Characterization Of The Gene Encoding 28 S Large Subunit Ribosomal RNA Of The Lung Stage Of Schistosoma Mansoni (7-Days Schistosomula)

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ABSTRACT

The parasitic helminth ***Schistosoma mansoni*** is a major public health concern in many developing countries. Over 200 million people have, and another 600 million are at risk of contracting, schistosomiasis, one of the major neglected tropical diseases. For this dangerous disease the development of long - lasting immunity through vaccination may be the real solution to control the spread of the disease. As molecules on the surface or associated with the tegument of Schistosoma ***mansoni*** are a major focus as potential vaccine candidates, but in the present study we screened surface and internal proteins to increase the chances for the discovery of a unique protein of the parasite to be targeted by the immune system of the host and used as a vaccine candidate. In the recurrent study pooled sera collected from ***Schistosoma mansoni*** chronically infected patients was purified over a column made of soluble extract of lung stage (7-days schistosomula) of ***Schistosoma mansoni*** , then , used to immunoscreen λgt11 cDNA library of 7-days schistosomula , a number of cDNA clones were identified after three rounds of immuno-screening and plaques purification. The phage DNAs of the isolated clones were amplified with polymerase chain reaction (PCR) using λgt11 forward and reverse primers, then, cloned in PCR^{TM}II plasmid vector. The isolated clone 4-65 was fully sequenced and found to encode the gene of 28S ribosomal RNA of 7-days schistosomula of Schistosoma ***mansoni.*** The 0.9 kb cDNA clone was found to have a single open reading frame (ORF) encoding 269 amino acids exhibited 97% identity to the gene of 28S large subunit ribosomal RNA of Schistosoma ***mansoni*** and a number of eukaryotic species.

Key Words: ***Schistosoma mansoni;*** 7-days schistosomula***;*** immuno-screening

INTRODUCTION

Schistosoma mansoni is a blood-dwelling parasitic worm that causes schistosomiasis in humans throughout Africa and parts of South America. A vaccine would enhance attempts to control and eradicate the disease that currently relies on treatment with a single drug (**Gary *et al.,* 2008**). An effective schistosome vaccine is a desirable control tool but progress towards that goal has been slow (Alan *et al.,* 2006). An effective vaccine would be a useful adjunct and if sufficiently potent, a replacement for chemotherapy but the development of such a product has proved elusive (Alan *et al.,* 2006). The first attempts to develop a schistosome vaccine began half a century ago. By analogy with successful microbial and viral vaccines, they

involved the vaccination of mice with crude worm extracts or purified components, followed by a cercarial challenge (Hayunga *et al.,* 1985**).** The results were equivocal with 20, 30, and even 50% reduction in worm burden recorded, but there was a lack of consistency, even in the same laboratory and it seemed apparent that crude extracts were inadequate vaccines. Perhaps there were a few key antigens that needed to be identified (Alan *et al.,* 2006). So, a particular attention was thus given to identify and characterize sensitive and specific ***Schistosoma mansoni*** antigens to obtain better diagnostic tool and vaccine development (Valili *et al.,* 1990**).** A few defined soluble antigens were separated to show high sensitivity and specificity in endemic areas (Valili *et al.,* 1990).

Schistosomes are truly a formidable adversary that won't easily be beaten. It has been shown that hosts can develop an acquired immunity against challenge infection either after primary infection, immunization with irradiated larvae, or with defined antigens (Smithers and terry 1990; Soisson *et al.,* 1969). The skin and lung stages of the parasite, termed schistosomula, from a challenge infection are the target of these immune responses (Capron *et al.,* 1987).

In the present research we reported the result of a cDNA clone isolated from 7-days schistosomula cDNA library which could be a target for immune attack. DNA, the genetic material, carries the information to specify the amino acid sequences of proteins. It is transcribed into several types of RNA functioning in protein synthesis , including messenger RNA (mRNA) which ultimately direct the process of protein synthesis , transfer RNA (tRNA) form the adaptor that select amino acids and hold them in a place on a ribosome for incorporation into protein and ribosomal RNA (rRNA) which has the dominant role in translation forming the core of ribosomes , determining the overall structure of the ribosome , forming the binding sites for the tRNAs , matching the tRNAs to codons in the mRNA, and providing the peptidyl transferase enzyme activity that links amino acids together during translation (Gesteland *et al.,* 1999).

Approximately 80% of the total RNA in rapidly growing mammalian cells is rRNA. The primary transcripts from most rRNA genes undergo extensive cleavage, processing and modification, so that the mature functional form of rRNAs contained within the precursor are liberated and incorporated into the correct ribosomal subunit (Von 1998; Kressler *et al.,* 1999; Venema *et al.,* 2000). Eukaryotic ribosomes contain four RNAs: 28S, 18S, 5.8S and 5S. The 5S rRNA is transcribed by RNA polymerase III, whereas the three remaining rRNA are transcribed as 45S polycistronic precursor by RNA polymerase I contained within the 45S precursor of the 18S rRNA , the RNA component of the small ribosomal subunit and the 5.8s and 28s rRNA which are the components of the large ribosomal subunit. Many of the processes leading to maturation of rRNA are carried out in the nucleolus by myriad of small nucleolar RNPs, maturation of 18S, 5.8S and 28S rRNA requires the box C/D small nucleolar RNPs, the box H/ACA small nucleolar RNPs and RNAse

mitochondrial RNA processing (Watkins *et al.,* 2000).To meet the need for transcription of large numbers of rRNA molecules, all cells contain multiple copies of rRNA genes. The human genome for example, contains about 200 copies of the gene that encodes the 5.8S, 18S and 28S rRNAs and approximately 2000 copies of the gene that encodes 5S rRNA. The genes for 5.8S , 18S and 28S rRNAs are clustered in tandem arrays on five different human chromosomes (chromosomes 13 , 14 , 15 , 21 and 22) , the 5S rRNA genes are present in a single tandem array on chromosome 1 (Gesteland *et al.,* 1999). The major goal of the present study is the identification of some cDNA clones from the lung stage of ***Schistosoma mansoni*** and the study of their vaccine potential in the future for finding out the possibility of using one/or all as vaccine candidates 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 (Besch 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 re-dissolved 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 λgtll cDNA Library with antibody (Huynh *et al.*, 1985):

To grow cells for transfect ion with schistosomula library, a single colony of ***E.Coli Y1090*** was incubated in 50 ml LB-ampicillin 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 ampicillin 100 mg/ml allowed to grow overnight with good aeration at 37°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°C for 15 min to allow the adsorption of the phage to the bacterial cells. Molten top agar, cooled to 50°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°C, then, and the plates were incubated at 40°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°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.

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′-GGTGGCCACGACTCCTGGAG GCGG-3′) and λ gt11 reverse (5′-TTG AC AC CAGACCAACTGGTAATC-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^{o}C for 1 min, then, the primers were annulled to the denatured templates at 55^{o}C for 2 min and finally extended at 72^{o}C for 10 min. The samples were maintained at 4^{o}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^{TM}II plasmid vector (Maniatis *et al.,* 1982):

The original TA cloning Kit (Invitrogen) was used for direct insertion of the amplicon into PCR^{TM}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^{o}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 β- mercaptoethanol (β-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^{o}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^{o}C for 1hr . Aliquot of 50 µl was spread onto LB-amp plate and the plate was placed inverted at 37^{o}C for at least 18 hrs finally the plate was shifted to 40^{o}C for 2-3 hrs for the proper color development. Positive transformants can be selected by using Cracking gel procedure, 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^oC 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^oC.

DNA sequencing using fmol DNA (Promega) Sequencing System (Moran *et al.,* 1990):

In four microfuge (0.5 ml) labeled (G, A, T, C), 2 µl of the appropriate d/dd NTPs. Then, to the 4 tubes, 1µg DNA template, 25 µg primer (M13 at 5$^/$-end and T7 a 3$^/$-end) 1µl of α-^{35}S, 5 µl sequencing buffer and dd H_2O up to 16 µl, then, to each tube 1µl of sequencing grade Taq DNA polymerare was added to the template/primer mix. The reaction was placed in a thermal cycler. The profile used in the reaction was 2 min at 95^oC, 30 sec at 90^oC ,then, 1 min at 70^oC for 30 cycle, then, the reaction was stopped by adding 3 µl stop solution to each tube. 3ul of each tube were loaded on a sequencing gel. After the electrophoresis, the gel was fixed, dried by heating, then, exposed to an X-ray film for 24 hrs at –70^oC. The film was developed and read from 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 polyadenylation signals AAATAA , AATTA and ATAA located +114 , +22 and +6 bp, 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 4-65) which was identified by affinity purified antibodies obtained from serum of the chronically infected patients contained a 0.9 kb insert. The full DNA sequence of the insert identified a single open reading frame (ORF) of 269 amino acids (aa) that showed high identity with the 28 rRNA gene of the large ribosomal subunit from a number of eukaryotic species (the % of homology was 97 , 97, 92, 93 , 93 , 94 , 94 , 93 , 94 and 93 to gene for 28S rRNA of *Schistosama mansoni, Schistosama rodhaina*, *Schistosama spindale, Schistosama hippopotami, Schistosama edwariense, Schistosama margrebowiei, Schistosama incognitum, Schistosama intercalatem Schistosama mattheei* and *Schistosama hematobium,* respectively . The 0.9 clone was completely sequenced in both directions after being inserted into a plasmid vector (PCRTMII); it did not contain the entire coding region. The 5$^/$- upstream region in the sequence we had gut considering the first initiation codon (ATG) is located -201

bp from the beginning of this region without any transcription activation TATA and CAAT boxes. There are three putative, respectively from the 3′-downstream region, there is no polyadenylation site (poly A tail) (Fig. 1). 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™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. 2), 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 was sequenced using two oligonucleotides (primers), M13 from the 5′- end and T7 from the 3′- end followed by another two pairs of primers till reaching the overlapping region for completing the sequence of the isolated clone, each sequence gel was exposed to an X- ray film for 24 hrs, then, developed and read from the bottom of the autoradiogram (Fig.3).

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1   CCGAAGUCUAAGCACGCAUGCAAGCGCCAUCUCCGACACACCGCACAGAUCCACUACAGC 60
      P  K  S  K  H  A  C  K  R  H  L  R  H  T  A  Q  I  H  Y  S
 61 CAGUUAGUAACACUCACGGCCCAUAAACUGAGCCAAAGAGCCACCUACCGACCCAACCAA 120
      Q  L  V  T  L  T  A  H  K  L  S  Q  R  A  T  Y  R  P  N  Q
121 GACCGACAGAAAUCAGACCUGAUCAGUUUGACCAUAGCAGACAGGCAGCAGUCGUCGUGG 180
      D  R  Q  K  S  D  L  I  S  L  T  I  A  D  R  Q  Q  S  S  W
181 UGCACACCUGAGAAAGUGCACAUGACAAGCAGACCCUCACACCAACAGUGCGCGCGUCGU 240
      C  T  P  E  K  V  H  M  T  S  R  P  S  H  Q  Q  C  A  R  R
241 AAGCAAACGAUUCACACAUACCACCCAAAAAGGAAGGCACCUGCGGUUAAACAGACACUG 300
      K  Q  T  I  H  T  Y  H  P  K  R  K  A  P  A  V  K  Q  T  L
301 AAGGCGGCCAGUAUGCCCAAGUACAAAUCACACUCAUCAGCUGAACUCCCAGAGCUUGCA 360
      K  A  A  S  M  P  K  Y  K  S  H  S  S  A  E  L  P  E  L  A
361 GUUCAACUCCACCCGUUUACCUUUGAGCGGUUUCACGCACUGUUUACUCUCUCUUCAAAG 420
      V  Q  L  H  P  F  T  F  E  R  F  H  A  L  F  T  L  S  S  K
421 UACUUUUCAACUUUCCCUCACGGUACUUGUUUGCUAUCGGACUCGUGUAAGUAUUUAGCC 480
      Y  F  S  T  F  P  H  G  T  C  L  L  S  D  S  C  K  Y  L  A
481 UUGGAUGGAGUUUACCACCCACUUUGGGCUGCAUUCACAAACAACCCGACUCCAAGGGUA 540
      L  D  G  V  Y  H  P  L  W  A  A  F  T  N  N  P  T  P  R  V
541 GCUCAGAGCAAAACUGUCACACUUGAUCUCUGCCCCCACGGGCCUUUCACCCUCUUUGGG 600
      A  Q  S  K  T  V  T  L  D  L  C  P  H  G  P  F  T  L  F  G
601 CCAGGAUGGGAAGCCGUACUCAUUGCUGGACUUGGGACAGAGCAGGUAAUGCCUGAAGCC 660
      P  G  W  E  A  V  L  I  A  G  L  G  T  E  Q  V  M  P  E  A
661 ACCCUAAACACCACAUUGCUUUACGAUCAAAUAACGGCAGGCUUCGGUGUUGGGCUAAUC 720
      T  L  N  T  T  L  L  Y  D  Q  I  T  A  G  F  G  V  G  L  I
721 CCUGUUCACUCGCAGUUACUAGGGGAAUCCUUGUUAGUUUCUUUUCCUCCCCUGAGUAUA 780
      P  V  H  S  Q  L  L  G  E  S  L  L  V  S  F  P  P  L  S  I
781 UGCUUAAGUUCAGCGGGUAAUCACGCCUGAUCGAGGUCGGGGUCAAUUAAAUAAUUCGUG 840
      C  L  S  S  A  G  N  H  A  *  S  R  S  G  S  I  K  *  F  V
841 AUCAUACACACACAAUCGGUACAAACCAUAGACCAAACCAGAGACAAGAUCAAGUGAUUA 900
      I  I  H  T  Q  S  V  Q  T  I  D  Q  T  R  D  K  I  K  *  L
901 ACGUAGCAUACGAUAGGUGCGAAUUAUCCCGAGGAUGUAUAAUGUCAG 948
      T  *  H  T  I  G  A  N  Y  P  E  D  V  *  C  Q
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Fig. 1: The Complete nucleotide sequence and deduced amino acids sequence of the gene encoding 28S large subunit rRNA isolated from λg11 cDNA library of 7-days schistosomula, start codon (ATG), stop codon (TGA), three polyadenylation signals (AAATAA), (AATTA) and (ATAA) are underlined.

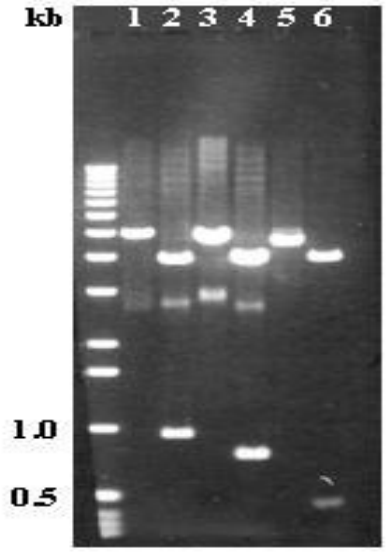

Fig. 2: 1% agarose gel showing the digestion pattern of three isolated inserts from λgt11 cDNA library of 7-day schistosomula, cloned in PCR™II plasmid vector , digested by two restriction enzymes EcoR1 and BamH1, the plasmid DNA samples were arranged in double, each represents from left to right, EcoR1 digested and BamH1 digested DNA. 1kb ladder was indicated on the left side of the gel. The selected clone (4-65) was run in lanes 1 and 2, the arrow points at the size of the insert (0.9 kb).

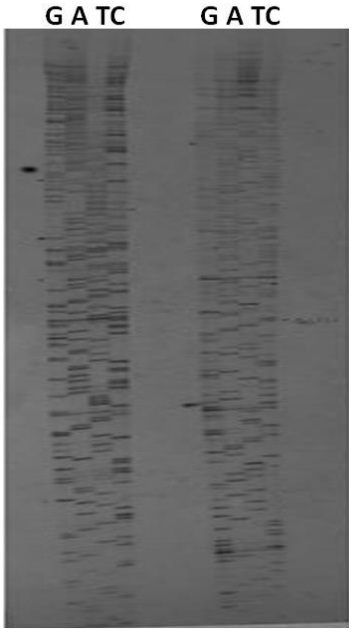

Fig 3: An autoradiogram showing sequence of the gene encoding 28S large subunit rRNA isolated from λ gt 11 cDNA library of 7-days schistosomula cloned in PCR™II plasmid vector from 5′- and 3′-ends using M13 and T7 primers.

DISCUSSION:

Over 200 million people have and another 600 million are at risk of contracting schistosomiasis. Transmission of infection which is caused by helminth parasites of genus ***schistosoma*** depends upon release of eggs from the human host (Tori *et al.,* 2007) .The recent studies on schistosomiasis have focused on identification and characterization of defined antigens that may have vaccine and/ or diagnostic potential. The development of vaccine against schistosomiasis would provide a powerful tool for the control of this important parasitic disease and it must be effective which should be confirmed by protection test (Thomas *et al.,* 2006). Vaccine development for complex parasite as *Schistosoma mansoni* is a great challenge. Given the persistant lack of an effective immunogen. Several vaccine strategies have been tried such as the use of synthetic peptides **(Fonseca *et al.,* 2004).** The tegument assoicated with certain antigens which are likely to be involved in important host-parasite interactions and may be important candidates for vaccine development.These antigens expressed on newly transformed and developing schistosomules have been shown to be protective (Miller *et al.,* 1989; Capron M and Capron D , 1986). In the recurrent study , we did not work on identification , characterization and study the vaccine potential of of tegumental antigens of 7-days schistosomula but, we tried to increase the possiblity for identifying new antigens (tegumental and/or internal) which could be vaccine candidates against schistosomiasis , this is why we did not apply the technique of extracting surface proteins , but we did sonicate all the parasite to be able immunoscreen most of the proteins f the parasite either those proteins are surface or internal. After three rounds of immunoscreening of λgt 11 cDNA library of 7-days schistosomula by affinity purified antibodies obtained from serum of the schistosomiasis chronically infected patients, a number of cDNA clones were isolated, one of them (clone 4-65) was amplified by PCR using λgt 11 forward and reverse primers , then , cloned in the EcoR1 site of PCR^{TM}II plasmid vector. The size of the selected clone was shown to be 0.9 kb by checking the pattern of restriction endonuclease digestion using two enzymes EcoR1 and BamH1, then , the result of digestion by the two enzymes was run on 1% agarose gel, the digestion by EcoR1 proved the size of the insert. The DNA sequence revealed that it did not contain the entire coding region which appears clear in both 5′-upstream and 3′-downstream regions, in the upstream region no transcription activation boxes (TATA and CAAT) can be found , the intiation codon (ATG) is located at -201 bp from the 5′-upstream region. The stop codon is located +138 bp , there are three putative three polyadenylation signals (AAATAA), (AATTA) and (ATAA) located + 114 , +22

and +6 bp, respectively from the 3/-downstream region , there is no polyadenylation site.

The DNA sequence using fmol sequencing system showed high identity with the 28 rRNA gene of the large ribosomal subunit from a number of eukaryotic species (the % of homology was 97 , 97, 92, 93 , 93 , 94 , 94 , 93 , 94 and 93 to gene for 28S rRNA of *Schistosama mansoni, Schistosama rodhaina*, *Schistosama spindale, Schistosama hippopotami, Schistosama edwariense, Schistosama margrebowiei, Schistosama incognitum, Schistosama intercalatem Schistosama mattheei* and *Schistosama hematobium,* respectively , and with other eukaryotes. The genes for 18S, 5.8S and 28S ribosomal RNA first sequenced and characterized by Ellis *et al.,* (1986) are found in a large tandem repeat of 100-150 copies on the right end of chromosome 1, each repeat contains one copy each of 18S , 5.8S and 28S genes (*C. Elegans* Sequencing Consortium, 1998). 18S, 5.8S and 25-28S ribosomal RNAs are transcribed in nucleolus of eukaryotic cells by RNA polymerase I in the form of a long precursor rRNA (pre-rRNA) that subsequently undergoes a number of processing cleavages to remove the external transcribed spacer sequences (EST) internal transcribed spacer sequences (ITS) and release the mature rRNAs involving a number of small nucleolar RNAs (Sno RNAs) (Borovjagin and Gerbi 1999).

In eukaryotes, nucleolus is the centre of ribosomes biogenesis, important proteins associates with rRNA and the mature ribosomal subunits are, then, exported to cytoplasm (Hughes *et al.,* 1991)**.**

Cloning and sequencing of DNA encoding pre-rRNA from many species showed that this DNA shares several properties in all eukaryotes. First, the pre- rRNA genes are arranged in long tandem arrays separated by non transcribed spacer regions ranging in length from 2 kb in frog to 30 kb in humans. Second, the genomic regions corresponding to the three finished rRNAs are always arranged in the same 5/---3/ order: 18S, 5.8S and 28S. Third, in all eukaryotic cells (and even in bacteria), the pre-rRNA gene as well as the primary transcript, is considerably longer than the sum of the three finished rRNA molecules (Venema and Tollervey 1995)**.** Once again new technologies and information open up the prospect of progress towards the elusive goal of an effective schistosome vaccine. Access to the genome, transcriptome and proteome provide a fantastic opportunity to search for new vaccine candidates. However, we should always bear in mind that schistosomes are not stupid. They have had tens of millions of years to evolve mechanisms that help them survive immune attack from the mammalian host, even an attack orchestrated by our vaccine strategies. The next step in this research is to apply technique of DNA hybridization using the isolated clone as a probe to screen genomic library of *Schistosama mansoni* for picking up the full length gene of 28S rRNA of the large ribosomal subunit, then, expressing it in an expression vector to obtain its protein product and to study its vaccine potential.

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