*Proc. Natl. Acad. Sci. USA*
Vol. 83, pp. 5544–5548, August 1986
Developmental Biology

# Characterization of a female-specific cDNA derived from a developmentally regulated mRNA in the human blood fluke *Schistosoma mansoni*

(subtractive hybridization/female development/DNA sequence/egg shell protein/gene expression)

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*Communicated by William Trager, April 11, 1986*

ABSTRACT **We have isolated and characterized a cDNA clone that is derived from a developmentally regulated mRNA found only in mature female schistosomes. The mRNA is approximately 950 nucleotides in length and is not detectable in immature female schistosomes isolated from single-sex infections, in male worms, or in eggs. During normal bisexual infections, the mRNA species is first detected 28 days after infection (the time of worm pairing) and increases to a high level 35 days after infection, coinciding with the start of egg production. The nucleotide sequence of the cDNA shows two large open reading frames in the coding strand. Several features of the clone, including the deduced sequence of the polypeptide encoded by one of the reading frames, suggest a relationship to the silk moth chorion (egg shell) gene family. The isolation of this clone provides us with a probe for further studies of female schistosome development and is a first step toward a detailed understanding of this process at the molecular level.**

Schistosomes are multicellular parasites whose life cycle includes both human and snail hosts. *Schistosoma mansoni*, as the other species that infect humans, inhabits the bloodstream of man. Infection begins when cercariae penetrate unbroken skin of humans who are in contact with fresh water containing *Schistosoma*. Once the skin is penetrated, the transformed parasite, termed a schistosomule, remains in the interstitial tissue for 2 days, then enters the circulation, and migrates to the lungs. By day 7–14 the parasites leave the lungs and migrate to the liver where male and female worms mate (approximately day 28), and *encopula* move to their final niche in the mesenteries. Egg laying commences about day 35 (1).

Unlike most trematode parasites, schistosomes are dioecious. *S. mansoni* has 8 pairs of chromosomes, and the female schistosome is heterogametic (2). Sex is determined in the zygote, which develops into an embryonated egg.

Earlier studies dealing with female maturation and development demonstrated that the presence of the male worm is necessary for full growth and complete sexual maturation of the female (3, 4). Female worms obtained from single-sex infections are stunted and not sexually developed (3, 5), although male worms from single-sex infections appear normal (6, 7).

Moreover, Clough (8) showed that male worms are necessary to maintain, as well as to initiate, female maturation and fecundity. When sexually mature female worms are removed from their male partners, they stop laying eggs and regress to a condition morphologically resembling immature female parasites, until they are reunited with mature male worms. The stimulus from the male schistosome responsible for female growth, maturation, and fecundity is independent of sperm transfer (6, 7, 9), but its exact nature is unresolved (4, 10–12).

The unusual interplay between male and female schistosomes in the regulation of female development provides a unique system to study sexual differentiation. Moreover, since the production of eggs by mature worm pairs (300–1000 per day) is responsible for most of the pathology in schistosomiasis, an understanding of schistosome female maturation and fecundity could bring about innovative modes of disease control.

This study presents our initial work in the definition of the molecular events involved in the maturation and development of the mature female schistosome.

## MATERIALS AND METHODS

**Parasites.** For bisexual infections hamsters were exposed to male and female cercariae of *S. mansoni* (NMRI) obtained from previously infected snails. For single-sex infections, hamsters were exposed to cercariae obtained from snails (*Biomphalaria glabrata*) previously exposed to a single miracidium. As sex has already been determined in the miracidium, the cercariae that are produced in the snail by asexual reproduction are all of the same genotype. Schistosome worms were obtained from infected hamsters by perfusing the hepatic portal system (13). To obtain either male or female adult worms from bisexual infections, worm pairs were placed in RPMI 1640 and gently Vortex mixed. The dislodged worms were manually separated according to sex under a stereomicroscope.

**RNA Isolation.** Schistosome worm and egg RNAs were prepared as described (14). For RNA dot blots an equal amount of total cell RNA from each worm preparation was denatured in 50% (vol/vol; deionized) formamide, 6% (vol/vol) formaldehyde, 20 mM sodium phosphate (pH 6.5) for 5 min at 60°C and bound to nitrocellulose. Filters were prehybridized and hybridized according to standard procedures (15). For RNA gel blots, total cell RNA was denatured in 50% (vol/vol) formamide, 6% (vol/vol) formaldehyde, 1× Mops buffer (20 mM Mops, sodium salt, 5 mM sodium acetate, 1 mM sodium EDTA, pH adjusted to 7.0 with acetic acid) for 15 min at 60°C. RNA was then size-separated on a 1.4% agarose gel containing 6% (vol/vol) formaldehyde and transferred to a GeneScreen (New England Nuclear) membrane by capillary blot procedure with 0.025 M phosphate buffer (pH 6.5) as described by the supplier. The hybridization procedure using dextran sulfate (method I) described by the supplier was followed.

**DNA Techniques.** Schistosome worm DNA was prepared as described (14). Colony hybridization and preparation of plasmid DNAs and DNA blots were by standard procedures

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Abbreviations: ss, single stranded; bp, base pair(s).

(15). Details of hybridization conditions were as described in van Keulen *et al.* (14).

To prepare a "mature female-specific" single-stranded (ss) cDNA probe, the procedure described by Sargent and Dawid (16) with minor modifications was followed. The first strand of ss cDNA was synthesized by starting with 2 $\mu$g of poly(A)$^+$ RNA isolated from *S. mansoni* mature female worms obtained from bisexual infections. The cDNA was labeled to a high specific activity ($2 \times 10^9$ cpm/$\mu$g of DNA) using [$\alpha$-$^{32}$P]dCTP. Labeled cDNA was mixed with a 20 molar excess (40 $\mu$g) of male poly(A)$^+$ RNA. The male schistosomes used to make RNA were obtained from animals with unisexual infections to be certain that there was no contamination of RNA sequences from female schistosomes. Hybridization was conducted at an RNA concentration of 3.8 mg/ml, in 1 M NaCl at 65°C to an equivalent $R_0t$ of approximately 4000 mol per liter per sec. At this concentration of RNA, to achieve a $R_0t$ of 4000, hybridization proceeded for 36 hr. The hybridized mixture was then applied to hydroxylapatite column at 60°C, and ss cDNA was eluted with 0.12 M $NaH_2PO_4$, pH 6.8.

For nucleotide sequencing both the dideoxy sequencing method of Sanger *et al.* (17) in M13mp19 bacteriophage (18) and the Maxam and Gilbert chemical sequencing method (19) were used.

The Messing computer programs were used to organize and analyze the data (18). The Protein Identification Resource (PIR) of the National Biomedical Research Foundation (Georgetown University Medical Center)‡ was accessed to identify homologies of the deduced amino acid sequence of clone pSMf 61-46 with other known protein sequences.

## RESULTS

**Preparation and Assessment of a Mature Female-Specific ss cDNA Probe.** To create a probe specific for mRNA sequences present in mature female schistosomes but not in males, a subtractive hybridization procedure was used (16). During the hybridization, sequences common to both female and male schistosomes were expected to form cDNA–RNA hybrids, making the portion of the cDNA which remained ss or unhybridized, female specific. In some instances, the resulting ss cDNA probe was purified away from the double-stranded hybrids by hydroxylapatite column chromatography before being used as a probe; however, this step was not necessary as the unfractionated probe retained its specificity. The specificity of the nonpurified probe was tested by RNA dot blot analysis. Fig. 1 shows that the probe was indeed specific for sequences present only in the mature female, since it hybridized strongly to RNA isolated from mature female worms and to RNA from adult worm pairs but only weakly or not at all to RNA isolated from male worms (from both bisexual or unisexual infections) or to RNA isolated from immature female worms obtained from animals with unisexual infections.

**Isolation of a Female-Specific cDNA Clone.** To isolate recombinant plasmids containing schistosome female-specific inserts, a cDNA library was screened by colony hybridization with the female-specific probe described above. The cDNA library was constructed from poly(A)$^+$ RNA, obtained from *S. mansoni* adult worm pairs, that was reverse transcribed and cloned into the *Pst* I site of pBR322 after G/C tailing. Screening of approximately 5000 colonies resulted in the identification of 120 positive colonies. Analysis on agarose gels of plasmids isolated from 60 of these, after digestion with *Pst* I, showed cDNA inserts ranging in size from 300 to 1500 base pairs (bp). A representative sampling of the 60 clones is shown in Fig. 2 (*Upper*).

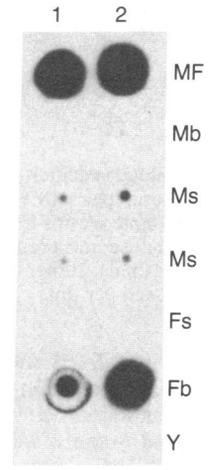


FIG. 1. Dot-blot analysis of schistosome worm RNA. A ss cDNA obtained by subtractive hybridization was used as a probe. Total cellular RNA was obtained from schistosome worm pairs from mixed sex (MF) infections, male worms from bisexual (Mb) or unisexual (Ms) infections, female worms from bisexual (Fb) or unisexual (Fs) infections, or from yeast (Y). Either 2.5 $\mu$g (column 1) or 5.0 $\mu$g (column 2) of RNA was used per dot.

To determine if the selected clones represented cDNA from more than one mRNA sequence, *Pst* I-digested DNA from each of the clones was blotted onto nitrocellulose and hybridized to a nick-translated insert from clone pSMf 61-46 that was chosen because it strongly hybridized to the ss cDNA probe in colony hybridization and had a large insert (780 bp). Fig. 2 (*Lower*) shows that most of the clones analyzed cross-hybridized with the insert of pSMf 61-46 (54 of 60 clones).

To assess the specificity of each of these clones, RNA dot-blot hybridizations were performed using each clone separately as a hybridization probe. Fig. 3 shows that clone pSMf 61-46 was female specific, since it showed strong hybridization to RNA isolated from adult worm pairs and from mature female worms but not to RNA isolated from male worms from unisexual infections. In addition, all of the

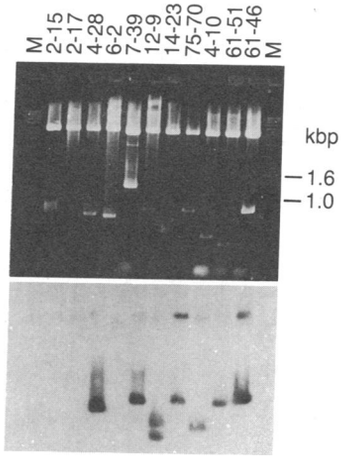


FIG. 2. Identification of cross-hybridizing female-specific cDNA clones. A series of cDNA clones identified by female-specific ss cDNA probe were digested with *Pst* I, separated on an agarose gel (*Upper*), transferred to nitrocellulose, and hybridized with the nick-translated insert of clone pSMf 61-46 (*Lower*). The Bethesda Research Laboratories 1-kilobase ladder (M) was used for size markers.

‡National Biomedical Research Foundation (1986) *Protein Sequence Data Base of the Protein Identification Resource* (Washington, DC), Release No. 7.0.

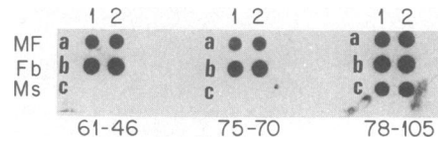


FIG. 3. Determination of stage-specificity of cDNA clones. Total cellular RNA from adult worm pairs (MF), female worms from bisexual infections (Fb), and male worms from unisexual infections (Ms) was applied to nitrocellulose and probed with nick-translated cDNA inserts of indicated plasmids. Either 2.5 μg (column 1) or 5.0 μg (column 2) of RNA was used per dot.

clones tested that cross-hybridized with clone pSMf 61-46 were also shown to be female specific (e.g., pSMf 75-70 in Fig. 3) whereas all of the noncross-hybridizing clones were not, since they hybridized equally well to all three RNA preparations (e.g., pSM 78-105 in Fig. 3).

**Identification of an RNA Complementary to pSMf 61-46 in *S. mansoni*.** To determine the nature and size of the specific RNA complementary to pSMf 61-46 in females of *S. mansoni*, total RNA was isolated from adult worm pairs, from mature female worms, or from male worms and size-separated by electrophoresis on an agarose gel under denaturing conditions. A replica of the gel, created by blotting to GeneScreen, was then hybridized to the nick-translated insert of pSMf 61-46 (Fig. 4). The blot revealed an RNA species of about 950 bases that, as expected, was present in the RNA isolated from females or worm pairs but was absent in the RNA isolated from males.

**Developmental Regulation of RNA Complementary to Clone pSMf 61-46.** Fig. 5*A*, lane 1, shows that the probe pSMf 61-46 is again specific for RNA expressed only in mature female worms, since RNA from mature male worms isolated from bisexual infections did not hybridize. Furthermore, RNA isolated from immature female worms whose development was arrested because of unisexual infection also did not hybridize, nor did RNA from *S. mansoni* eggs.

Since RNA complementary to clone pSMf 61-46 could not be detected in immature female worms or male worms, the time of appearance of complementary RNA in worms from animals with bisexual infections was determined. *S. mansoni* worms were recovered from hamsters starting at 23 days after infection, then at 26, 28, 30, 32, 35, and 45 days, and RNA from each sample was isolated. Before RNA was extracted, the worms were scored for their pairing status by observation under a stereomicroscope. No worm pairing was detected

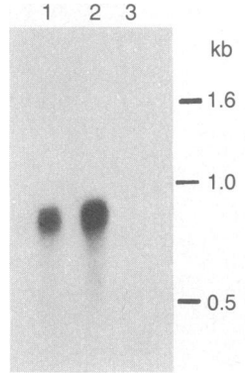


FIG. 4. Identification of RNA complementary to pSMf 61-46. Total cellular RNA from worm pairs (lane 1), mature female worms (lane 2), or male worms (lane 3) was denatured, size-separated, and transferred to GeneScreen. The blotted RNA was hybridized with the nick-translated insert of pSMf 61-46. Glyoxylated 1-kilobase (kb) DNA fragments (Bethesda Research Laboratories) were used as size markers.

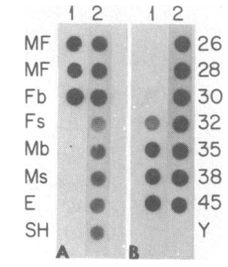


FIG. 5. Developmental regulation of mRNA complementary to pSMf 61-46. (*A*) Nick-translated insert of pSMf 61-46 (lane 1) or pSM 78-105 (lane 2) was hybridized to RNA dotted onto nitrocellulose from worm pairs (MF), from mature female worms (Fb), from 45-day-old immature female worms (Fs), from male worms from bisexual (Mb) or unisexual (Ms) infections, from schistosome eggs (E), or from immature *Schistosoma haematobium* worm pairs (SH). (*B*) RNA was obtained from worms from mixed sex infection and dot blotted onto nitrocellulose. Worms were of various days after infection. Y represents RNA isolated from yeast. pSMf 61-46 (lane 1) or pSM 78-105 (lane 2) was used as a probe. In *A* and *B* each dot contained 1 μg of total cellular RNA.

until day 28, and at that time only a few worms were paired. By day 35 all the worms were paired. Egg production by some female worms (fecundity) had started by 35 days after infection. Dot-blot analysis of the RNA was then performed using several probes. In these experiments equal amounts of total RNA from each time point as measured by optical density were blotted.

Fig. 5*B*, lane 1, shows that RNA complementary to clone pSMf 61-46 was first detectable in 28-day-old worms. Density scanning of the x-ray film (not shown) indicated that the amount of hybridizing RNA increased dramatically between 30 and 35 days after infection and continued to increase until it reached a plateau level. To rule out the possibility that the increase in abundance of this RNA molecule was merely a reflection of an increase in abundance of all RNA molecules relative to rRNA, clone pSM 78-105 was used as a hybridization probe. This clone has already been shown to recognize an RNA sequence present in both males and females (see Fig. 3). Fig. 5 *A* and *B*, lanes 2, demonstrates that this probe, unlike pSMf 61-46, hybridized equally well to all RNA preparations. A cDNA clone believed to encode actin (data not shown) and a genomic ribosomal DNA clone (data not shown) also hybridized equally well to the RNA isolated at every time point.

Thus the RNA sequence complementary to pSMf 61-46 appears temporally regulated in female worms from hamsters with mixed-sex infections. The appearance of the complementary RNA in females is clearly dependent on the presence of male worms, since the RNA was barely detectable in immature females from unisexual infections, even if RNA was isolated from 45-day-old worms (Fig. 5*A*). Furthermore the appearance of the RNA correlated well with worm pairing, and maximal levels were found at the time of egg production.

**DNA Sequence of Clone pSMf 61-46.** The nucleotide sequence of the insert of pSMf 61-46 shows two tandem repeats of 47 nucleotides that are identical except for a single guanosine to adenosine substitution (residues 258–304 and 306–352 in Fig. 6). On one of the strands there is a classical polyadenylylation signal ("AATAAA"; ref. 20) 13 nucleotides before the start of a tract of 33 adenosines (Fig. 6). This strand also contains two large open reading frames (RF1 and RF2) capable of encoding a polypeptide of 177 or 186 amino acids, respectively, as measured from the first ATG in each reading frame.

The existence of two large open reading frames on the coding strand makes it difficult to predict with absolute

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            12                  24                  36                  48                  60                  72
TCA ACA TCT GAG CAT AAA GTC ATC ACA CCC AGT ACA ACA ACA CCA ACA ATT TGA AAA ATG AAA CAG TCA CTC
                                                                                MET Lys Gln Ser Leu

            84                  96                 108                 120                 132                 144
ACA CTC GTC TTC TTA GTA GCC ATT GGT TAC GCC ACC GCC TAC ACC ACA TCA CAT GAC TAT TCG GGT GGG TAC
Thr Leu Val Phe Leu Val Ala Ile Gly Tyr Ala Thr Ala Tyr Thr Thr Ser His Asp Tyr Ser Gly Gly Tyr
                                                                  MET Thr Ile Arg Val Gly Thr

           156                 168                 180                 192                 204                 216
GGT GGC GGT TGC TAT GGT AGC GAT TGT GAT AGC GGT TAT GGC GAT AGT GGA TAT GGT GGA GGC TGT ACT GGC
Gly Gly Gly Cys Tyr Gly Ser Asp Cys Asp Ser Gly Tyr Gly Asp Ser Gly Tyr Gly Gly Gly Cys Thr Gly
 Val Ala Val Ala Met Val Ala Ile Val Ile Ala Val Met Ala Ile Val Asp Met Val Glu Ala Val Leu Ala

           228                 240                 252                 264                 276                 288
AGT GAC TGT GGC GGC GGC TAT GGT GGT GGC TAT GGT GGA GGT TGC AGT GGT GGA GAT TGT GGT AAT TAC GGT
Ser Asp Cys Gly Gly Gly Tyr Gly Gly Gly Tyr Gly Gly Gly Cys Ser Gly Gly Asp Cys Gly Asn Tyr Gly
 Val Thr Val Ala Ala Ala Met Val Val Ala Met Val Glu Val Ala Val Val Glu Ile Val Val Ile Thr Val

           300                 312                 324                 336                 348                 360
GGT GGC TAT GGT GGT GAT TGC AAT GGT GGA GAT TGT GGT AAT TAC GGT GGT GGC TAT GGT GGT GGG AAT GGT
Gly Gly Tyr Gly Gly Asp Cys Asn Gly Gly Asp Cys Gly Asn Tyr Gly Gly Gly Tyr Gly Gly Gly Asn Gly
 Val Ala Met Val Val Ile Ala Met Val Glu Ile Val Val Ile Thr Val Val Ala Met Val Val Gly Met Val

           372                 384                 396                 408                 420                 432
GGT GGT TGC AGT GGT GGC AAT TGT GGA GGT GGC TTC GAT GAG GCC TTA CCT GCC CCC TAT GGC GGT GAT TAT
Gly Gly Cys Ser Gly Gly Asn Cys Gly Gly Gly Phe Asp Glu Ala Leu Pro Ala Pro Tyr Gly Gly Asp Tyr
 Val Val Ala Val Val Ala Ile Val Glu Val Ala Ser Met Arg Pro Tyr Leu Pro Pro Met Ala Val Ile Met

           444                 456                 468                 480                 492                 504
GGT AAC GGT GGC AAC GGC TTT GGA AAA GGT GGT AGT AAA GGC AAC AAT TAT GGA AAG GGT TAT GGC GGT GGT
Gly Asn Gly Gly Asn Gly Phe Gly Lys Gly Gly Ser Lys Gly Asn Asn Tyr Gly Lys Gly Tyr Gly Gly Gly
 Val Thr Val Ala Thr Ala Leu Glu Lys Val Val Val Lys Ala Thr Ile Met Glu Arg Val Met Ala Val Val

           516                 528                 540                 552                 564                 576
AGC GGT AAG GGT AAG GGT GGT GGC AAA GGT GGC AAA GGC GGC AAA GGT GGC ACT TAC AAA CCC AGC CAT TAT
Ser Gly Lys Gly Lys Gly Gly Gly Lys Gly Gly Lys Gly Gly Lys Gly Gly Thr Tyr Lys Pro Ser His Tyr
 Ala Val Arg Val Arg Val Val Ala Lys Val Ala Lys Ala Ala Lys Val Ala Leu Thr Asn Pro Ala Ile Met

           588                 600                 612                 624                 636                 648
GGA GGC GGT TAC TGA GGC ACC AGT TGA GTT GTG GAT CAT TCT AAT TTG TTT GTG TCA CAC TCT CCA CTG TCC
Gly Gly Gly Tyr ***
 Glu Ala Val Thr Glu Ala Pro Val Glu Leu Trp Ile Ile Leu Ile Cys Leu Cys His Thr Leu His Cys Pro

           660                 672                 684                 696                 708                 720
TAT TTT TCT ACA CAC CTC TCA ATT CAA CTC ACT GTA ATA TAG TCG TGT TTG AAT TCG AGA TGA ATA AAA CCT

 Ile Phe Leu His Thr Ser Gln Phe Asn Ser Leu ***

           732                 744                 756
ATT CAT TCT AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA
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FIG. 6. DNA sequence and translation of pSMf 61-46. The DNA sequence of clone pSMf 61-46 and translation of two reading frames corresponding to RF1 and RF2 is shown. Note the "AATAAA" (boxed) at residues 711–716, the poly(A) tail (residues 730–762), and the direct repeats (underlined).

certainty how the mRNA is actually translated. From the amino acid composition of the deduced protein sequence, it is clear that a protein encoded by RF1 would be extremely rich in glycine (44%) and tyrosine (11%), while a protein encoded by RF2 would be rich in valine (29%), alanine (17%), isoleucine (9%), and methionine (9%). In addition, analysis of the sequence from RF1 reveals many repeats of Gly-Gly-Gly-Tyr, Gly-Gly-Gly-Cys, Gly-Gly-Asp, Gly-Gly-Asn, and Gly-Gly-Lys, which are regularly distributed throughout the protein.

The following arguments suggest that RF1 is in fact translated into a protein. (*i*) The only ATG in RF1 is at its start and is nearer the 5′ end of the mRNA than the first ATG of RF2. Existing data suggest that the first ATG from the 5′ end is the one actually used (21). In addition, this ATG is proceeded by an adenosine at position −3 conforming with the consensus sequence preceding initiator ATGs (22). However, an adenosine in the −3 position is not found until the 13th ATG of RF2. (*ii*) The amino acid sequence in the portion of RF1 that overlaps RF2 precludes the occurrence of stop codons in RF2. However, the reverse is not true, since stop codons can be introduced into RF1 without altering the translated sequence of RF2. (*iii*) It seems highly unlikely that the codon usage in RF2 would generate the repeated structure of RF1 by chance, without repeats at the DNA level. (*iv*) The putative protein sequence from both reading frames was used to search the Protein Sequence Data Bank‡ for homology with known sequences. The sequence from RF2 gave no significant homologies with proteins that would be biologically relevant within the context of this system. However, the sequence from RF1 showed striking homology (51.1%) with the carboxyl-terminal 121 amino acids of mouse cytoskeletal keratin. Even more intriguing was the 53% homology seen with 36 amino acids of the silk moth chorion A (egg shell) protein and 48% homology with 58 amino acids of the silk moth chorion B (egg shell) protein.

## DISCUSSION

We have characterized a mRNA species present in the mature female schistosome that is developmentally regulated. The mRNA cannot be detected in immature females, males, or eggs. It is first detectable in female worms in bisexual infections 28 days after infection, a time that coincides with the time when schistosomes begin to form pairs. It therefore, seems likely that the appearance of this mRNA in the female is somehow influenced by the presence of the male. The most probable mechanism for this is regulation at the transcriptional level. However, as we have only analyzed steady-state mRNA, we cannot formally exclude mRNA stability as a factor. That the mRNA was not detected in the egg stage was not surprising as the vitellaria in the female worm are thought to produce the proteins necessary for egg shell formation. The egg shell is formed before the eggs are deposited by the female worm (6).

Several laboratories have attempted to show sex-specific differences at the protein level by direct analysis on polyacrylamide gels but few reproducible differences have been observed and correlation of the results of one group with any other is difficult (23–27). Furthermore, an antigenic analysis of male and female worms has also shown no differences (28) although another report has identified gender-specific and pair-dependent glycoprotein antigens (29). Atkinson and Atkinson (30) also reported a protein that appeared to be synthesized in males and transferred to females; however, these results could not be substantiated (31).

The identification of a specific gene in female schistosomes that seems to be activated in response to the presence of males provides the first clear confirmation at the molecular level of a classical morphological observation: female schistosomes do not complete physical or reproductive development unless worm pairing takes place (3, 4). Since the changes that occur as a result of pairing are many, it seems likely that several genes could be regulated in this manner. The probe used in the present study should theoretically be representative of all of these. However, all of the female-specific clones isolated cross-hybridized. A possible reason for this is that hybridization of the probe to a cDNA library from adult worm pairs favored the detection of an abundant species, since that sequence would contain a high proportion of the radioactivity. To isolate less abundant sequences, the probe could be used to construct a library in a fashion similar to that used for the isolation of rare stage-specific sequences during *Xenopus* development (16).

Analysis of the DNA sequence of pSMf 61-46 revealed the two following unusual features: the presence of an almost perfect 47-nucleotide tandem repeat (one mismatch) and two large open reading frames on the coding strand (RF1 and RF2). Since the nucleotide repeat is contained within both open reading frames, a putative protein made from either frame would contain a repeated stretch of 13 amino acids. Further analysis at the amino acid level revealed numerous smaller repeats especially in RF1 that are rich in glycine. The glycine-repeat motif has striking similarity to repeats found in the silk moth chorion (egg shell) proteins. It is striking that the amino acid composition of RF1 is similar to the reported composition of purified schistosome egg shells in that both are rich in glycine and depleted in tryptophane, methionine, isoleucine, valine, and arginine (32). Thus, although we cannot be certain that pSMf 61-46 encodes a schistosome chorion-like protein, several features of the clone argue in favor of this hypothesis, as does the size and abundance of the mRNA hybridizing to it and the biological context in which it is found.

Chorion genes have been extensively studied in *Drosophila* (33) and in silk moths (34). In the former case, the chorion genes are amplified during development; in the latter, they consist of a large multigene family clustered in a giant locus probably exceeding $10^6$ bp (34).

Southern blot analysis of genomic DNA probed with pSMf 61-46 from both female (immature and mature) and male worms gave a simple pattern suggesting the absence of a large gene family and showed no evidence for gene amplification. From reconstruction experiments, measurements of copy number suggest 1–5 copies per haploid genome (data not shown).

As this work was nearing completion, we became aware that two other laboratories had isolated a female-specific clone using different procedures. Studies in our laboratory comparing one of the clones with pSMf 61-46 have shown cross-hybridization (data not shown). However, as the sequence of this clone was not reported (35), we cannot be certain that it represents the same gene. The other cDNA clone was derived from an mRNA that is distinctly different in size and sequence from pSMf 61-46 (K. S. Johnson, D. W. Taylor, and J. S. Cordingly, personal communication).

The authors are indebted to Ms. Lynette Spencer for technical service, Dr. Marie-Louise Hammarskjold for discussions, and Ms. Theresa Wnuk for clerical service. This work was funded by a grant from the National Institute of Allergy and Infectious Diseases (R22AI-18867), a grant from the Edna McConnell Clark Foundation, and the Research Career Development Award A000905 (D.M.R.) from the National Cancer Institute. This work was conducted as part of the Center for Applied Molecular Biology and Immunology supported by the State University of New York at Buffalo.

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