**: 253–64.

Tabor S, Richardson CC. (1989) Selective inactivation of the exonuclease activity of bacteriophage T7 DNA polymerase by *in vitro* mutagenesis. *J Biol Chem.*; **264**: 6447–58.

Towbin H, Staehelin T, Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A.*; **76**: 4350–4.

WHO (1993) *The control of schistosomiasis.* 2nd report of the WHO expert committee. Technical report series 830. WHO, Geneva.

Waine GJ, Becker MM, Scott JC, Kalinma BH, Yang W, McMarus DP. (1994) Purification of a recombinat *Schistosoma japonicum* antigen homologous to the 22-kDa membrane-associated antigen of *S. mansoni,* a putative vaccine candidate against schistosomiasis. *Gene.*; **142**: 259–63.

Xiaochuan XC, Jean-Marie G, Raymond P, Mireille R, Frederic M, Farid Z, Jean-Pierre D, Simone P, Jinli L, Josette F, Sophia L, Andre C, Lille CF. (1997) Expression of a *S. mansoni* 28-kDa glutathione *S*-transferase in the livers of transgenic mice and its effect on parasite infection. *Infect Immun.*; 3867–74.

Young RA, Davis RW. (1983) Efficient isolation of genes by using antibody probes. *Proc Natl Acad Sci U S A.*; **80**: 1194–8.