Schistosoma mansoni: Partial Molecular Characterization of The Gene Encoding Zinc Finger Protein ,The Transcriptional Regulatory Protein Of Lung Stage (7-Days Schistosomula)

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ABSTRACT: Schistosomiasis is a serious parasitic disease with world-wide distribution, causing an estimated 200 000 deaths per year. Despite the fact that the global distribution of schistosomiasis has changed significantly in the past 50 years, particularly in regions where control strategies have been successfully employed, the disease remains endemic in over 70 developing countries and more than 200 million people are estimated to be harbouring the disease. Schistosomes also infect livestock and cause serious economic hardship in many developing nations. Chemotherapy, does not provide a satisfactory solution since, although effective, it does not prevent re-infection, and in addition, partial drug resistance to the most commonly used chemotherapeutic agent against schistosomiasis, Hence, immunological intervention in the form of a vaccine would contribute to the success of the present efforts if added to existing control strategies. Most of the trials in the development of antischistosomiasis vaccine were involving membrane-associated antigens contained in the adult *Schistosoma mansoni* tegument because they are capable of stimulating protective immunity , but in the recurrent study we tried to find another antigens which could be vaccine candidates by incorporating internal antigens of the parasite lung stage (7-days schistosomula) and not depending on tegumental proteins only , so , instead of extracting surface proteins ,we obtained the soluble extract of 7-days schistosomula which was coupled to Sepharose-4B column for affinity purification of pooled sera obtained from patients with chronic infection of schistosomiasis. The purified sera were used to immunoscreen λgt11 cDNA library of 7-days schistosomula. The plaques purification after the three rounds of immune-screening gave a number of cDNA clones. Phage DNA of one of the isolated clones (clone 2-4) was amplified by polymerase chain reaction (**PCR**) using λgt11 forward and reverse primers , then , cloned in plasmid vector **(PCR™II)**. The cloned insert was partially sequenced 270 bp from the 5′- end using Sp6 primer and 187 bp from the 3′-end using T7 primer and was found to encode the gene of **Zinc Finger** protein (the transcriptional regulatory protein) having two open reading frames (ORF) , the sequenced part of the insert showed 31-36% identity to the gene of **Zinc Finger** protein from a number of eukaryotic species including human , rat and mice. [New York Science Journal. 2008;1(4):77-88]. (ISSN: 1554-0200).

Key Words: 7-days schistosomula; **Zinc Finger** protein; antischistosomiasis; *Schistosoma mansoni*

INTRODUCTION

Most current viral and microbial vaccines were developed empirically, but in the knowledge that first exposure to the pathogen generated a strong immunity to re-infection. For parasites the situation is altogether more complex, not least because they have evolved efficient mechanisms to evade host immune responses. In the case of

schistosomiasis mansoni, the result is a chronic debilitating infection that may persist for more than 30 years (***Harris et al., 1984***). In these circumstances the development of a schistosome vaccine was always going to be a difficult task. In what might be termed the classical approach, the strategy is to identify protected individuals in an endemic population. The immune mechanisms that such people deploy to limit or prevent establishment of invading cercariae should form the basis of a successful vaccine. In the last two decades great progress has been made in characterizing human responses to schistosomes (Dunne & Mountford 2001), but no immune mechanisms or specific antigens *strongly* associated with a protected status have been identified . In recent years, considerable effort has been made to develop a protective vaccine against schistosome infection and several potential DNA constructs encoding several candidate molecules have been identified (***Yang et al., 1995, Waine et al., 1999, Nascimento et al., 2002, Sidiqui et al. 2003., Shalaby et al., 2003, Sidiqui et al., 2005, Fonseca et al., 2006, Zhu et al., 2006***). Schistosome tegumental antigens have been shown to play a pivotal role on the evasive mechanisms of ***Schistosoma mansoni*** in a mammalian host. Additionally, the principal membrane-associated antigens contained in the adult ***Schistosoma mansoni*** tegument do not cross-react with egg antigens of the parasite, which are involved in immunopathology (***Smithers et al. 1990***). Thus, the characterization of proteins within the tegument is relevant in a more basic level to improve the understanding of the function of this structure and in a more applicative level to identify molecules that are useful for diagnosis, or may act as targets of protective immunity and/or chemotherapy (Abath *et al.,* 2000). Part of the problem may be due to the ability of the parasite to evade host immune mechanisms. In the case of schistosomiasis, a sterilizing vaccine, although desirable, is not essential. Since schistosomes do not multiply within the final host, a vaccine that induces even a partial reduction in worm burdens could considerably reduce pathology, limit parasite transmission and be less expensive than repetitive drug treatment (Chitsulo *et al.,* 2004). Many world health agencies agree that the development of an antischistosomiasis vaccine should be seeked. Several studies are in progress in this field, testing different antigens of the parasite and different vaccination strategy (*Chitsulo et al., 2004*) . Vaccine candidate antigens are often secreted by or anchored on the surface of pathogens. Proteins that are secreted or anchored on the surface of schistosomes are exposed to host tissues and thus present as potential candidate molecules for the development of new vaccines. It has been shown that isolated tegumental membranes are capable of stimulating protective immunity in mice (*Smithers et al., 1990*).

The expression of eukaryotic genes is controlled primarily at level of transcription initiation , although in some cases , transcription may be attenuated and regulated at subsequent steps. As in bacteria , transcription in eukaryotic cells is regulated by proteins that bind to specific regulatory sequences and modulate the activity of RNA polymerase (*Hochschild and Dove, 1998, Nikolove and Burley,1997*) , in addition the packing of DNA into chromatin and its modification by methylation impart further levels of complexity to the control of eukaryotic gene expression *(Felsenfeld ,1996 , Kadonaga , 1998 , Wolffe, 1998 , Razin and Cedar, 1994).*

Many different transcription factors have now been identified in eukaryotic cells, as might be expected, given the intricacies of tissue-specific and inducible expression in complex multi-cellular organisms . DNA binding domains in eukaryotic transcription factors exhibit variety of structure, among the most common structural motifs are the homeodomain, basic zipper (leucine zipper), helix-loop-helix and several types of zinc fingers (*Pabo and Sauer, 1992*). Zinc finger domains contain repeats of cystiene and histidine that bind central Z^{+2} ions and fold producing a compact domain from a relatively short length of the polypeptide chain termed a zinc finger , this structural motif was first recognized in DNA- binding domains , but now is known to occur in proteins that do not bind to DNA. These domains were initially identified in the polymerase II factor TFIIIA but, are also common among transcription factors that regulate polymerase II promoters, including Sp1. Other examples of transcription factors that contain domains are the steroid hormone receptors , which regulate gene expression in response to hormones such as estrogen and testosterone (*Pabo and Sauer, 1992 , Mitchell and Tjian, 1989, Ptashne and Gann, 1997, Johnson and McKnight, 1989 , Burley and Roeder, 1996*).

The first zinc finger structure is C_2H_2 zinc finger, containing three or more repeating finger units and bind as monomers, it is the most common DNA binding motifs in eukaryotic transcription factors. The second type is designated C_4 zinc finger , found in more than 100 transcription factors , identified as specific intracellular high affinity binding proteins or receptors for steroid hormones , it contains only two finger units and bind to DNA as homodimers or heterodimers. The DNA binding domain in the yeast Ga14 proteins exhibits a third type of zinc finger motif, known as C_6 zinc finger , binds to DNA as homodimer in which the monomers associate through hydrophobic interactions along one face of their α-helical regions (*Pabo and Sauer, 1992, Kustu et al,. 1991 , Burley and Roeder, 1996*).

In the recurrent study we aimed to isolate some clones from λgt11 cDNA library of 7-days schistosomula , after cloning in a plasmid vector and sequencing the isolated clones , one of them showed 31-36% identity to zinc finger protein (the transcriptional regulatory protein) from some eukaryotic species and although it is not a surface protein but, we hoped to provide a new potential vaccine antigen against schistosomiasis.

MATERIALS AND METHODS

Soluble extract of 7- days schistosomula:

Schistosomula mansoni NMRI strain was maintained in the laboratory of Theodore Bilharz Researsh Institute using Biomphalaria glabrata snails, cercariae were obtained from infected animals (*Fletcher et al., 1981*). Schistosomula were obtained by mechanical transforming cercariae where cercarial bodies were separated from tails by centrifugation 2000 rpm for 15 min over 70% percoll gradient (*Lazdins et al., 1982*). Cercarial bodies were recovered from tube bottom and washed three times with Minimum Essential Medium (MEM) containing 10% fetal calf protein , then , incubated in Modified MEM at 37^0C in a humidified 5% CO_2 incubator for 7 days. Finally, the medium was collected and living schistosomula were separated by centrifugation at 2000 rpm for 15 min over 60% percoll gradient (*Basch, 1981***).** The soluble extract was made by sonication of the parasites in a buffer containing 20 mM Tris, pH 7.2 and 2 mM phenyl methyl sulphonyl fluoride (PMSF) , then , centrifuged at 6000 rpm for 20 min. The supernatant was removed and stored at -70^{o}C.

Affinity purification of sera:

Sera used in immunoscreening experiment were pooled from schistosomiasis chronically infected patients admitted to Department of Tropical Medicine, Zagazig University Hospitals. Cyanogen bromide – activated Sepharose 4B was used to purify sera according to instructions of manufacturer by coupling 6-8 mg of 7- days schistosomula soluble extract to the column. Pooled sera were precipitated with 45% ($NH_4)_2SO_4$, the precipitate was redissolved in phosphate buffered saline (PBS) (0.4 g NaCl, 1.44g Na_2HPO_4 and 0.24 g KH_2PO_4/L) and dialyzed against PBS overnight. The dialysate was ,then, passed onto the column containing the NP-40 schistosomular extract. The flow through from the column was collected and tested using ELISA for reactivity to the extracted proteins. The column was washed with 30 ml PBS. Antibodies bound to the column were eluted by 0.1 M glycine–HCl, pH 2.6 and collected as 1 ml fractions. The pH of the elute was immediately adjusted to 7.0 with 100 µl 1M Tris-base., then, they were dialyzed against PBS over night to be ready for immunoscrening.

Screening of 7-days schistosomula λ gtll cDNA Library with antibody (*Huynh, 1985*):

To grow cells for transfection with schistosomula library , a single colony of *E.Coli* Y1090 was incubated in 50 ml LB-ampicilline medium (LB-amp) (10 g Bacto-tryptone, 5 g Bacto-yeast extract, 10 g NaCl, and distilled H_2O up to 1L , pH 7.0) containing 0.2% maltose and ampicilline 100 mg/ml allowed to grow overnight with good aeration at 37^{o}C, to used as hosts for plating the library. For the primary screening of the library, 150 mm LB-amp plates were used and 90 mm plates were used for secondary and tertiary screenings. An overnight bacterial culture, about 0.6 ml for each large plate and 0.2 ml for the small one , was incubated with 0.1 ml of SM medium (5.8 g NaCl, 2.0 g $MgSO_4$.7 H_2O, 50 ml 1M Tris ; pH 7.5 and 5 ml of 2% gelatin solution/ L). The cell suspension was incubated at 37^{o}C for 15 min to allow the adsorption of the phage to the bacterial cells. Molten top agar, cooled to 50^{o}C was added to the infected cells, 7 ml / large plate and 3.5 ml / small plate are poured onto the LB-amp plates pre-warmed to 37^{o}C, then, the plates were incubated at 40^{o}C for 3-4 hours (hrs). Dry nitrocellulose (132 mm and 82 mm) circular filters were used for large and small plates, respectively. The filters were saturated in 10 mM IPTG and air dried, then, placed onto the plates. The plates were transferred to a 37^{o}C incubator for another 3 hrs. then, removed from the plates and transferred to the Blotto buffer (non fat dry milk 5g in 100 ml PBS-0.05% Tween-20) to block the non-specific binding protein sites and shook at room temperature for 30 min. The filters were then washed 3 times in TBST (37.5 ml 4M NaCl, 10 ml 1M Tris; pH 8.0, double distilled H_2O up to 1L and 0.05% Tween-20) for 10 min each, followed by incubation for 3 hrs with primary antibody (the purified sera over schistosomula soluble extract column) ,then, washed 4 times at room temperature in TBST for 20 min each. The anti-rabbit IgG alkaline phosphatase conjugate, diluted in TBST according to the data sheet, was used to bind the

primary antibody-antigen complex. Following 1 hr incubation at room temperature in the secondary antibody, the filters were washed 4 times in TBST as before for 10 min each , dried and transferred to the color development substrate solution [33 µl of 50 mg/ml Nitro Blue Tetrazolium (NBT) + 16.5 µl of 50 mg/ml BCIP per ml AP buffer (10 ml of 1 M Tris; pH 9.5, 2 ml of 5 M NaCl 0.5 ml of 1 M MgCl , distilled H_2O up to 100 ml)]. The filters were incubated in dark until the desired color intensity had been developed , then, rinsed in distilled water. The developed filters were used to pick up agar plugs containing phage particles corresponding to the signals on the filters (the positive plaques) to be suspended into 0.5 ml of SM medium (5.8 g NaCl, 2 g $MgSo_4.7\ H_2O$, 50 ml 1 m Tris, pH 7.5 , 5 ml 2% gelatin solution and distilled H_2O up to 1 L) and placed on a shaker for 1 hr at 37°C. the purified phage plaques were used for the next round of screening.

Small scale preparation of bacteriophage DNA (*Maniatis et al., 1982*):

A bacteriophage suspension in ***E.Coli Y1090*** culture (O/N) culture , incubated at 37°C without shaking for 15 min, then to which 4 ml of NZCY-ampicilline medium (10 g NZ amine, 5 g bacto-yeast extract, 5 g NaCl, 2 g $MgSo_4.7\ H_2O$ and distilled H_2O up to 1 L ,then , autoclaved at 121°C and ampicilline 100 mg/ml). The culture was agitated at 37°C for 9 hours (hrs), followed by adding 0.1 ml chloroform. The lysate was , then , centrifuged at 8000 rpm for 10 min. Ribonuclease A and DNase I (Sigma) were added to supernayant to final concentration 1 µg/ml of each. An equal volume of ice cold solution containing 20% PEG-8000 and 2 M NaCl in SM medium were added to lysate and chilled on ice for 1 hr, followed by centrifugation at 10000 rpm at 4°C to pellet the phage particles which were suspended in 0.5 ml SM medium. To the suspension 5 µl of each 10% SDS and 0.5 M EDTA, pH 8 were added and incubated at 68°C for 15 min. The solution was , then , extracted with phenol, with phenol-chisam and with chiasm each extraction was done once, then , precipitated by adding 1/10 volume of sodium acetate and 2.5 volume of ice-cold absolute ethanol and stored at -20°C O/N , then, dissolved in 100 µl distilled H_2O , then , checked by running an aliquot of 10 µl using 0.7% agarose gel electrophoresis.

Polymerase Chain Reaction (PCR) (*Saiki et al., 1988*):

The isolated phage DNA from plaques was amplified using a pair of primers, λ gt11 forward (5′-GGTGGCCACGACTCC TGGA GGCGG-3′) and λ gt11 reverse (5′-TTGACACCAGACCAACTGGTAATC-3′). Taq DNA polymerase (Perkin-Elmer Cetus and Stratagene) was used in this reaction to synthesize the new strands generated by that process. A typical PCR reaction mix (100 µl reaction) was prepared (10 µl 10 X Taq DNA polymerase buffer, 16 µl of 1.25 mM dNTP, 5 µl forward primer , 5 µl reverse primer , 2 µl (100 ng) phage DNA template, 0.5 µl Taq DNA polymerase, sterile distilled H_2O up to 100 µl). The reaction components were mixed in 0.5 ml microfuge and a drop of mineral oil was added on top of the reaction mix. The samples were amplified using a programmable thermal cycler Gene Amp 9600, Perkin-Elmer, using a 3-file program. Samples were denatured in the first file at 94°C for 1 min , then , the primers were annulled to the denatured templates at 55°C for 2 min and finally extended at 72°C for 10 min. The samples were maintained at 4°C. The amplification products (amplicons) were withdrawn from underneath the oil and 10 µl aliquots were separated on 1% agarose gel.

Subcloning of the recombinant gene in PCR™II plasmid vector (*Hanahan, 1983*):

The original TA cloning Kit (Invitrogen) was used for direct insertion of the amplicon into PCR™II vector at EcoR1 site. A typical ligation reaction was prepared as follows (1 µl PCR product , 1 µl of 10X ligation buffer, 2 µl plasmid vector, sterile H_2O up to 9 µl , 1 µl DNA ligase). The ligation reaction was incubated overnight at 15°C till ready for transformation. the readymade INV competent cells of the original TA cloning kit were used. The vial containing the ligation reaction was spun down briefly and placed on ice. Two µL of 0.5M B- mercaptoethanol (B-ME) and 2 µl ligation reaction were pipetted into each vial of the competent cells and mixed by gentle stirring with the pipette tip , then, the vial was incubated on ice for 30 min , and exactly 30 sec in 42°C water bath. The vial was removed from the water bath and placed on ice for 2 min 450 µl of SOC medium were added to the vial which was shaked at 37°C for 1hr . Aliquot of 50 µl was spread onto LB-amp plate and the plate was placed inverted at 37°C for at least 18 hrs finally the plate was shifted to 40°C for 2-3 hrs for the proper color development. Positive transformants can be selected by using Cracking gel procedure (*Maniatis et al., 1982*), where the non-recombinant transformants migrate faster than the recombinant ones when checked by 1% agarose gel electrophoresis.

Small scale preparation of plasmid DNA (*Sambrook et al., 1989*):

A single bacterial colony that contains the desired plasmid was used to inoculate 100 ml of LB-amp medium incubated at 37^{o}C with vigorous shaking overnight (O/N). The bacterial cells were harvested by centrifugation at 10000 rpm for 10 min. The cells were lysed using solution I (50 mM glucose, 25 mM Tris HCl, pH 8, 10 mM EDTA, pH 8), freshly prepared lysozyme was added , then , followed by solution II [0.2 M NaOH , 1% sodium dodecyl sulphate (SDS)] , the suspension was incubated at room temperature (RT) for 10 min. 20 ml of solution III was added (3 M potassium acetate, 2 M glacial acetic acid). DNA can be recovered by adding equal volume of isopropanol and precipitated by centrifugation at 10000 rpm for 10 min at (RT). The pelleted DNA was dissolved in 100 µl distilled H_2O to which RNase (10 mg/ml) was added, then, left for incubation at 37^{o}C for 2 hrs. The DNA solution was , extracted with phenol-chisam , then , precipitated by ethanol 2.5 volumes and 0.1 volume of 3 M sodium acetate and dissolved in 50 µl distilled H_2O. The plasmid DNA was quantitated by determining the **O.D$_{260}$** , then , stored at – 20^{o}C.

DNA sequencing (***Sanger et al., 1977***):

5 µl of the plasmid DNA were denatured by 20 µl of 0.2 M NaOH for 5 min , neutralized by 8 µl of 5 M ammonium acetate pH 7.4 , incubated at -70^{o}C for 30 min after precipitation with 100 µl ice-cold absolute ethanol. The DNA was pelleted and dried , then, dissolved in 4 µl distilled H_2O , 4 µl dNTPs and 2 µl of 5X sequanase reaction buffer were added to the denatured template , this mixture was boiled , then , cooled gradually to room temperature (RT). 2 µl of labeling mix (0.1 M DTT , ^{35}S-dATP and 2 µl 1:4 diluted sequanase enzyme were added to the reaction mix and the tube was incubated at RT for 5 min . 3.5 µl aliquots were added to 4 different tubes each containing 2.5 µl of each of the ddNTPs termination mixes. The reaction was stopped by adding 4 µl stop dye. The 4-tube set were labeled G,A,T,C was heated for 5 min , then , chilled on ice and loaded onto sequencing gel (8% polyacrylamide-8 M urea gel), the run was continued for 2-8 hrs. After electrophoresis , the gel was fixed in a solution of 10% acetic acid and 105 methanol for 30 min , dried and exposed to X-ray film. After 24 hrs exposure , the film was developed and read from the bottom. The informations obtained from DNA sequence were analyzed using the Genetics Computer Group Sequence analysis Software package.

RESULTS

Sera obtained from *Schistosama mansoni* chronically infected patients was purified over an antigen column made from soluble extract of 7-days schistosomula coupled to Sepharose-4B beads. The affinity purified eluted antibodies were , then , used to immunoscreen 7-days schistosomula λgt11 cDNA library. One of the isolated cDNA clones (clone 2-4) which was identified by affinity purified antibodies obtained from serum of the chronically infected patients contained a 0.7 kb insert. The partial DNA sequence (270 bp from 5$^{/}$-end and 187 bp from 3$^{/}$-end) of the insert identified two open reading frames (ORFs) of 90 amino acids (aa) that showed 31-36 % identity with zinc finger protein from a number of eukaryotic species (including rat, mice and human) , The 0.7 clone was partialy sequenced in both directions using Sp6 at 5$^{/}$-end and T7 at 3$^{/}$-end (Fig. 1). After the three rounds of immune-screening bacteriophage DNA was prepared by small scale procedure and checked by running the prepared phage DNA on 0.7 % agarose gel (Fig. 2). The original TA cloning Kit (Invitrogen) was used to provide a quick, one-step cloning strategy for direct insertion of the PCR products into a plasmid vector (PCR^{TM}II vector) at EcoR1 site. Some of the isolated clones were checked for size after being inserted in the desired plasmid vector using two restriction enzymes EcoR1 and BamH1 (Fig. 3), which showed no BamH1 site in the insert , while the plasmid DNA was digested by EcoR1 giving the actual size of each insert . The cloned insert (2-4) was sequenced using two oligonucleotides (primers), Sp6 from the 5$^{/-}$ end and T7 from the 3$^{/}$-end, the sequence gel was run for 2.5 and 6.5 hours , then , exposed to an X- ray film for 24 hrs , then , developed and read from the bottom of the autoradiogram (Fig. 4).

Sp6:

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1   GCUAUGAUUCAAGCUUGGUACCGAGCUCGGAUCGCACUAGUAACGGCCGCCAGUGUGCUG 60
      A  M  I  Q  A  W  Y  R  A  R  I  A  L  V  T  A  A  S  V  L
61  GAAUUCGGCUUGGUGGGCAGGACUCCUGGAGCCCGUCAGUAUCGGCGGAAUUCCUCACAC 120
      E  F  G  L  V  G  R  T  P  G  A  R  Q  Y  R  R  N  S  S  H
121 CAACAGUGCGGCGUCGUAAGCAAACAAUUCACCCAUACCCACCAAAAAGGAAGGCACCUU 180
      Q  Q  C  G  V  V  S  K  Q  F  T  H  T  H  Q  K  G  R  H  L
181 CGGUUAAACAGACCCUGGAGUUUUCCAGUAUGCCCAGGUACAAAUCACAUCUCAUCAGCC 240
      R  L  N  R  P  W  S  F  P  V  C  P  G  T  N  H  I  S  S  A
241 GUGAAUUCCCAGAGUCUUUCAGUUCAAUCU270
      V  N  S  Q  S  L  S  V  Q  S
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T7:

GCC GCC AGT GTG ATG GAT ATC TGC AGA ATT CCG GCT TTT CGC ACC AGA CCA ACT GGT AAT GGT AGC GAC CAG TTT CAG CTG GAA TTC CAG CGG AGG AAA AGA AAC GTA ACA AGG ATT CCC CTA GTA ACT GCG AGT GAA CAG GGA TTA GCC CAA CTC CGA AGC CTG CGT TAT TTG ATC GTA AGG CAA T

Fig. 1: The partial nucleotide and deduced amino acids sequence of the gene encoding zinc finger protein isolated from λ g11 cDNA library of 7-days schistosomula, sequenced by Sp6 from 5′-end and T7 from 3′-end.

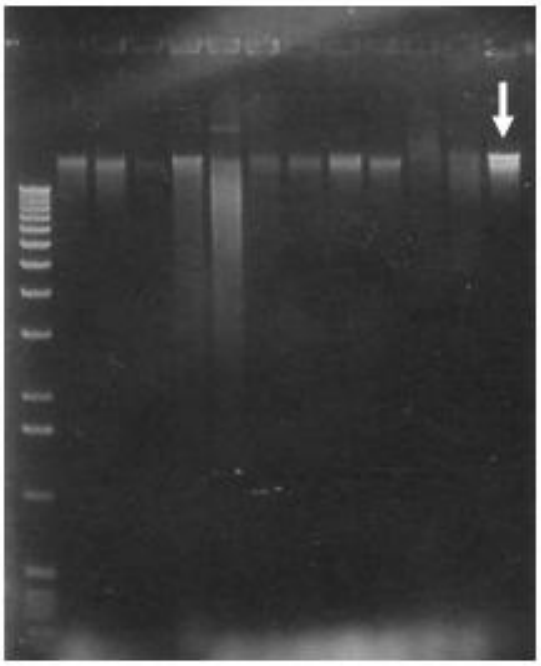

Fig. 2: 0.7% agarose gel representing the preparation of phage DNA of the plaques isolated from λ g11 cDNA library of 7-days schistosomula , the last lane is the selected clone.

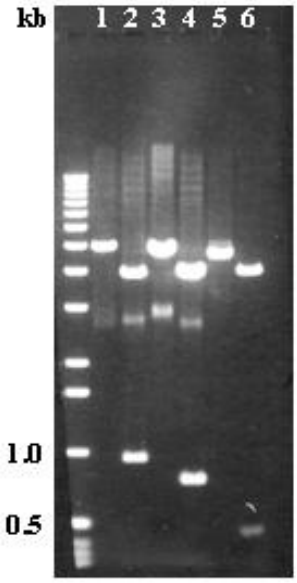

Fig. 3: 1% agarose gel showing the digestion pattern of three isolated inserts from λgt11 cDNA library of 7-day schistosomula, cloned in PCR^{TM}II plasmid vector , digested by two restriction enzymes EcoR1 and BamH1, the plasmid DNA samples were arranged in double, each represents from left to right, EcoR1 digested and BamH1 digested DNA. The selected clone (2-4) was run in lanes 5 and 6 The size of the insert is 0.4 kb.

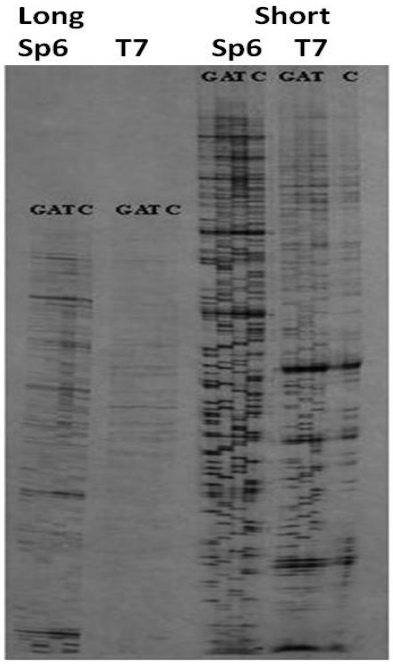

Fig. 4: An autoradiogram showing sequence of the gene encoding zinc finger protein isolated from λ gt 11 cDNA library of 7-days schistosomula cloned in PCR^{TM}II plasmid vector from 5$^{/}$- and 3$^{/-}$ends using Sp6 and T7 primers.

DISCUSSION

Schistosomiasis remains a serious public health problem with an estimated 200 million people infected in 76 countries. It is a major strategy to develop vaccines against schistosomiasis recommended by the World Health Organization (*Bergquist et al., 2002*) . In recent years, studies on vaccines have progressed rapidly, and a series of vaccine candidates have been identified and tested against schistosome infection in experimental models (***Carpron et al., 2001***). Nevertheless, these vaccines provide only 20-40% protection against the challenge of schistosoma cercariae. Scientists have studied different types of schistosomiasis vaccines. Despite the progress achieved, a feasible anti- schistosomiasis vaccine for humans or livestocks has not been found (*Min et al., 2005*). A schistosome vaccine would provide a useful tool for the control and eradication of ***Schistosoma mansoni*.** In spite of several decades of research an effective vaccine remains elusive. Current advances in post-genomic techniques are providing new avenues to identify the secreted and surface exposed antigens that mediate protection (*Curwen et al., 2004, Dillon et al., 2006*).

The sequencing of the *Schistosoma mansoni* transcriptome (*Verjovski-Almeida et al., 2003*) and genome has opened up exciting new possibilities for antigen discovery. In the present study we reported the discovery of the gene encoding zinc finger protein of 7-days schistosomula, isolated from cDNA library of the lung stage of *Schistosoma mansoni* using sera obtained from schistosomiasis chronically infected patients and purified over Sepharose-4B column made of soluble extract of sonicated 7-days schistosomula. By sonicating the lung stage to obtain the soluble extract for increasing the chances for developing a schistosome vaccine ,although what we have discovered is not a surface associated proteins. Transcriptional factors are classified according to the type of DNA-binding domain they contain. Most of the structural classes of DNA-binding domains have characteristic consensus amino acids sequences.

The genomes of higher eukaryotes may encode dozen of classes of DNA-binding domains and literally hundreds of transcription factors as homeodomain , zinc finger , leucine zipper and helix loop helix proteins (*Patikoglou and Burley , 1997*). Zinc finger proteins are the most popular DNA binding proteins in mammals (*Yuko , 2008*), involved in protein-DNA interactions and is also known to be involved in binding of RNA, lipids and proteins (*Gamsjaeger et al., 2007*). The most common zinc finger proteins are C_2H_2 finger proteins whose structure is consisted of ˜30 aa stabilized by a Zn ion bound to two cystiene and two histidine residues of each finger containing two β- sheets and one α- helix (*Wolfe et al., 2000*). C_2H_2 finger proteins can bind to different target sequences depending on the amino acid sequence of the fingers, the number of the fingers and the combination of fingers (*Luchi, 2001*), originally thought to be confined to the eukaryotic kingdom could be wide spread throughout the living kingdom from eukaryotic, both animal and plant to prokaryotic (*Gaetano et al., 2007*). Zinc finger proteins participate in a variety of cellular activities, including development, differentiation, cell cycle and tumor suppression. It has been estimated that up to 1% of the genes in human genome may encode proteins with zinc finger domains (*Hoovers et al., 1992*). Recently, there has been a great deal of progress in the development of modular protein domains that recognize specific DNA triplets. The C_2H_2 zinc finger motif is the ideal structural scaffold on which a sequence specific protein may be constructed (*Lee et al., 2003*). DNA structural domain of zinc finger proteins usually consist of 3 or 6 zinc fingers, artificial zinc finger proteins technology allows DNA sequences to be selected directionally and a DNA binding domain to be constructed (*Dreier et al., 2005*). The structural studies accomplished on classical zinc finger protein-DNA complexes have revealed the sequence-specific recognition is achieved by contacts between the α- helix of the zinc-finger and bases in the major groove of the DNA. A single zinc-finger domain in itself is not sufficient for high-affinity binding to a specific DNA target sequence. In fact , proteins containing multiple zinc finger domain usually require a minimum of two zinc-fingers for high-affinity DNA binding (*Klug and Schwabe, 1997*). Various screening procedure and artificial design strategies have also been attempted to make zinc finger proteins to bind to desired sequences (*Mandell and Barbas, 2006 , Papworth et al., 2006*). Such artificial zinc finger proteins are expected to be artificial transcriptional factors and nucleases (*Varshavsky, 2007*).

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