

Molecular Characterization of a 20.8-kDa *Schistosoma mansoni* Antigen

SEQUENCE SIMILARITY TO TEGUMENTAL ASSOCIATED ANTIGENS AND DYNEIN LIGHT CHAINS*

(Received for publication, February 13, 1997, and in revised form, March 31, 1997)

Karl F. Hoffmann‡ and Mette Strand§

From the Department of Pharmacology and Molecular Sciences, Center for Monoclonal Studies, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Survival of *Schistosoma mansoni* within the infected host requires the parasite to actively maintain its protective tegument. The components responsible for this maintenance are therefore attractive targets for immunoprophylaxis or chemotherapy. Here we report the molecular characterization of a 20.8-kDa tegumental antigen with sequence similarity to dynein light chains and tegumental associated antigens. A cDNA encoding the 20.8-kDa polypeptide contains an open reading frame of 181 amino acids and predicts an isoelectric point of 7.27. Expression of the 20.8-kDa antigen is developmentally regulated, with the highest concentration found in cercariae. Our data show that the 20.8-kDa polypeptide specifically interacts with a *S. mansoni* 10.4-kDa dynein light chain that we have previously described (Hoffmann, K. F., and Strand, M. (1996) *J. Biol. Chem.* 271, 26117–26123). Velocity sedimentation analysis of a parasite extract demonstrated that this 10.4-kDa dynein light chain and the 20.8-kDa polypeptide were present in a complex that sedimented at 4.4 Svedberg units. We have also shown by antibody cross-reactivity that a 20.8-kDa homolog of the *S. mansoni* antigen is present in *Schistosoma japonicum*, but not in *Schistosoma hematobium* or *Fasciola hepatica*. Because the 20.8-kDa polypeptide displays ideal characteristics of a potential vaccine candidate, including (i) expression in the tegument, (ii) significant divergence from mammalian brain cytoplasmic dynein, and (iii) a conserved homolog in *S. japonicum*, we are currently evaluating its immunoprophylactic efficacy.

More than 200 million people worldwide are affected by the disease schistosomiasis (2). The helminths responsible for this disease are unusual in that they inhabit the blood, are dioecious and digenetic, and possess a double layered outer membrane characteristic only of blood flukes. Survival of the parasite in the infected host's bloodstream depends upon the maintenance of its tegument and heptalaminate membrane. Several studies have demonstrated active repair of the hepta-

laminate membrane after damage, reviewed in Ref. 3, but the components that regulate and participate in this repair have not been elucidated. Knowing how this protective barrier is actively maintained is critical to understanding how schistosomes survive in the infected host. One approach toward studying the mechanisms that govern schistosome tegument maintenance is to identify the antigens involved. We have used monoclonal antibodies (mAbs)¹ raised against components of the tegument as tools to isolate and molecularly characterize the antigens responsible for these processes.

We have previously characterized the target antigen of one of these antibodies, mAb 709A2/2, and reported its molecular similarity to cytoplasmic dynein light chains (DLCs) from various organisms (1). Cytoplasmic DLCs have recently been implicated in embryonic *Drosophila* lethality (4) and inhibition of neuronal nitric-oxide synthase (5), strongly suggesting their importance in normal cellular homeostasis. Cytoplasmic dynein complexes (reviewed by Bloom (6), Vallee (7), Holzbaur *et al.* (8), and Vallee and Sheetz (9)) are large, macromolecular (containing heavy, intermediate, and light chains) motors involved in the movement of membranous organelles toward the minus ends of microtubules, as well as mitotic spindle positioning and elongation in yeast. Because it is not known how cytoplasmic dynein discriminates among various classes of membranous organelles or how it positions mitotic spindles, a number of research groups have been interested in molecularly characterizing the components of this complex and in identifying other interacting proteins as a step toward understanding the roles they play in dynein function and/or regulation.

Toward this end, we now present the molecular characterization of a 20.8-kDa polypeptide that we previously found to be coprecipitated with SmDLC by mAb 709A2/2 (1). The sequence encoding this antigen (Sm 20.8) is similar to those of the 8-kDa family of DLCs (of which SmDLC is a member), an 18-kDa calcium-binding outer arm DLC from *Chlamydomonas reinhardtii* (10), and a family of schistosome proteins termed tegumental associated antigens (11–14)). Members of this family all (i) have conservative calcium binding EF hand-like motifs, but none (including Sm 20.8) binds calcium experimentally; (ii) display developmentally regulated expression; and (iii) have as of yet no identified functions or significant sequence homologies.

Much effort has been devoted to the molecular and functional characterization of these putative cytoplasmic DLCs with the goal of understanding the role they play in dynein function. The results presented in this report underscore our continued

* This work was supported by National Institutes of Health Grant AI-19217. Parasite material used in this study was provided by NIAID, National Institutes of Health, Supply Contract AI-55270. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U87243.

‡ Supported by NIGMS, National Institutes of Health, Pharmacology Training Grant 5732GM07626.

§ To whom correspondence should be addressed: The Johns Hopkins University School of Medicine, Dept. of Pharmacology and Molecular Sciences, Center for Monoclonal Studies, 725 North Wolfe St., Baltimore, MD 21205. Tel.: 410-955-8477; Fax: 410-955-1894.

¹ The abbreviations used are: mAb, monoclonal antibody; pAb, polyclonal antibody; GST, glutathione S-transferase; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis; PIPES, 1,4-piperazinediethanesulfonic acid; NMS, normal mouse serum.

interest in determining the importance of molecules similar to DLCs for schistosome survival in the infected host. We believe that these findings may have broad applicability to the dynamics of dynein regulation and association in other systems.

EXPERIMENTAL PROCEDURES

Parasites—Parasite materials derived from *Schistosoma mansoni* (Puerto Rican strain) *Schistosoma japonicum*, *Schistosoma hematobium*, and *Fasciola hepatica* were used throughout this study. Adult worms were obtained as described previously (15). The schistosomular life stage was obtained by mechanical transformation as described by Dalton *et al.* (16). 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 (17) obtained as described by Oaks *et al.* (18). A monospecific mouse polyclonal antibody (pAb 20.8) was generated against the native 20.8-kDa polypeptide following the immunization protocol described by Cianfriglia *et al.* (19). The 20.8-kDa antigen was obtained from worm lysates by immunoaffinity chromatography using mAb 709A2/2 as outlined by Harlow and Lane (20), followed by SDS-PAGE separation. The Coomassie Blue-stained band corresponding to the 20.8-kDa polypeptide co-immunoprecipitated by mAb 709A2/2 was cut, emulsified with complete Freund's adjuvant, and used for immunization. A mouse polyclonal serum (pAb SmdLc-GST) was raised against the previously described SmdLc-glutathione *S*-transferase (GST) fusion protein (1) as follows. 50 µg of SmdLc-GST emulsified in complete Freund's adjuvant was used for primary intraperitoneal immunization on day 0, followed by boosting with SmdLc-GST in incomplete Freund's adjuvant on day 10.

cDNA Cloning—A cDNA clone encoding the 20.8-kDa antigen (clone 3A-1), the recombinant form termed Sm 20.8, was identified by expression screening, using standard methods (21), of an oligo(dT)-primed adult worm schistosome UNIZAP-XR (Stratagene) cDNA library. After three rounds of plaque purification with pAb 20.8, the phagemid containing clone 3A-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 748-bp *EcoRI/XhoI* restriction fragment was sequenced in both directions using the 7-deaza-dGTP Sequenase sequencing kit (U. S. Biochemical Corp.) and dideoxy chain termination (22), as well as by automated sequencing performed with a Li-Cor sequencer.

Computer Analysis—Analysis of sequence assembly and comparisons was performed using software developed by the Genetics Computer Group. Searches of the GenBank™ data base to identify sequences similar to Sm 20.8 were performed at the National Center for Biotechnology Information (NCBI) using the BLAST network server. Primary sequence motifs were identified using the PROSITE network server at the European Molecular Biological Laboratory (EMBL). Secondary structure predictions were made using MacVector® software. Sequence alignments were generated by the Multiple Sequence Alignment program developed at Washington University.

Northern Blot—Adult worm total RNA was isolated, electrophoresed, and capillary-transferred to a nylon membrane (Zeta-Probe, Bio-Rad) as described previously (23). Clone 3A-1 containing the 748-bp insert was linearized by *EcoRI*, transcribed by T3 RNA polymerase, and radiolabeled by incorporation of [α -³⁵S]dUTP (Amersham Corp., 800 Ci/mmol). The nylon membrane containing RNA was incubated for 8 h at 42 °C in prehybridization solution before the addition of the radiolabeled probe. Incubation with the radiolabeled probe continued overnight at 42 °C, after which the membrane was washed according to standard conditions before exposure to Amersham Hyperfilm-MP autoradiographic film.

Protein Sequencing—Protein samples submitted for sequencing were prepared by immunoaffinity purification over the mAb 709A2/2 affinity column. Adult worm lysates were passed over a mAb 709A2/2-coupled Protein G-agarose (Pharmacia Biotech Inc.) affinity column essentially as described by Harlow and Lane (20). The isolated 20.8-kDa antigen was fractionated by SDS-PAGE and transferred to a Bio-Rad microsequencing polyvinylidene difluoride membrane (Bio-Rad Laboratories) by electrophoresis at 70 V for 2 h. The 20.8-kDa polypeptide was subjected to *in situ* trypsin digestion, purification of the eluted peptides by reversed-phase chromatography, mass spectrophotometric analysis of the purified eluted peptides, and N-terminal amino acid sequencing at the Wistar Sequencing Facility by David Reim.

Solubilization of Proteins—Extracts of cercariae, 3-h schistosomula, and solubilized egg antigens were prepared according to the procedure

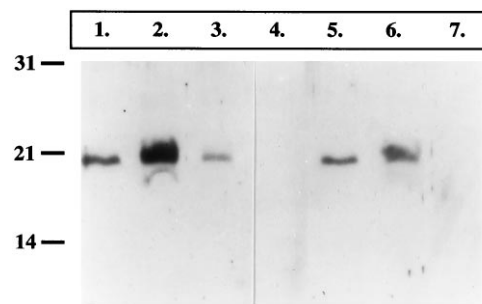


FIG. 1. *In vitro* protein interaction between SmdLc and native Sm 20.8. Native Sm 20.8 obtained from a 0.2% Triton X-100 *S. mansoni* worm extract specifically binds to SmdLc-GST but not to GST. Lanes 1, 4, and 5 (controls) represent electrophoresis of 4 µg of *S. mansoni* solubilized worm protein preparation. Lanes 2 and 6 represent native Sm 20.8 that failed to bind to SmdLc-GST (lane 2) and GST (lane 6). Lanes 3 and 7 represent native Sm 20.8 that did bind to SmdLc-GST (lane 3) but not to GST (lane 7). Lanes 1–3 and 5–7 were probed with pAb Sm 20.8, while lane 4 was probed with NMS. Molecular mass standards (in kilodaltons) are indicated to the left.

described by Hawn and Strand (15) in the presence of 1% Triton X-100. Adult worms were solubilized in disruption buffer (1.25 M Tris-HCl, pH 6.8, 0.7 M 2-mercaptoethanol, 0.002 M EDTA, 5% glycerol, 2% SDS, and 8 M urea) by sonication (10 s at maximum output in a cup cell disrupter, SmithKline Beecham) prior to SDS-PAGE.

Source of Recombinant Proteins—The recombinant SmdLc (1) used for *in vitro* protein-protein interaction assays was expressed in *Escherichia coli* DH 5α cells as a fusion protein with GST of *S. japonicum* and obtained as described by Soisson *et al.* (24). Recombinant GST was purified in exactly the same manner as recombinant SmdLc-GST.

In Vitro Sm 20.8-Sm DLC Protein Interaction—Ten micrograms of SmdLc-GST or GST were incubated overnight at 4 °C with 50 µl of glutathione conjugated to agarose beads (Sigma). The recombinant proteins conjugated to glutathione-agarose beads were then incubated with a 0.2% Triton X-100 adult tegumental membrane extract (Oaks *et al.* (18)) overnight at 4 °C to allow for protein-protein interactions. The beads were washed, and the 20.8-kDa polypeptide captured by SmdLc-GST was eluted into SDS-PAGE sample buffer and then detected by gel electrophoresis and Western blotting.

Velocity Sedimentation Analysis—Velocity sedimentation analysis of *S. mansoni* adult worm extracts was performed essentially as described by Echeverri *et al.* (25) and Schroer *et al.* (26). Briefly, ~200 worms were solubilized on ice in 2 ml of a homogenization buffer containing 35 mM PIPES, pH 7.4, 5 mM MgCl₂, 1 mM EGTA, 0.5 mM EDTA, pH 7.4, and 500 mM phenylmethylsulfonyl fluoride using a Dounce homogenizer and type A pestle. The extract was microcentrifuged for 15 min at 16,400 × *g* to remove any insoluble material. Sucrose gradients (4.8 ml, 5–20% in homogenization buffer) were prepared, and 200 µl of the soluble supernatant was carefully layered on top. The gradients were subjected to velocity sedimentation in a SW50.1 rotor (Beckman Instruments, Palo Alto, CA) at 26,500 rpm for 18 h at 4 °C, after which 250-µl fractions were collected. Sedimentation standards, which included thyroglobulin (669 kDa), catalase (232 kDa), aldolase (158 kDa), and chymotrypsinogen A (25 kDa), were diluted in homogenization buffer and subjected to velocity sedimentation with the worm extracts. Equal volumes of all fractions were analyzed by gel electrophoresis followed by Western blotting.

Gel Electrophoresis—Gel electrophoresis was carried out as described previously (27), with minor modifications. One-dimensional gels used for analysis of SmdLc or both SmdLc and Sm 20.8 were 10–20% (w/v) gradient polyacrylamide. One-dimensional gels used for analysis of Sm 20.8 alone were 15% (w/v) polyacrylamide.

Western Blot Analysis—Western blot analysis was carried out as described previously (28) with the following modifications: the primary pAb was used at a dilution of 1:5,000, and the secondary donkey anti-mouse horseradish peroxidase (Jackson Immunoresearch Laboratories) antibody was diluted 1:10,000.

RESULTS

Cloning of the cDNA Encoding Sm 20.8—We have previously reported the co-immunoprecipitation of an approximately 18.4-kDa protein (now designated Sm 20.8 on the basis of the mass predicted by primary sequence information; see below) with

SmDLC by mAb 709A2/2 (1). Confirmation of the specific protein-protein interaction between these molecules was sought using a different experimental condition (Fig. 1). The native Sm 20.8-kDa polypeptide interacted with the SmDLC-GST fusion protein (Fig. 1, lane 3) but not with recombinant GST (Fig. 1, lane 7). This result provides additional evidence for a specific association between SmDLC and Sm 20.8.

Because Sm 20.8 may represent an additional component of schistosome dynein, we wished to isolate the cDNA encoding this polypeptide. A mouse polyclonal serum against the 20.8-

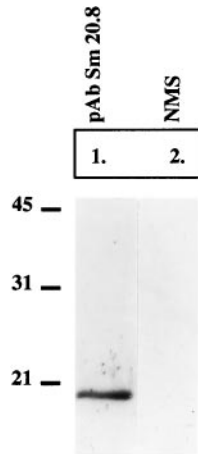


FIG. 2. **Recognition of native Sm 20.8 by pAb Sm 20.8.** The monospecificity of pAb Sm 20.8 is shown in a Western blot analysis of 10 μ g of *S. mansoni* solubilized worm antigen preparation. Lane 1, recognition of the 20.8-kDa polypeptide by pAb Sm 20.8; lane 2, negative control NMS. Molecular mass standards (in kilodaltons) are indicated to the left.

kDa polypeptide was generated and shown to be monospecific for an antigen of similar size by Western blot analysis of solubilized worm proteins (Fig. 2, lane 1). Because of the monospecificity of this serum, it was used to screen a UNIZAP-XR adult worm cDNA expression library from which a positive clone, 3A-1, was identified and sequenced. Clone 3A-1 contained an insert of 748 bp encoding an open reading frame of 181 amino acids (Fig. 3) predicting a polypeptide of 20.8 kDa and a pI of 7.27. A strong Kozak (29) consensus start site present at nucleotides 54–60 and a conserved poly(A)⁺ signal sequence (AATAAA) occur 127 bp upstream of the poly(A)⁺ tail. Two EF hand-like motifs were predicted for Sm 20.8 by the BLOCKS program algorithm (30), but neither of these sites was sufficient to bind calcium *in vitro* (data not shown). No potential post-translational modifications were predicted by PROSITE at EMBL for Sm 20.8. Native Sm 20.8 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 16 amino acids (positions 84–99, LHPDVVVIYEQLPLDR) contained within the open reading frame of clone 3A-1 (Fig. 3). *In vitro* transcription and translation of clone 3A-1 resulted in a polypeptide whose migration on SDS-PAGE was similar to that of the native Sm 20.8 polypeptide (data not shown). These two independent lines of evidence support the contention that the native protein and the polypeptide product of the open reading frame contained within clone 3A-1 are identical.

The 181-amino acid open reading frame contained within clone 3A-1 showed significant similarities to SmDLC as well as to a calcium-binding 18-kDa outer arm dynein light chain of *C. reinhardtii* (Fig. 4, A and B). Sm 20.8 displays a 53% sequence similarity to SmDLC (and other members of the 8-kDa DLC

1	CT	CAT	TAT	CAT	TAT	TAT	CAT	CAA	TAA	AGT	ACT	TTG	GAA	AAC
	AAA	ATA	TAG	GCA	AAA	ATG	GAA	CCA	TTT	GTA	CAA	GTA	TTC	TTT
1					M	E	P	F	V	Q	V	F	F	
10	GCT	ATT	GAT	AGA	GAT	GGA	ACT	GAA	ACT	ATC	ACA	GTA	GAA	GAA
	A	I	D	R	D	G	T	E	T	I	T	V	E	E
24	TTG	AAA	AAG	TAT	GTT	GCT	GAA	AAT	AAA	CTT	GAT	GAT	ATG	ATG
	L	K	K	Y	V	A	E	N	K	L	D	D	M	M
38	GTT	ACA	AAA	TGG	AAA	TCA	TTA	TTT	GAT	CCC	AAA	GGT	ACT	GGA
	V	T	K	W	K	S	L	F	D	P	K	G	T	G
52	AGA	ATA	ACA	TTT	AAA	ACT	TTT	TGT	GAT	GTA	TTG	GGT	TTG	AGT
	R	I	T	F	K	T	F	C	D	V	L	G	L	S
66	CCT	GCA	CAA	GCA	GTA	GCC	ATG	AAA	ACA	CAA	CAT	CAG	CAA	GCA
	P	A	Q	A	V	A	M	K	T	Q	H	Q	Q	A
80	TCT	TCA	TTG	AAA	CTT	CAT	CCA	GAT	GTT	GTT	GTA	ATC	TAT	GAA
	S	S	L	K	L	H	P	D	V	V	V	I	Y	E
94	CAA	CTT	CCA	TTA	GAT	AGA	CAA	GTC	GCT	ATT	AGT	AAT	AAA	GCA
	Q	L	P	L	D	R	Q	V	A	I	S	N	K	A
108	ATT	GAA	CTA	TTA	ACA	TCT	TCA	AAG	AAA	TTC	GAT	GAG	AAA	GAT
	I	E	L	L	T	S	S	K	K	F	D	E	K	D
122	CAA	GCT	GTT	CAA	TTG	AAA	CAA	TGG	TTA	GAT	ATC	ACT	TAT	GGT
	Q	A	V	Q	L	K	Q	W	L	D	I	T	Y	G
136	AAA	GCA	TGG	CAC	ATT	GTC	ATA	GTT	AAA	GGA	TCA	TTT	TGG	AGT
	K	A	W	H	I	V	I	V	K	G	S	F	W	S
150	AGT	TAT	TCA	CAT	AGT	GCT	AAT	AAA	TGT	TTT	ATA	TTT	CGT	GTA
	S	Y	S	H	S	A	N	K	C	F	I	F	R	V
164	CGT	GAT	GTA	TCT	TAT	CTT	GTA	TGG	AGA	ACA	CCA	GAT	GAA	GAA
	R	D	V	S	Y	L	V	W	R	T	P	D	E	E
178	ATA	ACC	AGT	GCA	TAA	ATA	AAT	AAT	TAG	ATA	ACT	TGA	AGA	GAA
	I	T	S	A	.	.	.	.	.	.	.	.	.	.
	ACA	AAC	AAT	TTA	AAA	AGA	TAG	TTA	ACA	GTA	AAT	GCT	TTG	TTA
	AAA	CAT	ATG	AAA	ACC	AAT	ATT	ATT	ATG	ATA	AAG	ATT	AAA	TAC
	AGG	GAT	AAT	TAC	TTT	TTC	TTA	AAA	AAA	AAA	AAA	AA	748	

FIG. 3. **Sequence of Sm 20.8.** Nucleic acid and deduced amino acid sequence of clone 3A-1 encoding Sm 20.8. **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 a peptide sequence obtained from the purified native Sm 20.8 (LHPTVVVIYEQLPLDR) polypeptide.

A

SmDLC (35-89) EKDI AANIKKDFDRKYHPTWHCI VGR-HF-GSYVTHETHNFIYFY LDDRAFLLFKSGD-----
 Sm 20.8 (120-184) EKDOAVOLKQWLDITYGKAWHIVIVKGSFWSSYSHSANKCFIFR-V-RDVSYLWVRTPDEEITSA

B

1 75
 Sm 20.8 MEPFVQVFFAIDRDGTETITVEELKKYVAENK LDDMMVTKWKS LFDPKGTGRITFKTFCDVLGLSPAQAVAMKTO
 Cr 18 MAAKVDIE-----AANLPN-LTDEEMD--MCRKAFAMEDKDGSGTIDTKE LRTALS-ALGQNPT EEDM

76 152
 Sm 20.8 HQQASS LK LHPDVVVIYEOLPLDROVAISNKAIELLTSSKKFDEKDOAVOLKQWLDITYGKAWHIVIVK-GSFW
 Cr 18 FVMISQVDODGSRCTEFKEFV--RVIOINKQM-----SAKDADEADTLDAFVA-EGGNLDKTRILVDKLRSLC

153 189
 Sm 20.8 SSYSHSANKCFIFRV--RDV-SYLWVRTPDEEITSA
 Cr 18 EEFELTYNVDRLEVKDADRD LNGFLSYDEFRALLS--

C

1 75
 Sm 20.8 -----MEPFVQVFFAIDRDGTETITVEELKKYVAENK LDDMMVTKWKS LFDPKGTGRITFKTFCDVLGLS
 Sj 22 MATTEYRLSLMEQIRAFIEIDKDNNEIDKQELTKYCOQONMDMKQIDPWIA RFDTKDGKVGLEEF CRGFG LK
 Sm 22 MA-TETKLSOMEEFIRAFLEIDADSNEMIDKQELIKYCQYRLDMKLIDPWIA RFDTKDNKISIEEF CRGFG LK
 Sm 21.7 MD-----SPMEKFIQTYLTLLRDGDET VETSKLS ES CRKEKLD MKQVNEWI ALFDV D K D Q K I T F E E F C R G F G L K

76 150
 Sm 20.8 PAQAVAMKTO---HQQASS LK LHPDVVVIYEOLPLDROVAISNKAIELLTSSKKFDEKDOAV--OLKQWLDITYG
 Sj 22 VWEVRRKEELKRDKEGKVSTLPLDIQIIAATMSKAKQYNI CCKFEKELDKTSRTGDEVRAVANDLKAF LDSEYG
 Sm 22 VSEIRREKDELKKERDGGKFKLPNIEIIAATMSKTQOYEICCOFKEYVDNTRSRTGNDMRE VANKMKSLLDNTYG
 Sm 21.7 QNEMRIERNH IKT VQSGREQSLPEGVSI IASTMPKPKQVEVTLQFKDIYNEVKKDPDMNKVY--KTFKSE LERRYG

151 197
 Sm 20.8 KAWHIVIVKGSFWSSYSHSANKCFIFRV RDVSYLWVRTPDEEITSA
 Sj 22 RVWQVILLTGSYWMNFSHEPFLSMQFKYSNYVCLLWRT PSS-----
 Sm 22 RVWQVLLTGSYWMNFSHEPFLSIOFKYNNYVCLAWRT PSE-----
 Sm 21.7 RVWQVNAVTHSYWASF SHEPFSIQFOYDNKIL L W R T P S N-----

FIG. 4. Alignment of Sm 20.8 with SmDLC, *C. reinhardtii* 18-kDa DLC, and members of the tegumental associated antigen family. A, alignment of Sm 20.8 (residues 120–184) with SmDLC (residues 35–89). B, alignment of Sm 20.8 with the *C. reinhardtii* 18-kDa DLC. C, alignment of Sm 20.8 with members of the tegumental associated antigen family including Sj 22, Sm 22, and Sm 21.7. The shaded boxes represent residues conserved between two or more family members. The alignment was generated by the multiple sequence alignment program developed at Washington University.

family not shown), but this similarity is limited to the carboxyl termini. This degree of conservation is consistent with other reports of two molecularly characterized DLCs from the same organism, particularly the 8- and 11-kDa DLCs from *C. reinhardtii* that show a 41.8% identity. Sm 20.8 is also 44% similar to the *C. reinhardtii* 18-kDa outer arm dynein light chain; this similarity extends the full length of both polypeptides. This lower degree of similarity is not unexpected because the overall sequence similarity between cytoplasmic and axonemal dynein heavy chains can be as low as 27% (7). In addition, the 74-kDa cytoplasmic dynein intermediate chain is only 47.7% similar to the 70-kDa intermediate chain of *C. reinhardtii* axonemal dynein (31), further supporting the notion that Sm 20.8's sequence similarity to the 18-kDa *C. reinhardtii* molecule is significant.

Despite the sequence similarity of Sm 20.8 to the DLCs, the smallest Poisson probability distribution ($P(n)$; the probability of the match occurring solely by chance) for Sm 20.8 is 4.7×10^{-31} to 1.9×10^{-20} (calculated by the BLAST server at NCBI using the default matrix). These scores indicate the similarity of Sm 20.8 to a group of previously characterized polypeptides from schistosomes termed tegumental associated antigens (11–14). Comparison of the alignment between Sm 20.8 and this group of proteins (Fig. 4C) indicates that 17% of the residues are invariant among members, but several instances of conservative substitutions increase the overall sequence similarity to approximately 66%.

Secondary structure predictions for Sm 20.8 made with MacVector® software indicate that this polypeptide is most

likely α -helical with a carboxyl-terminal extended β -sheet. Sm 20.8 lacks both a definable transmembrane-spanning sequence and a signal sequence, suggesting that this polypeptide probably is not a membrane or secreted protein. Furthermore, the native 20.8-kDa polypeptide associates with the aqueous phase in a Triton X-114 phase separation analysis (data not shown), strongly suggesting that the antigen is hydrophilic and not hydrophobic.

The size of the mRNA encoding Sm 20.8 was determined to be ~ 740 bp by Northern blot analysis (Fig. 5). The width of the band observed made it impossible to determine whether there was more than one message encoding Sm 20.8 or, alternatively, whether the Sm 20.8 probe hybridized to mRNA messages encoding other members of the tegumental associated antigen family. In either case, the signal observed was the correct size for the message encoding Sm 20.8, suggesting that the full-length cDNA had been isolated.

Developmental Expression of Sm 20.8 and SmDLC—The expression pattern of Sm 20.8 was examined in four life stages of *S. mansoni* (miracidia, cercariae, 3-h mechanically transformed schistosomula, and adults) by Western blot analysis (Fig. 6B). Sm 20.8 was most abundant in cercariae (Fig. 6B, lane 2), with decreasing levels being detected in the 3-h mechanically transformed schistosomula (Fig. 6B, lane 3) and adults (Fig. 6B, lane 4). There appeared to be no detectable expression of Sm 20.8 in the miracidial stage (Fig. 6B, lane 1) at the level and sensitivity of this assay. Sm 20.8 migrated faster under SDS-PAGE in the cercaria and schistosomula stages than the adult stage (Fig. 6B, compare lanes 2, 3, and 4),

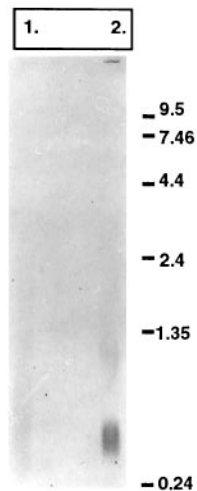


FIG. 5. **Northern blot analysis.** Shown is a Northern blot of total RNA isolated from adult worms and uninfected mouse spleen. Lane 2, a broad message of ~ 740 bp is observed for Sm 20.8; lane 1, no reactivity occurred with the mouse spleen RNA. Molecular size standards (in kilobase pairs) are indicated to the right.

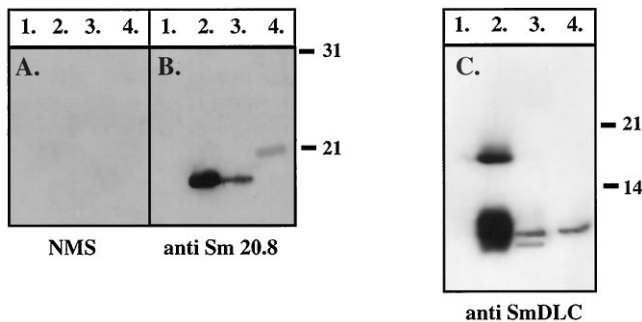


FIG. 6. **Developmental expression of Sm 20.8 and SmDLC.** The developmental expression of Sm 20.8 and SmDLC was assessed by Western blot analysis of solubilized parasite antigens from miracidia (lane 1), cercariae (lane 2), 3-h mechanically transformed schistosomula (lane 3), and adults (lane 4). Approximately 25 μ g of parasite antigen were loaded in each lane. A, reactivity of NMS with each of the four parasite extracts. B, Reactivity of pAb Sm 20.8 with the four parasite extracts. C, reactivity of pAb SmDLC-GST with the four parasite extracts. Molecular mass standards (in kilodaltons) are indicated to the right.

suggesting a mass difference that could be attributable to a post-translational modification not predicted by PROSITE. Normal mouse serum (NMS) did not react with any specific proteins (Fig. 6A) in any of the life stages examined.

Because of the high level of Sm 20.8 expression in cercariae as well as the observation that Sm 20.8 and SmDLC interact (Fig. 1), we reexamined the developmental expression of SmDLC (Fig. 6C). Previously, we had been unable to detect SmDLC in either miracidia or cercariae using mAb 709A2/2 in indirect immunofluorescence or enzyme-linked immunosorbent assays (1). Use of the newly generated reagent, pAb SmDLC-GST, in a Western blot assay resulted in the identification of SmDLC in cercaria (Fig. 6C, lane 2) but not in miracidia (Fig. 6C, lane 1). As was true for Sm 20.8, SmDLC was most abundant in cercariae with decreasing levels of expression detected in schistosomula (Fig. 6C, lane 3) and adults (Fig. 6C, lane 4). There also appeared to be a larger molecular mass polypeptide (17 kDa) that was recognized in the cercariae by pAb SmDLC-GST but not present in any other life stage (Fig. 6C, lane 2). The lower molecular mass polypeptide recognized in the schistosomula stage (Fig. 6C, lane 3) was not always observed.

Identification of Possible Sm 20.8/SmDLC Homologs in

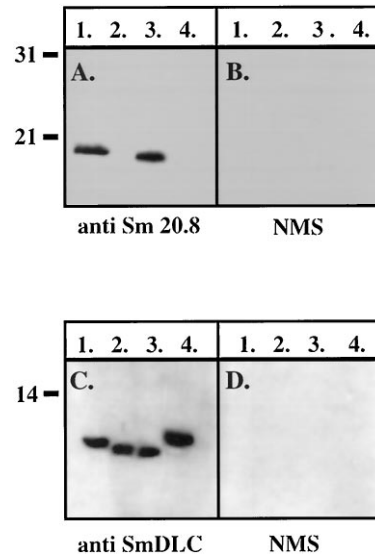


FIG. 7. **Identification of possible Sm 20.8 and SmDLC homologs in other trematodes.** To identify possible Sm 20.8 and SmDLC homologs in other trematodes, Western blot analysis was performed on extracts of *S. hematobium* (lane 2), *S. japonicum* (lane 3), and *F. hepatica* (lane 4) using pAb Sm 20.8 (A) and pAb SmDLC-GST (C). *S. mansoni* extract (lane 1) was included as a positive control in all experiments. Panels B and D were probed with NMS as a negative control. Four times as much *S. japonicum* extract (100 μ g) was used in all experiments to equalize signal observed between trematodes. Molecular mass standards (in kilodaltons) are indicated at the left.

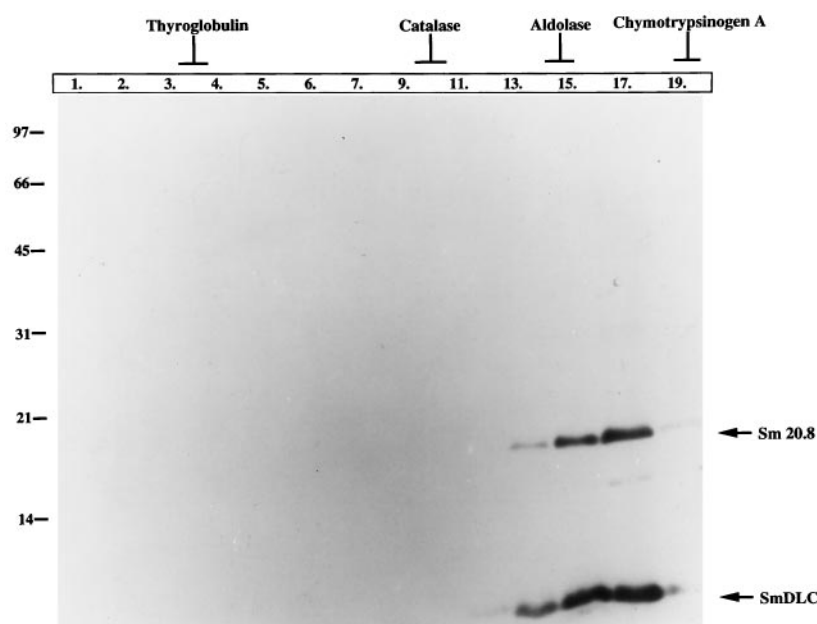
Other Trematodes—Because tegumental associated antigens have been identified in other schistosome species (12) and 8-kDa DLCs have been found in almost every eukaryotic organism, we wanted to determine whether potential Sm 20.8 and SmDLC homologs existed in adults of *S. japonicum*, *S. hematobium*, and *F. hepatica* by Western blot analysis (Fig. 7). A specific polypeptide of approximately 20.8-kDa from *S. japonicum* was cross-reactive with the polyclonal Sm 20.8 serum, suggesting that this schistosome species does contain a potential Sm 20.8 homolog (Fig. 7A, lane 3). Neither *S. hematobium* (Fig. 7A, lane 2) nor *F. hepatica* (Fig. 7A, lane 4) extracts contained a similar molecular mass antigen that was cross-reactive with the polyclonal Sm 20.8 serum. In contrast, SmDLC homologs appeared to exist in all three trematodes examined, with only minor differences in molecular mass observed (Fig. 7C). NMS did not recognize any specific polypeptides in any of the trematode extracts (Fig. 7, B and D) investigated.

Velocity Sedimentation—Cytoplasmic dynein, by definition, behaves as an 18–22 S macromolecular complex during velocity sedimentation analysis (7). To address whether Sm 20.8 and SmDLC are associated with a similar size complex in schistosomes, velocity sedimentation of a soluble worm extract through a 5–20% sucrose gradient was performed (Fig. 8). Sm 20.8 and SmDLC co-sedimented, but these polypeptides were most abundant in a fraction that is consistent with a 90-kDa complex rather than a 669-kDa complex. Co-sedimentation of Sm 20.8 and SmDLC as a complex of 90-kDa was also observed in the presence of 1% Nonidet P-40, a condition tested to increase the solubilization of worm carcasses (data not shown).

DISCUSSION

We describe in this report the molecular characterization of a *S. mansoni* protein that shares sequence similarity with dynein light chains and tegumental associated antigens. The association of this polypeptide with SmDLC (1) suggests that a cytoplasmic dynein complex exists in schistosomes. The observation that recombinant SmDLC-GST, but not GST, was able

FIG. 8. **Velocity sedimentation analysis of soluble *S. mansoni* proteins.** A 5–20% sucrose gradient was used to fractionate soluble schistosome proteins during velocity sedimentation. Sm 20.8 and SmDLC co-sediment as a 90-kDa macromolecular complex at fraction 17. Velocity sedimentation standards are thyroglobulin (669 kDa), catalase (232 kDa), aldolase (158 kDa), and chymotrypsinogen A (25 kDa). Molecular mass standards during SDS-PAGE (in kilodaltons) are indicated at the left.



to capture a 20.8-kDa polypeptide from worm extracts in an *in vitro* assay (Fig. 1) confirmed the *in vivo* association of SmDLC and Sm 20.8 that we had previously observed by co-immunoprecipitation using either mAb 709A2/2 (1) or pAb 20.8 (data not shown). Since we have shown in Fig. 1 that the pAb 20.8 does not cross-react with SmDLC, the appearance of SmDLC in the co-immunoprecipitation experiment with pAb 20.8 indicates a specific interaction between these two components. The interaction displayed between Sm 20.8 and SmDLC is similar to that reported by King *et al.* (32), who have shown that there is an association of a 9-kDa light chain with a 19-kDa light chain in outer arm dynein isolated from rainbow trout spermatozoa. The sequence similarity between Sm 20.8 and this 19-kDa dynein light chain is not known at this time, since the trout protein has not been molecularly characterized. However, Sm 20.8 is 44% similar to an 18-kDa outer arm dynein light chain from *C. reinhardtii* (10). This degree of sequence similarity is in the same range as that reported between axonemal and cytoplasmic dynein homologs (7, 31) and, thus, would classify Sm 20.8 as a member of the dynein light chain family of proteins.

Sm 20.8 also displays sequence similarity to the family of 8-kDa DLCs (of which SmDLC is a member) that have recently been reported to occur in most eukaryotic organisms. Although this sequence similarity is concentrated at the carboxyl termini, the conservation between Sm 20.8 and SmDLC is 55% (Fig. 4A). It is possible that the sequence conservation over this restricted region represents a motif that indicates a shared function, whereas the more divergent amino termini encode sequences unique to the specific functions of the two proteins. Since it has been shown that there are 8- and 20-kDa light chains associated with the base of cytoplasmic dynein (32, 33) and this is the base that is thought to interact with organelles (7, 9), it is not unreasonable to suggest that a common sequence motif may be an essential aspect of organelle recognition.

Sm 20.8 is also highly similar to the members of a family of tegumental associated antigens previously described (11–14) (Fig. 4C). Although it is not clear what role these proteins play in the tegument of schistosomes, some investigators believe that they help to maintain this organ (11). Because all of the other members of the family of tegumental associated antigens are known to undergo developmental regulation (11–14), we also examined the developmental expression pattern of Sm

20.8. Sm 20.8 was approximately 5 times more abundant in the cercariae than in the 3-h mechanically transformed schistosomula and adults (Fig. 6B). This result is similar to the developmental expression pattern that has been observed for another tegumental associated antigen, Sm 22, a potential schistosome vaccine candidate (12, 34). Like Sm 22, Sm 20.8 was not detected in the developing miracidia, suggesting that both antigens may be unimportant in penetration of the intermediate host.

Taking into consideration the developmental expression pattern seen for Sm 20.8, we reexamined that of SmDLC (Fig. 6C). Previously we had been unable to detect SmDLC in miracidia and cercariae using mAb 709A2/2 (1). This mAb did not react in Western blot, whereas the newly generated pAb against the recombinant SmDLC did. This pAb indicated a pattern of SmDLC expression that was identical to that observed for Sm 20.8; however, approximately 10 times more protein was present in the cercariae than in the 3-h mechanically transformed schistosomula or adult worms. These results strongly suggest that the up-regulation of SmDLC and Sm 20.8 occurs at the most likely time and place at which the presence of cytoplasmic dynein would be essential for the parasite's continued survival. During the developmental switch from cercaria to schistosomulum, the parasite is actively synthesizing the components necessary for the dynamic transformation of the tegument from a trilaminar to a heptalaminar membrane, a change that coincides with definitive host infection. The apparently diminished expression of Sm 20.8 and SmDLC after mechanical transformation of the cercariae may represent the steady state level of each protein in the more developed schistosomulum. The 17-kDa polypeptide observed in the cercariae extract (Fig. 6C, lane 2) probably represents a protein that contains a conserved epitope cross-reactive with pAb SmDLC-GST. This cross-reactive protein may be expressed at a higher level in cercariae than in schistosomula and adults and, therefore, was not observed in these more developed life-stages by Western blot analysis. The smaller molecular mass polypeptide seen in the schistosomulum stage was not always observed, but it probably represents the previously described 7.6-kDa DLC that is recognized by mAb 709A2/2 (1).

The identification of a possible Sm 20.8 homolog in *S. japonicum*, but not in *S. hematobium* or *F. hepatica*, is intriguing (Fig. 7A). It is possible that the closely related African schisto-

some, *S. hematobium*, contains other as yet uncharacterized tegumental associated antigens but lacks one similar to Sm 20.8. Since pAb Sm 20.8 cross-reacted with a protein of similar mass in the more distantly related Asian schistosome, *S. japonicum*, this explanation seems likely. The apparent lack of pAb 20.8 cross-reactivity in *F. hepatica* also suggests that this trematode either does not contain a Sm 20.8 homolog or contains a significantly divergent form of the antigen. In contrast, pAb SmDLC-GST cross-reacted with all three trematode species examined (Fig. 7C), further demonstrating the ubiquity and conservation of this DLC in parasitic trematodes.

The co-sedimentation of Sm 20.8 and SmDLC during velocity sedimentation (Fig. 8) confirmed the *in vitro* interaction observed for SmDLC-GST and native Sm 20.8 (Fig. 1). However, these proteins were detected in a fraction corresponding to ~4.4 S and not the 18–22 S that is typically observed for cytoplasmic dynein (7). There are several possible explanations for this result: (i) Sm 20.8 and SmDLC associate with multiple copies of themselves or other proteins, and this complex sediments at a fraction with an apparent molecular mass of 90 kDa; (ii) the conditions used to solubilize the worm carcasses were not amenable to isolation of intact schistosome dynein, and only the portion containing Sm 20.8 and SmDLC or these two molecules and other unidentified components sedimented at a position consistent with a mass of 90 kDa; (iii) schistosome dynein is not as large a complex as are other reported dyneins; or (iv) these dynein complexes undergo developmental regulation in schistosomes. We are currently in the process of addressing these questions by attempting to isolate intact cytoplasmic dynein from schistosome adult worms and cercaria using microtubule affinity columns and velocity sedimentation.

We do have preliminary evidence that pAb SmDLC-GST weakly recognizes an ~8-kDa component in purified cytoplasmic dynein preparations from bovine brain extracts (data not shown). However, the schistosome pAb 20.8 reagent failed to recognize a 20.8-kDa polypeptide from the same bovine brain cytoplasmic dynein complex (data not shown). It is possible that the cross-reactivity of this antiserum is not very high. An alternative explanation for this finding is that the 20.8-kDa DLC is less abundant in bovine brain cytoplasmic dynein than is the 8-kDa DLC. This second possibility seems more likely because the stoichiometry of the 8-kDa DLC and the 20-kDa DLC in bovine brain was estimated to be 10:1 by King *et al.* (33). The ratio of SmDLC to Sm 20.8 in adult worms is in good agreement with this calculation. Therefore, based on the weak reactivity of pAb SmDLC and bovine brain cytoplasmic dynein, it is not unexpected that pAb 20.8 fails to recognize a cross-reactive antigen from the same preparation.

We report here that a 20.8-kDa *S. mansoni* tegumental antigen may be related to the growing family of DLCs. The evidence supporting this statement includes its sequence similarity to two characterized DLCs (SmDLC and the *C. reinhardtii* 18-kDa DLC) and the association of Sm 20.8 with SmDLC. However, it is also equally possible that these two polypeptides may be associated with macromolecular complexes other than cytoplasmic dynein, as our velocity sedimentation analysis suggests. It has been reported that a rat protein with sequence similarity to the 8-kDa family of DLCs (SmDLC being a member of this family) has potent neuronal nitric-oxide synthase-inhibitory activity (5); this protein, designated PIN (protein inhibitor of nNOS), exerts its inhibitory activity alone, and not as part of a dynein complex. This observation supports the association of DLCs with other, non-dynein-like macromolecu-

lar structures.

Because Sm 20.8 is related to a tegumental associated antigen that currently is being considered as a vaccine candidate against schistosomiasis (12, 34), we are also actively exploring the immunoprophylactic efficacy of this polypeptide. The lack of cross-reactivity observed between pAb Sm 20.8 and bovine brain dynein makes this an attractive vaccine candidate. Further biochemical and immunological studies of Sm 20.8 and SmDLC should expand our understanding of the components that are involved in schistosome tegument maintenance.

Acknowledgments—We thank Alvin M. George for excellent technical assistance in all aspects of this work. *F. hepatica* flukes were graciously provided by Drs. Jack Malone and Stan Zukowski. We also thank Drs. Trina Schroer, James Bingham, and Steven King (Department of Biology, The Johns Hopkins University) for providing us with the purified bovine brain cytoplasmic dynein. We thank Dr. Deborah McClellan for excellent editorial assistance.

Note Added in Proof—Through personal communication, Drs. Amr M. Karim and Philip T. LoVerde have informed us that they have identified an antigen with the same sequence as the one reported in this manuscript.

REFERENCES

- Hoffmann, K. F., and Strand, M. (1996) *J. Biol. Chem.* **271**, 26117–26123
- Berquist, N. R. (1995) *Parasitol. Today* **11**, 191–194
- Hockley, D. J., and McClaren, D. J. (1973) *Int. J. Parasitol.* **3**, 13–25
- Dick, T., Ray, K., Salz, H. K., and Chia, W. (1996) *Mol. Cell. Biol.* **16**, 1966–1977
- Jaffrey, S. R., and Snyder, S. H. (1996) *Science* **274**, 774–777
- Bloom, G. S. (1992) *Curr. Opin. Cell Biol.* **4**, 66–73
- Vallee, R. B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 8769–8772
- Holzbaumer, 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
- Vallee, R. B., and Sheetz, M. P. (1996) *Science* **271**, 1539–1544
- King, S. M., and Patel-King, R. S. (1995) *J. Cell Sci.* **108**, 3757–3764
- Stein, L. D., and David, J. R. (1986) *Mol. Biol. Parasitol.* **20**, 253–264
- Jeffs, S. A., Hagan, P., Allen, R., Correa-Oliveira, R., Smithers, S. R., and Simpson, A. J. R. (1991) *Mol. Biol. Parasitol.* **46**, 159–168
- Francis, P., and Bickle, Q. (1992) *Mol. Biol. Parasitol.* **50**, 215–224
- Waine, G. J., Becker, M., Scott, J. C., Kalinna, B., Yang, W., and McManus, D. P. (1994) *Gene (Amst.)* **142**, 259–263
- Hawn, T. R., and Strand, M. (1994) *J. Biol. Chem.* **269**, 20083–20089
- Dalton, J. P., Strand, M., Mangold, B. L., and Dean, D. A. (1986) *J. Immunol.* **136**, 4689–4694
- Lewis, S. A. (1988) *Schistosoma mansoni: Characterization of Proteins and Immunogens Released by Adult Parasites*, Ph.D. dissertation, The Johns Hopkins University
- Oaks, J. A., Cain, G. D., Mower, D. A., and Raj, R. K. (1981) *J. Parasitol.* **67**, 761–775
- Cianfriglia, M., Armellini, D., Massone, A., and Mariani, M. (1983) *Hybridoma* **2**, 451–457
- Harlow, E., and Lane, D. (1989) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 5463–5467
- Andresen, K., Tom, T. D., and Strand, M. (1991) *J. Biol. Chem.* **266**, 15085–15090
- Soisson, L. M., Masterson, C. P., Tom, T. D., McNally, M. T., Lowell, G. H., and Strand, M. (1992) *J. Immunol.* **149**, 3612–20
- Echeverri, C. J., Paschal, B. M., Vaughan, K. T., and Vallee, R. B. (1996) *J. Cell Biol.* **132**, 617–633
- Schroer, T. A., Steuer, E. R., and Sheetz, M. P. (1989) *Cell* **56**, 937–946
- Norden, A. P., and Strand, M. (1985) *Am. J. Trop. Med. Hyg.* **34**, 495–507
- Hawn, T. R., Tom, T. D., and Strand, M. (1993) *J. Biol. Chem.* **268**, 7692–7698
- Kozak, M. (1984) *Nucleic Acids Res.* **12**, 857–872
- Henikoff, S., and Henikoff, J. G. (1994) *Genomics* **19**, 97–107
- Paschal, B. M., Mikami, A., Pfister, K. K., and Vallee, R. B. (1992) *J. Cell Sci.* **118**, 1133–1143
- King, S. M., Gatti, J.-L., Moss, A. G., and Witman, G. B. (1990) *Cell Motil. Cytoskel.* **16**, 266–278
- King, S. M., Barbarese, E., Dillman, J. F., III, Patel-King, R. S., Carson, J. H., and Pfister, K. K. (1996) *J. Biol. Chem.* **271**, 19358–19366
- Webster, M., Fulford, A. J. C., Braun, G., Ouma, J. H., Kariuki, H. C., Havercroft, J. C., Gachuihi, K., Sturrock, R. F., Butterworth, A. E., and Dunne, D. (1996) *Infect. Immun.* **64**, 4042–4046