

Molecular Identification of a *Schistosoma mansoni* Tegumental Protein with Similarity to Cytoplasmic Dynein Light Chains*

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The tegument of *Schistosoma mansoni* contains a number of proteins that presumably function in its maintenance and/or repair against damage incurred from host-mediated humoral immune responses. Here, we show that the schistosome antigen identified by monoclonal antibody 709A2/2 is a cytoplasmic dynein light chain. Dynein light chains are components of dynein, an enzyme complex involved in various aspects of microtubule-based motility. Monoclonal antibody 709A2/2 recognizes two polypeptides, one of 8.9 kDa and a second of 7.6 kDa, as determined by SDS-polyacrylamide gel electrophoresis. We find that expression of *S. mansoni* dynein light chain is developmentally regulated and localized to the tegument in the schistosomula, lung stage worms, and adult worms, but is not present in the cercariae or ciliated miracidia. By Northern blot analysis of adult worm RNA, *S. mansoni* dynein light chain is encoded by a single message of ~600 base pairs. A cDNA encoding this polypeptide contains an open reading frame of 89 amino acids with a deduced molecular mass of 10.4 kDa. Coprecipitation of an apparent 18.4-kDa antigen with *S. mansoni* dynein light chain by monoclonal antibody 709A2/2 illustrates that this molecule has an affinity for other proteins. Such interactions may play a role in *S. mansoni* dynein light chain participation in organelle trafficking in *S. mansoni*.

Current World Health Organization estimates indicate that >600 million people are endangered by schistosomiasis, with ~250 million people infected (1). There presently is no immunoprophylactic therapy against this parasitic infection, but age-dependent natural immunity has been demonstrated (2), which suggests that vaccination against this trematode may be possible. Several potential candidate vaccine antigens have been identified and are currently in preclinical evaluations (1). As the efficacy of any of these vaccine candidates against schistosomiasis remains uncertain, the identification of new target antigens is an ongoing process.

The life cycle of this parasite is complex and involves six different developmental stages: (i) the cercaria, (ii) the schis-

tosomulum, (iii) the lung stage worm, (iv) the adult worm, (v) the miracidium, and (vi) the sporocyst (3). The tegument covering the cercariae, schistosomula, lung stage worms, and adult worms is an anucleated syncytium that serves as the protective barrier between the parasite and the host. The tegument in three of these four life stages (not cercariae) is bound by a unique double lipid bilayer structure called the heptalaminate membrane. Organelles within the tegument include mitochondria, discoid bodies, and membranous bodies (4). The function of the specialized organelles (discoid bodies and membranous bodies) is not exactly known, although there is some evidence that they may continually maintain the integrity of the heptalaminate membrane and tegument (5). The organelles found in the tegument are made in the subtegumental cell, which contains all of the necessary components for protein synthesis and organelle development (5). These organelles are transported into the tegument via connecting cytoplasmic channels; however, it is still not understood how this transport occurs.

As antigens in the tegument are likely to come in contact with the host immune system and the proposed humoral dependent immune mechanisms associated with protection against *Schistosoma mansoni* appear to be directed against the tegument of schistosomula, we have generated mAbs¹ against antigens associated with isolated tegumental membranes (6). In this study, we have characterized the antigen recognized by one of these mAbs, mAb 709A2/2. The sequence encoding this antigen was found to be highly similar to the 8-kDa light chain common to dynein. However, the developmental expression of this antigen in *S. mansoni* suggests that SmDLC is a member of cytoplasmic and not axonemal dynein.

Dyneins are large (native masses between 1000 and 2000 kDa), multisubunit (heavy, intermediate, and light chains), molecular motors involved in various types of microtubule-based motility. These motors are currently divided into two groups: (i) axonemal (reviewed by Witman (7) and Witman *et al.* (8)), which drive flagellar and ciliary beating; and (ii) cytoplasmic (reviewed by Bloom (9), Vallee (10), and Holzbaur *et al.* (11)), which are involved in the movement of membranous organelles toward the minus ends of microtubules as well as spindle assembly and dynamics in mitosis and, in fungal cells, nuclear movements. Light chains having masses of 6–30 kDa are known to be associated with axonemal dyneins, but only two have been functionally characterized (12–14). Until recently, cytoplasmic dyneins were not believed to contain light chains, but a report by King and Patel-King (15) describing the characterization of two axonemal DLCs from *Chlamydomonas*

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U55992.

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¹ The abbreviations used are: mAbs, monoclonal antibodies; pAb, polyclonal antibody; DLC, dynein light chain; SmDLC, *S. mansoni* dynein light chain; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s); GST, glutathione *S*-transferase; PBS, phosphate-buffered saline.

reinhardtii verified that these molecules have sequence homologs in organisms that contain neither flagella nor cilia. Therefore, much interest has been generated in the molecular and functional characterization of these putative cytoplasmic DLCs in the hopes of understanding what role they play in dynein function.

MATERIALS AND METHODS

Parasites—Parasite material derived from *S. mansoni* (Puerto Rican strain) was used throughout this study. Adult worms were obtained as described previously (16). The schistosomular life stage was obtained by mechanical transformation as described by Dalton *et al.* (17). Eggs containing miracidia were collected from infected mouse livers, and cercariae were shed from the intermediate snail host *Biomphalaria glabrata*.

Antibodies—mAb 709A2/2 (IgG_{2a}) was derived from spleen cells of mice immunized with adult male *S. mansoni* tegumental membranes (6) obtained as described by Oaks *et al.* (18). mAb 654B2 (IgG_{2a}) was prepared from spleen cells of mice vaccinated twice with γ -irradiated cercariae as described (19). mAb 781.2 (IgG_{2a}) was prepared from mice immunized with human immunodeficiency virus type 1 recombinant gp160 (20). A monospecific mouse polyclonal antibody (pAb 709) was generated against native 8.9-kDa SmdLC following the immunization protocol described by Cianfriglia *et al.* (21). 8.9-kDa SmdLC was obtained from worm lysates by immunoaffinity chromatography using mAb 709A2/2 as outlined by Harlow and Lane (22), followed by SDS-PAGE separation. The Coomassie Blue-stained band corresponding to the 8.9-kDa polypeptide immunoprecipitated by mAb 709A2/2 was cut, emulsified with Freund's adjuvant, and used for immunization. mAb 128C3/3 (IgG_{2a}) was derived from spleen cells of mice immunized with *S. mansoni* cercarial glycoproteins (23). The concentration of each mAb within individual ascites fluids was found to be highly variable as judged by SDS-PAGE analysis (data not shown). Therefore, the ascites fluids were diluted to equalize the concentrations of immunoglobulin for each immunoassay.

cDNA Cloning—Expression screening, using standard methods (24), of an oligo(dT)-primed adult worm schistosome UNIZAP-XR (Stratagene) cDNA library identified a cDNA clone encoding SmdLC (clone 1B-1). After three rounds of plaque purification with pAb 709, the phagemid containing clone 1B-1 was rescued using helper phage, and the size of the insert contained within pBluescript II SK[−] (Stratagene) was determined by *EcoRI* and *XhoI* digestion. The 469-bp *EcoRI/XhoI* restriction fragment was sequenced in both directions using the 7-deaza-dGTP Sequenase sequencing kit (U. S. Biochemical Corp.) and the methodology of dideoxy chain termination (25).

Computer Analysis—Analysis of sequence assembly and comparisons was performed using software developed by the Genetics Computer Group. Searches of GenBankTM to identify sequences similar to SmdLC were performed at the National Center for Biotechnology Information using the BLAST network server. Primary sequence motifs were identified using the PROSITE network server at EMBL. Secondary structure predictions were made using a trial version of DNASTAR[®] developed by LasergeneTM. Sequence alignments were generated by the multiple sequence alignment program developed at Washington University.

Northern Blotting—Adult worm total RNA was isolated, electrophoresed, and capillary-transferred to a nylon membrane (Zeta-Probe, Bio-Rad) as described previously (26). The 469-bp insert contained within clone 1B-1 was radiolabeled with random primers and [α -³²P]dATP (800 Ci/mmol; Amersham Corp.) using a kit supplied by Life Technologies, Inc. The nylon membrane containing RNA was prehybridized for 2 h at 65 °C in Rapid Hybridization Buffer (Amersham Corp.) before addition of the radiolabeled denatured probe. Incubation with the radiolabeled probe was continued overnight at 65 °C, with the membrane subsequently washed according to the manufacturer's (Amersham Corp.) instructions before exposure to Kodak XR film.

Protein Sequencing—Protein samples submitted for sequencing were prepared by immunoaffinity purification over the mAb 709A2/2 affinity column. Briefly, adult worm lysates were passed over a mAb 709A2/2-coupled protein G-agarose affinity column (Pharmacia Biotech Inc.) essentially as described by Harlow and Lane (22). Isolated SmdLC was fractionated by SDS-PAGE and transferred onto a Bio-Rad microsequencing polyvinylidene difluoride membrane (Bio-Rad) by electrophoresis at 70 V for 2 h. The 8.9-kDa polypeptide was subjected to direct N-terminal amino acid sequencing by Dr. Wu-Schyong Liu at the Johns Hopkins Protein Sequencing Facility (Applied Biosystems 475A protein sequencer). The 7.6-kDa polypeptide was subjected to *in situ* trypsin

digestion, purification of the eluted peptides by reverse-phase chromatography, mass spectrophotometry analysis of the purified eluted peptides, and N-terminal amino acid sequencing at the Wistar Sequencing Facility by David Reim.

Radioisotope Labeling and Solubilization of Proteins—Adult male worm proteins were metabolically labeled with [³⁵S]methionine (Amersham Corp; 1200 Ci/mmol, 0.500 mCi/ml) as described previously (16). Conditions for optimizing protein-protein interactions during worm solubilization described by Oaks *et al.* (18) were used to prepare samples for coprecipitation experiments. Extracts of cercariae and solubilized egg antigens were prepared by the procedure described by Hawn and Strand (16) in the presence of 1% Triton X-100.

Triton X-114 Phase Separation—For Triton X-114 (Sigma) phase separation, the solubilized adult worm proteins were treated with precondensed Triton X-114 (final concentration of 1%), and phase separation was performed as described by Bordier (27). After the phase separation, the extracts were extensively dialyzed against 20 mM Tris-HCl, pH 7.6, containing 1 mM EDTA and 0.02% Triton X-100 in order to reduce the concentration of Triton X-114 before radioimmunoprecipitation.

Radioimmunoprecipitations—To decrease nonspecific binding during immunoprecipitation, radiolabeled worm extracts were preabsorbed with normal mouse and normal rabbit sera (1:100, v/v). To ensure precipitation of the normal sera, 10 μ g of rabbit anti-mouse immunoglobulins (Jackson ImmunoResearch Laboratories, Inc.)/2 μ l of sera was added, and the extract was absorbed with 10% *Staphylococcus aureus* (strain Cowan; Calbiochem). The precleared labeled extract was immunoprecipitated as described previously (28).

Gel Electrophoresis and Fluorography—Gel electrophoresis and fluorography were carried out as described previously (28) with minor modifications. One-dimensional gels used for analysis of radioimmunoprecipitation products and immune affinity eluents were 10–20% (w/v) gradient polyacrylamide. After SDS-PAGE, the radioimmunoprecipitation products were transferred to polyvinylidene difluoride paper (Millipore Corp.) by electrophoresis at 70 V for 2 h. After drying to completion, the membranes were sprayed with a coat of EN³HANCE (Amersham Corp.), dried to completeness again, and exposed to autoradiographic film (Amersham Corp.).

Western Blot Analysis—Western blot analysis was carried out as described previously (29) with the following modifications. Primary antibody dilutions of 1:200 and horseradish peroxidase-conjugated donkey anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc.) dilutions of 1:25,000 were used in this study.

Indirect Immunofluorescence—Adult worms, schistosomula, and lung stage parasites were embedded in O.C.T. Compound (Miles, Inc.). 6- μ m cryostat sections were cut and mounted on glass multitest slides (Flow), and indirect immunofluorescence was performed as described previously (16). Primary mAbs at ascites dilutions of 1:50 and fluorescein isothiocyanate-conjugated donkey anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, Inc.) at a 1:100 dilution were used in this study.

SmdLC-GST Fusion Protein Construction and Expression—To obtain SmdLC as a C-terminal fusion to *Schistosoma japonicum* glutathione S-transferase, the SmdLC coding region was restricted out of pBluescript II SK[−] and subcloned into the *XbaI* and *XhoI* sites of the vector pGEX-KG (30). *Escherichia coli* strain DH5 α was transformed with the resulting construct, and protein expression was subsequently induced by addition of isopropyl-1-thio- β -D-galactopyranoside to 0.5 mM final concentration. Cell lysates obtained by French press were affinity-purified over a glutathione-agarose column (Sigma), and SmdLC was eluted from the column with 10 mM reduced glutathione (Sigma) as described (31).

Enzyme-linked Immunosorbent Assay—2 μ g of solubilized egg antigen, cercarial extract, or adult worm lysate in 50 μ l of 0.2 M sodium carbonate/bicarbonate buffer, pH 9.4, was added per well and dried overnight at 37 °C in a 96-well microtiter plate. The wells were blocked with 5% dry milk, PBS, and 0.1% Tween 20 for 2 h at 37 °C; washed twice in PBS and 0.1% Tween 20; and incubated in primary antibody dilutions made in 1% bovine serum albumin and PBS. Unbound primary antibodies were removed by washing five times with PBS and 0.1% Tween 20, followed by secondary antibody addition (horseradish peroxidase-conjugated donkey anti-mouse) at a 1:2500 dilution in 1% bovine serum albumin and PBS. The wells were again washed five times, and bound secondary antibody was detected by addition of a 1:1 (v/v) ratio of ABTS peroxide substrate to hydrogen peroxide (Kirkegaard & Perry Laboratories, Inc.). The plate was then incubated for 1 h at room temperature in the dark and subsequently read at 415 nm with a Bio-Rad model 3350 microplate reader.

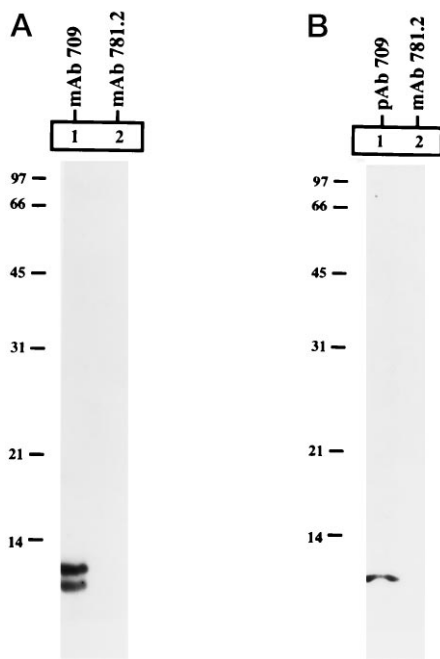


FIG. 1. Recognition of SmDLC by mAb 709A2/2 and pAb 709. A, radioimmunoprecipitation of solubilized [^{35} S]methionine-labeled worm proteins. Lane 1, SmDLC recognized by mAb 709A2/2; lane 2, negative control mAb 781.2. B, Western blot analysis of 10 μ g of solubilized worm antigen preparation. Lane 1, recognition of the 8.9-kDa polypeptide by pAb 709; lane 2, negative control mAb 781.2. Molecular mass standards (in kilodaltons) are indicated to the left.

RESULTS

Cloning of the cDNA Encoding SmDLC—mAb 709A2/2 immunoprecipitated two polypeptides with apparent molecular masses of 8.9 and 7.6 kDa (Fig. 1A, lane 1). To establish the identity of the polypeptides recognized by mAb 709A2/2, a mouse polyclonal serum against the larger of the two antigens in Fig. 1A was generated. This serum, pAb 709, was shown to be monospecific for the 8.9-kDa polypeptide by Western blot analysis against solubilized worm proteins (Fig. 1B). There are several possible interpretations why pAb 709 failed to recognize the 7.6-kDa polypeptide: differences in epitope conformation (native *versus* denatured), sensitivity of assays (Western blot *versus* immunoprecipitation), or epitope unique to the 8.9-kDa polypeptide. Most important, because of the monospecificity of pAb 709, it was a useful reagent for screening a UNI-ZAP-XR adult worm cDNA expression library, which led to the identification of a positive clone, 1B-1. Clone 1B-1 contained an insert of 469 bp encoding an open reading frame of 89 amino acids (Fig. 2A), predicting a polypeptide of 10.4 kDa and a pI of 6.13. A moderately strong Kozak (32) consensus start site is present at nucleotides 3–9, and a relatively conserved poly(A)⁺ signal sequence (AATATA) is seen 17 bp upstream of the poly(A)⁺ tail. Potential post-translational modifications of this reading frame were predicted by PROSITE at EMBL and include three phosphorylation motifs: a protein kinase C phosphorylation site at amino acids 2–4 (SER) and two tyrosine kinase phosphorylation sites at amino acids 43–50 (KKD-FDRKY) and 44–50 (KDFDRKY). Amino terminal sequencing of the native 8.9-kDa polypeptide resulted in sequence confirmation of the open reading frame contained within clone 1B-1. This confirmation extends over 7 residues (VIKNADXXXXXQ, where X represents residues whose identity could not be determined) and is illustrated in Fig. 2A. *In vitro* transcription and translation of clone 1B-1 followed by immunoprecipitation of the protein products with mAb 709A2/2 resulted in a polypeptide whose migration on SDS-PAGE was similar to that of the

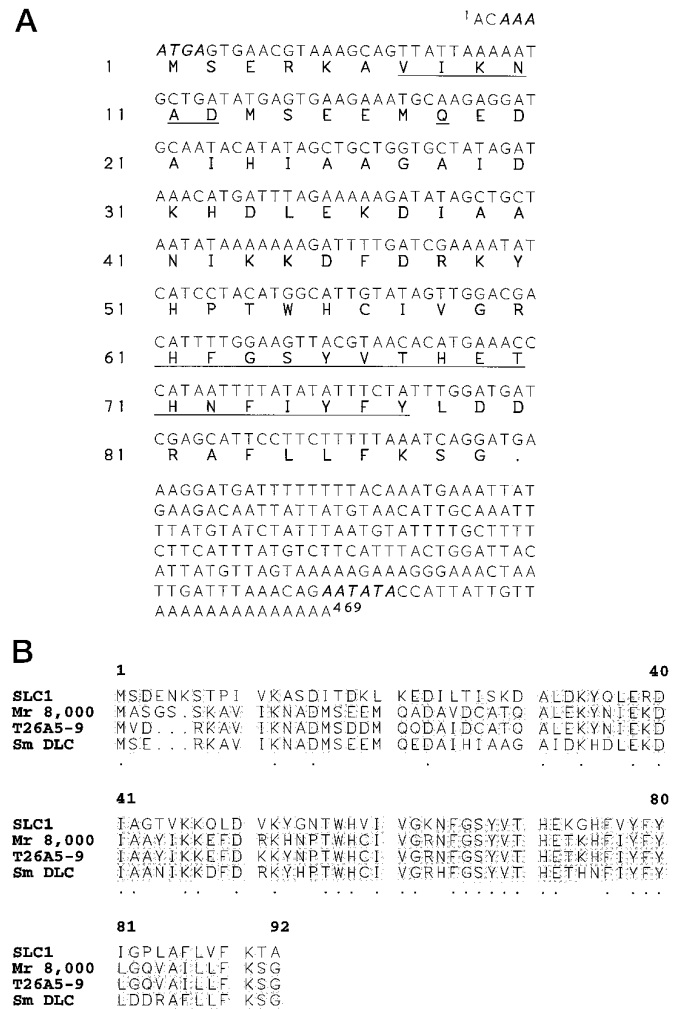


FIG. 2. Sequence of SmDLC and alignment of DLC family members. A, nucleic acid and deduced amino acid sequences of SmDLC. Boldface italic nucleic acids represent the predicted ribosomal binding site at the 5'-end and a poly(A)⁺ signal motif at the 3'-end. Underlined amino acids represent exact matches for peptide sequences obtained from the purified native 8.9-kDa (VIKNADXXXXXQ) and 7.6-kDa (HFGSYVTHETHNFIYFY) polypeptides. B, alignment of SmDLC with *S. cerevisiae* SLC1, *C. reinhardtii* 8-kDa DLC, and the predicted *C. elegans* protein (T26A5-9). The shaded boxes represent residues conserved between two or more family members. Invariant residues are indicated by dots. The alignment was generated by the multiple sequence alignment program developed at Washington University.

native 8.9-kDa polypeptide recognized by mAb 709A2/2 (data not shown). mAb 709A2/2 also reacted specifically with the fusion protein SmDLC-GST, but not with GST alone, as measured by enzyme-linked immunosorbent assay (data not shown). These three independent lines of evidence support the contention that the native protein and the polypeptide product of the open reading frame contained within clone 1B-1 are identical.

The smaller (7.6 kDa) of the two polypeptides recognized by mAb 709A2/2 (Fig. 1A) was digested with trypsin, and one peptide fragment was subjected to N-terminal microsequencing. The sequence obtained from this peptide matches exactly a continuous stretch of 17 amino acids (positions 61–77, HFG-SYVTHETHNFIYFY) contained within the larger polypeptide encoded by clone 1B-1 (Fig. 2A), demonstrating that the two polypeptides recognized by mAb 709A2/2 are related.

The 89-amino acid open reading frame contained within clone 1B-1 showed extensive similarities to dynein light chain homologs from various organisms and is illustrated in the generated alignment shown in Fig. 2B. SmDLC displays 71%

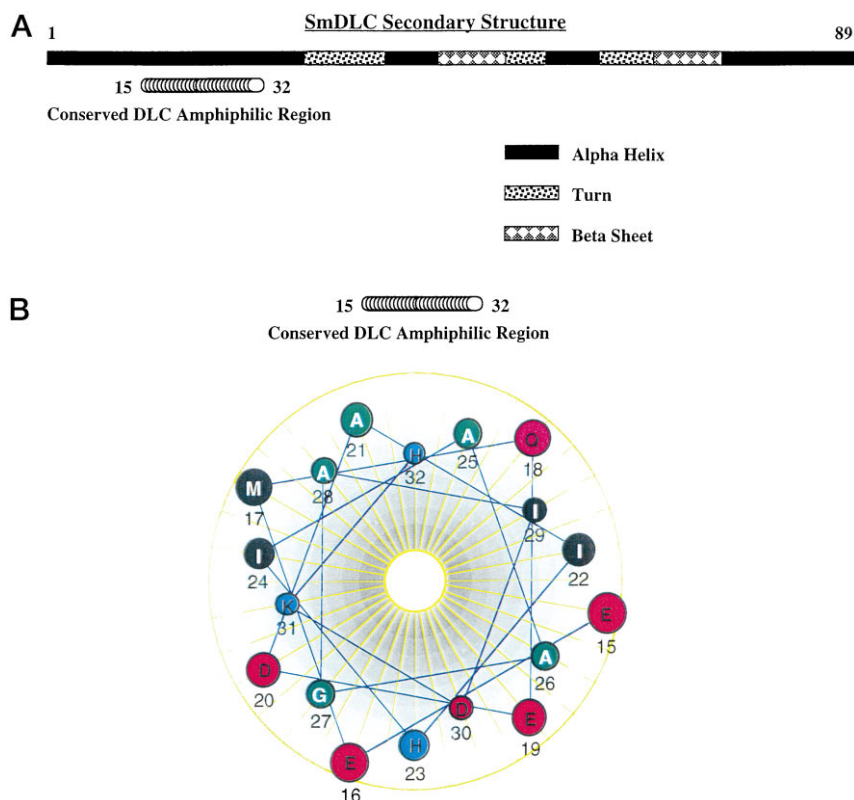


FIG. 3. **Secondary structure prediction of SmDLC.** A, secondary structure of SmDLC predicted by DNASTAR®. The indicated structures are conserved among other DLC family members only varying by length and repeat. The residues marked from 15–32 are part of an extended α -helix that is amphiphilic in nature. B, helical wheel illustration of residues 15–32 demonstrating amphiphilicity of the conserved segment.

identity and 87% similarity to a predicted open reading frame (T26A5–9) from chromosome III of *Caenorhabditis elegans* (33). An axonemal dynein light chain from *C. reinhardtii* (15), 8-kDa DLC is 74% identical to SmDLC and appears to be more divergent at the amino terminus than is T26A5–9 to SmDLC. SmDLC is also 50% identical to SLC1 (40), a putative cytoplasmic dynein light chain homolog from *Saccharomyces cerevisiae*. Like *C. reinhardtii* 8-kDa DLC, SmDLC also shares homology with 11-kDa DLC from this same organism (15). This sequence similarity is also high, but is restricted to two regions: amino acids 13–50 and 54–88 of SmDLC and amino acids 28–65 and 76–110 of 11-kDa DLC, respectively. This degree of conservation is expected since even in *C. reinhardtii* 8- and 11-kDa DLCs share 41.8% identity. Comparison of the alignment generated by multiple sequence alignment (at Washington University) for this novel protein family (Fig. 2B) indicates that 34% of the residues are invariant among members, with several instances of conservative substitutions, which increase the overall sequence similarity of these DLCs to ~65%.

SmDLC also showed significant sequence similarity to a number of expressed sequence tags in the data base that probably represent incomplete or uncharacterized members of the DLC family. Expressed sequence tag R95507 from *S. mansoni* is almost exactly identical to SmDLC cDNA, but contains an additional 19 nucleotides of novel sequence directly upstream of the predicted start site within SmDLC. The codon usage of these 19 nucleotides does not appear to be consistent with the codon bias of *S. mansoni*, and identity scores among the compared DLCs drop when these nucleotides are read in frame with SmDLC. Therefore, this expressed sequence tag sequence probably represents additional 5'-noncoding sequence upstream of the predicted start site in SmDLC.

The predicted secondary structure of SmDLC using the Garnier-Robson algorithm and DNASTAR® software (Fig. 3A) is consistent with other members of the DLC family and provides another level of structural similarity among this group of proteins. Residues 1–47 are predictive of an extended α -helix that

is directly followed by alternating β -sheets and α -helices. Behind the last β -sheet, another extended α -helical stretch (amino acids 77–86) continues to the carboxyl terminus. There is an amphiphilic stretch of residues contained within the first α -helix (amino acids 15–32) that appears to be conserved among other DLCs (15). The amphiphilicity is displayed as a helical wheel over this stretch of residues and is illustrated in Fig. 3B.

The size of the mRNA coding for SmDLC judged by Northern blot analysis was shown to be ~600 bp as compared with linear regression of the migration of the molecular mass standards through an agarose gel (Fig. 4). Because of the width of the band observed, we cannot exclude whether there is more than one message coding for SmDLC, possibly accounting for the 8.9- and 7.6-kDa polypeptides observed in Fig. 1A. Alternatively, the 7.6-kDa polypeptide could arise from leaky translation (a second, moderately strong ribosomal binding site exists at nucleotides 51–57, and initiation at this methionine would result in a protein reduced by ~1.9 kDa) as described by Kozak (34).

Coprecipitation of SmDLC-associated Antigens—Because dynein is a multisubunit enzyme containing a number of polypeptides (10), we reasoned that SmDLC might be associated with other proteins. To investigate this possibility, we performed a coprecipitation study on worm proteins that had been solubilized using a method that optimizes protein-protein interactions in schistosomes (18). A 18.4-kDa polypeptide was found to specifically coprecipitate with SmDLC by mAb 709A2/2 (Fig. 5, lane 2); negative control mAb 781.2 did not precipitate any specific protein (lane 1).

Triton X-114 Phase Separation of SmDLC—To address the influence of the conserved amphiphilic α -helix on the hydrophobic behavior of SmDLC, the native protein isolated from adult parasites was subjected to phase partitioning in the presence of Triton X-114 (27). The antigen Sm23, which is recognized by mAb 654, was used as a positive control (Fig. 6, lanes 2 and 5) in this experiment because of its established hydrophobicity (35, 36). After phase separation in Triton X-114, all of

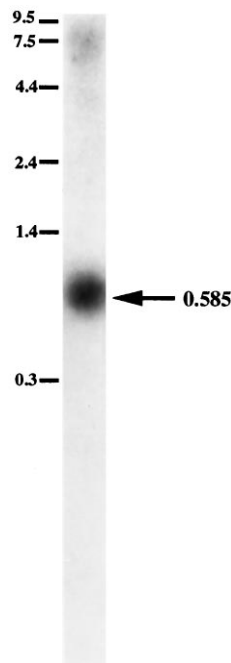


FIG. 4. **Northern blot analysis.** Shown is a Northern blot of total RNA purified from adult worms. A single message of ~600 bp is observed for SmDLC. Molecular mass standards (in kilobase pairs) are indicated to the left.

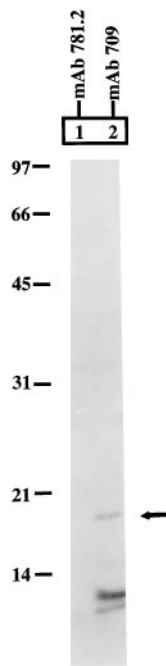


FIG. 5. **Coprecipitation of an 18.4-kDa antigen associated with SmDLC.** Detergent-solubilized, radiolabeled worm protein extracts were immunoprecipitated with mAb 709A2/2 (lane 2) or control mAb 781.2 (lane 1). mAb 709A2/2 specifically coprecipitates a 18.4-kDa polypeptide (indicated by the arrow) with SmDLC.

SmDLC was found associated with the detergent rather than the aqueous phase (Fig. 6, compare lanes 3 and 6). The background protein bands apparent in the aqueous phase of this experiment (Fig. 6, lanes 1–3) are due to nonspecific interactions with antibody since the pattern observed in these lanes is identical, regardless of mAb used.

Developmental Expression of SmDLC—The anatomical localization of SmDLC in five life stages of *S. mansoni* was determined by indirect immunofluorescent microscopy using mAb

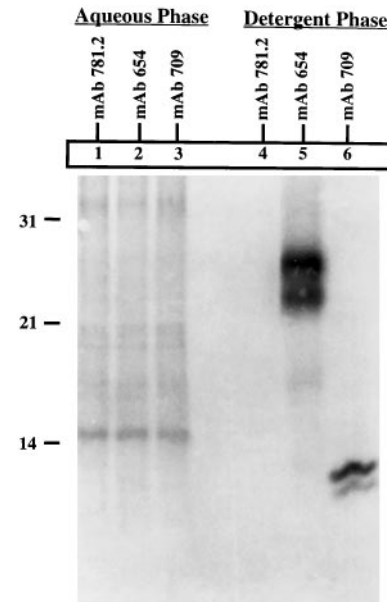


FIG. 6. **Triton X-114 phase separation of SmDLC.** SmDLC partitions into the detergent phase by Triton X-114 phase separation. Extracts of radiolabeled adult worm proteins were subjected to phase separation in the presence of Triton X-114. Lanes 1–3 represent immunoprecipitated proteins from the aqueous phase. Lanes 4–6 represent immunoprecipitated proteins from the detergent phase. Proteins were immunoprecipitated with mAb 781.2 (a negative control; lanes 1 and 4), mAb 654 (a positive control, Sm23; lanes 2 and 5), or mAb 709A2/2 (SmDLC; lanes 3 and 6).

709A2/2. Staining was observed only in three forms of the parasite found within the definitive vertebrate host (Fig. 7). In schistosomula, the form found in the infected host within the earliest hours of cercarial penetration, SmDLC is present in a diffusely stained rim surrounding the parasite (Fig. 7A), suggesting that it is concentrated in the tegument. Upon maturation into the lung stage, the localization of SmDLC becomes more concentrated within the tegument as is observed by a sharply defined line around the body of the lung stage worm (Fig. 7B). Tegumental staining continues to be observed into the adult worm. Here, SmDLC appears to be localized most strongly in the dorsal tegument, with less protein being concentrated ventrally (Fig. 7C). mAb 781.2, a negative control, did not demonstrate specific staining (Fig. 7D). Indirect immunofluorescence on cercariae and miracidia revealed no staining above the mAb 781.2 background levels (data not shown), suggesting that SmDLC is absent in these developmental stages. To confirm this observation, extracts from cercarial and miracidial life stages were probed by enzyme-linked immunosorbent assay. mAb 709A2/2 bound to extracts of adult worm parasites, but failed to bind to miracidial (solubilized egg antigen) or cercarial extracts even at the highest concentration of antibody used (data not shown). In contrast, positive control mAb 128C3 (which has previously been shown by immunofluorescent staining to bind to the surface of miracidial ciliated plates, to the cilia themselves, and to the surface of cercariae (37)) binds with high affinity to solubilized egg antigen and cercarial extracts (data not shown). These enzyme-linked immunosorbent assay data support the immunofluorescent microscopy observations and demonstrate that SmDLC is present in miracidia and cercariae below the detection limit of both assays.

DISCUSSION

In this report, we describe the molecular characterization of a cytoplasmic dynein light chain from *S. mansoni*. The identification of a DLC within the parasite's tegument, a layer that

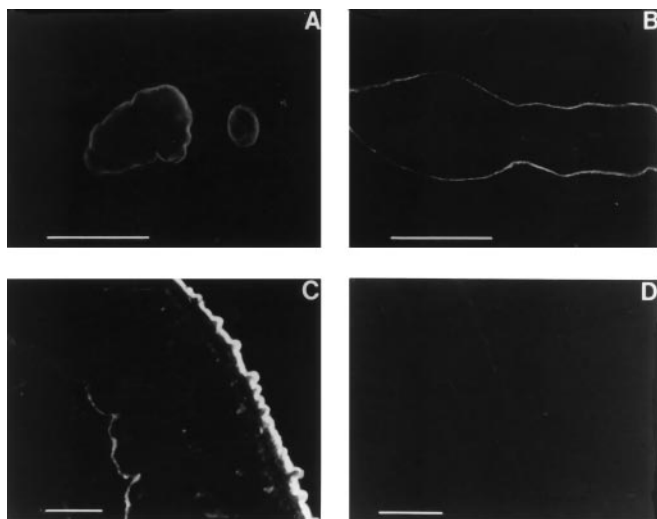


FIG. 7. Indirect immunofluorescent localization of SmDLC. 6- μ m cryostat sections were cut from fixed parasites and stained with mAb 709A2/2 or control mAb 781.2. A, schistosomula; B, lung stage schistosoma; C and D, adult male schistosome. A–C were treated with mAb 709A2/2; D was treated with negative control mAb 781.2. Bars in A and B = 100 μ m; bars in C and D = 20 μ m.

does not contain flagella or cilia, supports recent observations from other investigators (15) suggesting that cytoplasmic dyneins do, in fact, contain light chains. 8-kDa DLCs, of which SmDLC is a member, including SLC1 from *S. cerevisiae*, T26A5–9 from *C. elegans*, and 8-kDa DLC from *C. reinhardtii*, show remarkable primary sequence conservation as well as similar secondary structural organization. Additionally, this report presents novel characteristics that may be uniquely associated with schistosomes or may be applicable to other proteins in this growing family.

There is a discrepancy between the mass of the larger SmDLC isoform predicted from the cDNA sequence (10.4 kDa) and the observed migration of this polypeptide on SDS-PAGE (8.9 kDa). Aberrant migration of *C. reinhardtii* 8- and 11-kDa DLCs has also been observed (15). The cDNA encoding *C. reinhardtii* 8-kDa DLC predicts a protein with a molecular mass of 10.3 kDa, and the cDNA coding for *C. reinhardtii* 11-kDa DLC predicts a protein with a molecular mass of 13.8 kDa. These differences are probably attributable to the aberrant migration of DLCs through an SDS-polyacrylamide gel. Such anomalous behavior during SDS-PAGE has also been observed with pig heart calpastatin and has been attributed to the high proportion of basic amino acid residues (38).

Sequence identity and conservation between SmDLC and the other 8-kDa light chain family members are evident from multiple sequence alignment of SLC1, T26A5–9, and *C. reinhardtii* 8-kDa DLC with SmDLC (Fig. 2B). The overall structural organization of SmDLC is also highly similar to the secondary structures of other DLCs predicted by King and Patel-King (15) and is depicted by Fig. 3. The secondary structural prediction made for SmDLC suggests an amphiphilic α -helix between residues 15 and 32, which is conserved among other DLCs and might contribute to protein-protein interactions. King and Patel-King (15) first proposed this idea based upon their comparison of many DLC secondary structures and concluded that it was this amphiphilic α -helical region that may be responsible for the interactions of DLCs with other polypeptide components of dynein. Therefore, it is not surprising that a specific 18.4-kDa polypeptide coprecipitated with SmDLC when conditions were amenable for protein-protein interactions in schistosomes. Recently, we obtained data demonstrating that a cDNA encoding the 18.4-kDa polypeptide

shares sequence similarity with other DLCs from *S. cerevisiae*, *Homo sapiens*, *C. reinhardtii*, *Drosophila melanogaster*, and *C. elegans*.² This observation supports the findings described by King *et al.* (39), who showed that there is an association, or cosedimentation, of a 9-kDa light chain with a 19-kDa light chain in outer arm dynein isolated from rainbow trout (*Salmo gairdneri*) spermatozoa.

By the criteria of detergent phase separation, SmDLC behaved like a hydrophobic protein. However, the primary sequence does not predict a membrane-spanning sequence or lipid attachment motif. It is possible that association with Triton X-114 could be indirectly mediated by the hydrophobic residues spaced throughout SmDLC. Hence, hydrophobic interactions may contribute significantly to the association of SmDLC with other cytoplasmic dynein subunits. This may limit the ability of SmDLC to interact with the aqueous environment that is surely surrounding the cytoplasmic dynein-protein complex. The hydrophobicity of other 8-kDa cytoplasmic DLCs has not been determined, and it will be interesting to see if they display this same behavior under identical experimental conditions.

Immunolocalization studies demonstrate that expression of SmDLC is developmentally regulated. The most striking observation, however, was the absence of this DLC in ciliated miracidia. These cilia presumably are powered by axonemal dynein as in other ciliated organisms, although a detailed substructure architectural analysis of *S. mansoni* cilia has not been described. The presence of SmDLC in the tegument (a compartment that does not contain cilia or flagella) of schistosomula, lung stage worms, and adult worms supports the contention that this molecule is a cytoplasmic DLC. Sequence comparison of *C. reinhardtii* 8-kDa DLC with *C. elegans* T26A5–9 and a rice callus-expressed sequence tag performed by King and Patel-King (15) had raised the possibility that DLCs exist in organisms that do not contain cilia or flagella. From this observation, the authors suggested that these DLCs represent previously undescribed components of cytoplasmic dynein. The immunolocalization results presented in this study support the observation of King and Patel-King (15) and provide the first clear demonstration that DLCs do exist in the cytoplasm.

The results that mAb 709A2/2 does not recognize a 8-kDa DLC in ciliated miracidia or flame cells (subtegumental cells of the excretory pathway that contain cilia tufts) in adult worms suggest that *S. mansoni* contains two functionally and structurally distinct 8-kDa DLCs. These results also show that mAb 709A2/2 is specific for 8-kDa cytoplasmic DLC and does not recognize axonemal 8-kDa DLC. SmDLC, as part of the cytoplasmic dynein complex, may help function in the transport of membranous and discoid bodies from subtegumental cells to the tegument cytoplasm, where these specialized organelles can contribute to the integrity of heptalaminate boundary. The role SmDLC plays in the tegument, as well as the identification of associated proteins initiated in this study, is the focus of our future work. Elucidation of the function of SmDLC may help us better understand the protective role of the tegument as well as provide insight into the mechanisms of organelle trafficking in *S. mansoni* that may be applicable to other systems.

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² K. F. Hoffmann and M. Strand, manuscript in preparation.

REFERENCES

1. Bergquist, N. R. (1995) *Parasitol. Today* **11**, 191–194
2. Butterworth, A. E., and Hagan, P. (1978) *Parasitol. Today* **3**, 11–16
3. Stirewalt, M. A. (1974) *Adv. Parasitol.* **12**, 115–182
4. Hockley, D. J. (1973) *Adv. Parasitol.* **11**, 233–305
5. Hockley, D. J., and McLaren, D. J. (1973) *Int. J. Parasitol.* **3**, 13–25
6. Lewis, S. A. (1988) *Schistosoma mansoni: Characterization of Proteins and Immunogens Released by Adult Parasites*. Doctoral dissertation, The Johns Hopkins University
7. Witman, G. B. (1992) *Curr. Opin. Cell Biol.* **4**, 74–79
8. Witman, G. B., Wilkerson, C. G., and King, S. M. (1994) in *Microtubules* (Hyams, J. S., and Lloyd, C. W., eds) pp. 229–249, Wiley-Liss, Inc., New York
9. Bloom, G. S. (1992) *Curr. Opin. Cell Biol.* **4**, 66–73
10. Vallee, R. B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 8769–8772
11. Holzbaur, E. L. F., Mikami, A., Paschal, B. M., and Vallee, R. B. (1994) in *Microtubules* (Hyams, J. S., and Lloyd, C. W., eds) pp. 251–268, Wiley-Liss, Inc., New York
12. Stephens, R., and Prior, G. J. (1992) *J. Cell Sci.* **103**, 999–1012
13. Barkalow, K., Hamasaki, T., and Satir, P. (1994) *J. Cell Biol.* **126**, 727–735
14. Satir, P., Barkalow, K., and Hamasaki, T. (1995) *Biophys. J.* **68**, 222
15. King, S. M., and Patel-King, R. S. (1995) *J. Biol. Chem.* **270**, 11445–11452
16. Hawn, T. R., and Strand, M. (1994) *J. Biol. Chem.* **269**, 20083–20089
17. Dalton, J. P., Strand, M., Mangold, B. L., and Dean, D. A. (1986) *J. Immunol.* **136**, 4689–4694
18. Oaks, J. A., Cain, G. D., Mower, D. A., and Raj, R. K. (1981) *J. Parasitol.* **67**, 761–775
19. Dalton, J. P., Tom, T. D., and Strand, M. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84**, 4268–4272
20. Monell, C. R. (1992) *Mechanisms of HIV-1 gp41 Induced Immunosuppression*. Doctoral dissertation, The Johns Hopkins University
21. Cianfriglia, M., Armellini, D., Massone, A., and Mariani, M. (1983) *Hybridoma* **2**, 451–457
22. Harlow, E., and Lane, D. (1989) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Strand, M., McMillan, A., and Pan, X.-Q. (1982) *Exp. Parasitol.* **54**, 145–156
24. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467
26. Andresen, K., Tom, T. D., and Strand, M. (1991) *J. Biol. Chem.* **266**, 15085–15090
27. Bordier, C. (1981) *J. Biol. Chem.* **256**, 1604–1607
28. Norden, A. P., and Strand, M. (1985) *Am. J. Trop. Med. Hyg.* **34**, 495–507
29. Hawn, T. R., Tom, T. D., and Strand, M. (1993) *J. Biol. Chem.* **268**, 7692–7698
30. Guan, K., and Dixon, J. E. (1991) *Anal. Biochem.* **192**, 262–267
31. Soisson, L. M., Masterson, C. P., Tom, T. D., McNally, M. T., Lowell, G. H., and Strand, M. (1992) *J. Immunol.* **149**, 3612–3620
32. Kozak, M. (1984) *Nucleic Acid Res.* **12**, 857–872
33. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Laister, N., Latreille, P., Lightning, J., Lloyd, C., McMurray, A., Mortimore, B., O'Callaghan, M., Parsons, J., Percy, C., Rifken, L., Roopra, A., Saunders, D., Shownkeen, R., Smaldon, N., Smith, A., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson-Sproat, J., and Wohldman, P. (1994) *Nature* **368**, 32–38
34. Kozak, M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 2662–2666
35. Reynolds, S. R., Dahl, C. E., and Harn, D. A. (1994) *J. Immunol.* **152**, 193–200
36. Köster, B., and Strand, M. (1994) *Arch. Biochem. Biophys.* **310**, 108–117
37. Köster, B., and Strand, M. (1994) *Parasitology* **108**, 433–446
38. Takano, E., Maki, M., Mori, H., Hatanaka, N., Marti, T., Titani, K., Kannagi, R., Ooi, T., and Murachi, T. (1988) *Biochemistry* **27**, 1964–1972
39. King, S., Gatti, J.-L., Moss, A., and Witman, G. (1990) *Cell Motil. Cytoskeleton* **16**, 266–278
40. Dick, T., Uttam, S., and Chia, T. (1996) *Mol. & Gen. Genet.* **251**, 38–43