Cloning of a cuticular antigen that contains multiple tandem repeats from the filarial parasite *Dirofilaria immitis*

(repeated protein/nematode/cuticle)

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ABSTRACT An unusual antigen composed of tandemly repeated protein units was cloned from the filarial parasite *Dirofilaria immitis*. The antigen was initially identified by screening a λgt11 cDNA library with serum from dogs immunized with irradiated *D. immitis* third-stage larvae. DNA sequence analysis of the cDNA clone, Di5, revealed a continuous open reading frame composed of two 399-base-pair repeats arranged in tandem. Southern blot analysis of genomic *D. immitis* DNA showed that the gene coding for Di5 is composed of a tandem array of 25–50 copies of this same 399-base-pair repeat. Antiserum raised against recombinant Di5 protein detected a protein "ladder," from about 14 to >200 kDa with steps ≈15 kDa apart, on immunoblots of *D. immitis* extract. Metabolic labeling of adult parasites with [³⁵S]methionine showed that Di5 is synthesized as a large precursor that is subsequently cleaved to produce the ladder-like array. These results suggest that the characteristic ladder is created by proteolytic cleavage of the precursor at the same site in each monomer. The Di5 antigen was localized to the cuticle and hypodermis of adult *D. immitis* by immunoelectron microscopy. Both male and female parasites were found to release Di5 when cultured *in vitro*. DNA hybridization analysis demonstrated that Di5 is a member of a gene family present in many filarial parasites that infect both animal and human populations.

Filarial parasites are a major cause of disease in human and animal populations worldwide (1, 2). Transmission occurs through the bite of an insect harboring third-stage larvae. Third-stage larvae undergo two molts in the mammalian host to become adults. After mating, female parasites produce microfilariae, which circulate in the blood or cutaneous tissues. Insects injest microfilariae while taking meals from infected hosts. In the insect, the microfilariae undergo two molts to become third-stage larvae, the form of the parasite infectious for the vertebrate host.

The World Health Organization estimates that over 100 million people are infected with filarial parasites (3). People infected with the lymphatic filarial parasites *Brugia malayi* and *Wuchereria bancrofti* experience a range of symptoms including elephantiasis, the gross enlargement of limbs and genitals caused by blocked lymphatics. Onchocerciasis ("river blindness") is caused by *Onchocerca volvulus*. Another filarial parasite of clinical importance, *Loa loa*, can migrate through the eyes but generally does not cause permanent damage. *Dirofilaria immitis*, commonly known as dog heartworm, is a filarial parasite of global veterinary concern. Infections with *D. immitis* can damage many of a dog's vital

organs, including the heart, lungs, and kidneys. In some cases, 50 or more worms have been found in the heart (4).

Infection with filarial parasites can be prevented with the regular use of either diethylcarbamazine or ivermectin. Presently, no vaccine is commercially available. Experimentally however, protection has been obtained in dogs by immunizing with live irradiated third-stage larvae (5) or with chemically abbreviated infections of *D. immitis* (6). When dogs vaccinated with irradiated third-stage larvae are challenged with live parasites, the number of worms found in the heart is reduced by >80% (5).

As an initial step in identifying potentially protective antigens for *D. immitis*, a λgt11 cDNA library was screened with serum from vaccinated dogs. We report the characterization of a cuticular antigen, Di5, one of the major immunogens recognized by this serum. The gene coding for Di5 is composed of 25–50 direct tandem repeats of a 399-base-pair (bp) monomer.‖ Di5 protein is initially made as a large precursor, >200 kDa, that is cleaved to give a ladder-like array of peptides as shown by SDS/PAGE. Localization studies show that the antigen is in the cuticle and also released from the parasite. The Di5 gene is a member of a gene family that is present in a variety of parasitic nematodes.

MATERIALS AND METHODS

Isolation of Parasite DNA and Protein. Parasites were generously donated by the following: *Brugia timori* and *W. bancrofti*, F. Partono (University of Indonesia, Jakarta, Indonesia); *Brugia patei*, A. Vickery (University of Southern Florida); *L. loa*, T. Nutman (National Institutes of Health); *O. volvulus*, B. Duke (Armed Forces Institute of Pathology); *Onchocerca gibsoni*, L. Semprevivo (University of Massachusetts); *Mansonella ozzardi*, I. Petralanda (Centro Amazonico Investigacion Control Entermedades Tropicales, Puerto Ayacucho, Venezula); *Setaria cervi*, S. Dissanayake (Harvard School of Public Health). DNA samples from *Onchocerca cervicalis* and *Caenorhabditis elegans* were provided by E. James (Medical University of South Carolina) and P. Arasu (New England Biolabs), respectively. *B. malayi, Brugia pahangi, Litomosoides carinii, Acanthocheilonema vitea, Toxocara canis*, and *D. immitis* parasites were purchased from TRS Laboratory (Athens, GA). Human DNA was obtained from Sigma.

DNA was isolated from parasites by proteinase K digestion (7). *D. immitis* DNA was further purified by CsCl centrifu-

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gation (8). Protein extracts of adult *D. immitis* parasites were prepared in phosphate-buffered saline (PBS) containing protease inhibitors (9).

Cloning and DNA Sequencing. Restriction endonucleases, vectors, ligases, DNA size standards, and sequencing reagents were obtained from New England Biolabs and were used as instructed by the vendor. An adult female *D. immitis* λgt11 cDNA library (10) was screened with a 1:100 dilution of immune serum from dogs (TRS Laboratory) vaccinated with irradiated *D. immitis* third-stage larvae. The serum used for screening was taken from dogs prior to challenge, when the animals had been exposed only to third-stage and fourth-stage larvae (5). After plaque purification of the Di5 recombinant, the 868-bp *Eco*RI insert was subcloned into *Eco*RI-cleaved M13 mp19 and mp18 and sequenced in both directions by the dideoxy method (11).

Southern and Dot Blot Analysis. After agarose gel electrophoresis, DNA was blotted onto nitrocellulose (Schleicher & Schuell) (8). After baking, Southern blots were prehybridized and then hybridized in 6× standard saline citrate (SSC) (12) at 65°C overnight with ^{32}P-labeled Di5 fragment (10^6 cpm/ml of hybridization solution). After hybridization, blots were washed twice at room temperature and once at 58°C for 30 min each in 2× SSC/0.1% SDS. The *Eco*RI Di5 fragment was gel-purified with DEAE-paper (NA45, Schleicher & Schuell) (13) and then labeled with [α-^{32}P]dCTP (3000 Ci/mmol; NEN/DuPont; 1 Ci = 37 GBq) by oligonucleotide random priming (8).

DNA dot blots (12) were prehybridized and hybridized at 55°C with the Di5 fragment as described for Southern blots. Blots were washed twice at room temperature and once at 53°C for 30 min each in 2× SSC/0.1% SDS.

Production of Polyclonal Antiserum. The *Eco*RI Di5 fragment was fused in frame to the sequence encoding maltose-binding protein (MBP), and the fusion protein was affinity-purified over amylose resin by use of a MBP kit (New England Biolabs) as instructed by the supplier. Polyclonal antiserum was raised in BALB/c mice by intraperitoneal inoculation with 50 μg of MBP–Di5 fusion protein emulsified in Freund's complete adjuvant. Mice were given two intraperitoneal booster injections at 14-day intervals with 50 μg of protein emulsified in Freund's incomplete adjuvant. Animals were bled 1 week after the last boost.

Immunoblot Analysis. PBS *D. immitis* extract was subjected to SDS/PAGE in 4–20% gradient gels (ISS-Enprotech, Hyde Park, MA) under reducing conditions (14). After electrophoresis, proteins were electroblotted onto nitrocellulose (15). Blots were blocked in 1% nonfat dry milk and then probed with either mouse anti-MBP–Di5 (1:1000) or rabbit anti-MBP (1:10,000; New England Biolabs) serum for 1 hr. Bound antibody was detected (10, 16) with alkaline phosphatase-conjugated goat anti-mouse or goat anti-rabbit IgG (1:200; Cappel Laboratories) and the substrates nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega).

Metabolic Labeling of Parasites. Nine *D. immitis* adult females were preincubated in 100 ml of methionine-free RPMI 1640 (SelectAmine kit; GIBCO/BRL) containing penicillin/streptomycin (200 units/ml; GIBCO/BRL), amphotericin (25 μg/ml; GIBCO/BRL), and 1% (wt/vol) glucose at 37°C and 5% CO_2 for 2–4 hr to deplete them of endogenous methionine and then transferred to 100 ml of fresh medium containing 500 μCi of L-[^{35}S]methionine (1000 Ci/mmol; Amersham) (17). After 3, 6, or 20 hr of incubation at 37°C, three parasites were removed from culture and homogenized as described (9). A sample of each homogenate was precipitated with 10% (wt/vol) trichloroacetic acid (16) and the incorporation of [^{35}S]methionine was measured by liquid scintillation counting.

To monitor the metabolic processes responsible for generating the Di5 ladder seen on immunoblots, 10^6 acid-precipitable cpm from each time point was immunoprecipitated with anti-MBP–Di5 serum and protein A-Sepharose (Sigma) (17). Immunoprecipitates were subjected to SDS/PAGE in 10–20% gradient gels (ISS-Enprotech) under reducing conditions. After electrophoresis, gels were soaked in Enlightning (DuPont) for fluorographic enhancement, vacuum dried, and exposed to X-Omat film (Kodak).

Concentration and Analysis of Excretory/Secretory (E/S) Products. Adult male and female parasites were cultured as described above but in complete RPMI 1640 (GIBCO/BRL) for 7 days. At the end of the culture period, all parasites were viable and intact.

Parasites were transferred to fresh medium at 24-hr intervals. Spent female culture medium was filtered through a 5.0-μm-pore polycarbonate membrane (Nuclepore) to remove microfilariae and then passed through a 0.22-μm-pore, low-protein-binding filter (Millex GV sterilizing filter unit; Millipore) and frozen (17). Medium was pooled, brought to 1 mM EDTA and 1 mM EGTA, and then concentrated ≈2000-fold using an ultrafiltration stirred cell containing an M_r 10,000-cutoff Diaflo membrane (Amicon). Culture medium from male parasites was treated accordingly.

Concentrated female E/S products were subjected to SDS/PAGE in 4–20% gradient gels under reducing conditions. Typically, a lane of the gel was silver-stained (Gelcode kit; Pierce) to examine the protein profile. The remainder of the gel was electroblotted to nitrocellulose and examined for Di5 by immunostaining with anti-MBP–Di5 serum. Male E/S products were radiolabeled with ^{125}I-labeled Bolton–Hunter reagent (2200 Ci/mmol; NEN/DuPont) (18) and 1.5 × 10^6 cpm was examined for Di5 by immunoprecipitation followed by SDS/PAGE in 4–20% gradient gels under reducing conditions. After electrophoresis, gels were vacuum dried and exposed to x-ray film.

Immunoelectron Microscopy. *D. immitis* adult females were fixed, processed at low temperature, and embedded in LR gold (London Resin Company), and ultrathin sections (90 nm) were cut and mounted on Formvar-coated nickel grids (9). Sections were blocked, incubated, and washed in PBS containing 1% bovine serum albumin and 0.01% Tween 20. After blocking, sections were incubated with mouse anti-MBP–Di5 serum (1:1000) for 2 hr at room temperature. Control sections were incubated in rabbit anti-MBP serum (1:500) or mouse anti-yellow fever virus serum (1:1000; gift of E. Gould, London School of Health and Tropical Medicine, London, UK). Sections were washed three times and then incubated with gold (10 nm)-conjugated goat anti-mouse IgG/IgM or goat anti-rabbit IgG (1:10; Janssen Pharmaceutica) for 30 min. Sections were washed twice as above, then once in PBS containing 0.01% Tween 20 and finally in distilled water. Sections were counterstained for 10 min in saturated aqueous uranyl acetate and then for 2 min with lead citrate. Sections were examined on a Jeol 100CX transmission electron microscope.

RESULTS

An adult female *D. immitis* cDNA library (10) was screened with immune serum from dogs vaccinated with irradiated *D. immitis* third-stage larvae to identify immunogens that might be protective. This library was used because of difficulties in obtaining sufficient material from third-stage larvae for the construction of a cDNA library. Of the recombinants initially identified, three were found to be paramyosin (10) and one, Di5, was found to have a direct tandem repeat. DNA sequence analysis of the 868-bp insert revealed 2.2 copies of a 399-bp repeat. The second repeat (nucleotides 400–798) differs from the first by a single nucleotide at position 643

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      S Y F Q T Y L S W L T D A Q K D E I K K
  1  AGCTATTTTCAGACGTATCTGAGCTGGCTCACAGATGCTCAAAAAGATGAAATTAAAAAA   60
400  ......................................................   459

      M K E E G K S K M D I Q K K I F D Y F E
 61  ATGAAAGAAGAAGGAAAATCGAAAATGGATATTCAAAAAAAAATTTTTGATTATTTCGAA  120
460  ......................................................   519
                                        Pst I
      S L T G D K K K K A A E E L Q Q G C L M
121  AGTTTGACAGGTGATAAAAAGAAAAAGGCTGCAGAAGAACTTCAACAAGGTTGCTTAATG  180
520  ......................................................   579

      A L S E I I G N E K M L M L K E I K D S
181  GCTCTCAGTGAAATCATTGGTAATGAAAAGATGCTTATGTTGAAAGAGATTAAAGATTCA  240
580  ......................................................   639
                                              Hinc II
      G A D P E Q I R M K V E D M L K L V V D
241  GGCGCTGATCCAGAACAAATCAGAATGAAAGTCGAAGATATGTTGAAACTTGTCGTTGAC  300
640  ...H..................................................   699

      K E K K K R I D E Y A P V C R K I Y A A
301  AAAGAAAAGAAGAAAAGAATTGATGAATATGCTCCTGTATGCCGTAAAATTTATGCGGCA  360
700  ......................................................   759

      M N E R R K R N D H N L E
361  ATGAATGAACGGCGTAAGCGGAATGATCATAATTTAGAA  399
760  ...................................  798
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FIG. 1. Nucleotide sequence of the Di5 cDNA clone. The cDNA clone contains two complete members of a 399-bp repeat. The one-letter amino acid code of the translated sequence is shown above the second nucleotide of each codon. The second member of the repeat (nucleotides 400–798) is shown below the first sequence. Dots represent exact matches. A single nucleotide difference in the second repeat results in the substitution of a threonine for an alanine. Both *Pst* I and *Hinc*II cut once within each repeat; their recognition sites are boxed.

(Fig. 1). Translation of the DNA sequence revealed an open reading frame of 133 amino acids (aa) per repeat with each monomer in the same reading frame as the next. Di5 has an unusually high lysine content, 17% (Fig. 1), compared with the lysine content of eukaryotic proteins, which averages 6.5% (19).

Genomic organization of the Di5 gene was determined by digesting *D. immitis* DNA with *Pst* I, which cleaves each repeat once (Fig. 2A). Hybridization of the *Eco*RI insert to *D. immitis* DNA partially cleaved with *Pst* I identified a ladder-like array indicative of DNA repeats arranged in tandem (Fig. 2A, lane 1). The steps in the ladder are multiples of 400 bp, the approximate size of a repeat. Complete digestion of genomic DNA with *Pst* I (Fig. 2A, lane 2) produced three
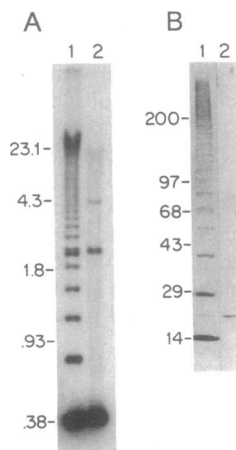
FIG. 2. (A) Restriction analysis of the Di5 repeat family. Southern blot of partial (lane 1) and complete (lane 2) *Pst* I digests of *D. immitis* DNA hybridized with ³²P-labeled Di5 fragment. Size standards in kilobases at left represent a combination of *Bst*NI-cut pBR322 and *Hind*III cut λ DNA. (B) Immunoblot of female *D. immitis* extract. Lanes 1 and 2 were probed with anti-MBP–Di5 and anti-MBP serum, respectively. Size standards (GIBCO/BRL) were myosin (200 kDa), phosphorylase *b* (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), β-lactoglobulin (18.4 kDa), and lysozyme (14.3 kDa).

bands of approximately 0.4, 2, and 4.3 kilobases. The larger fragments probably represent the 3' and 5' ends of the gene.

To determine the size of native Di5, a polyclonal antibody raised against MBP–Di5 fusion protein was used to probe an immunoblot of female *D. immitis* extract (Fig. 2B). Anti-MBP–Di5 serum identified a ladder of proteins ranging in size from 14 to >200 kDa (Fig. 2B, lane 1), whereas anti-MBP detected a single protein of about 20 kDa (lane 2). No protein was detected with normal mouse serum (data not shown). The estimated molecular mass of the monomer band from immunoblots is 14.3 kDa, whereas the predicted size from sequence data is 15.7 kDa. Steps in the protein ladder are multiples of ≈15 kDa. This ladder pattern was also detected on immunoblots of male parasites and microfilariae (data not shown).

Adult parasites were metabolically labeled with [³⁵S]methionine for 3, 6, or 20 hr to assess whether the protein ladder represented aggregation of small units or processing of a large precursor (Fig. 3). After labeling for 3 hr two high molecular weight bands were detectable, one at the origin of the gel and one slightly below this. After 6 hr, the dimer and larger forms were clearly visible, and after 20 hr, the ladder was complete, demonstrating that it resulted from the processing of a large precursor. Negligible radioactivity was immunoprecipitated from the ³⁵S-labeled homogenates with anti-MBP or normal mouse serum (data not shown).

To determine whether Di5 is secreted, culture medium from adult parasites was concentrated and analyzed for its presence. The E/S products from females comprised a mixture of proteins of 14.3–100 kDa (Fig. 4, lane a). The 14.3-kDa molecule is the most prominent E/S species. On immunoblots of female E/S proteins probed with anti-MBP–Di5 serum (Fig. 4, lane b), a 14.3-kDa band, the size of the Di5 monomer (lane c), was observed. Di5 dimer could also be detected on some immunoblots of female E/S products (data not shown).

To detect protein in male E/S products, it was necessary to iodinate the material. ¹²⁵I-labeled proteins (Fig. 4, lane d) ranged in size from 14.3 to >200 kDa. Unincorporated iodine ran at the gel front (lane d). Anti-MBP–Di5 immunoprecipitated a 14.3-kDa antigen (lane e), the size of the Di5 monomer, but anti-MBP serum did not (lane f). The 42- and 68-kDa bands immunoprecipitated with anti-MBP–Di5 were also precipitated with anti-MBP serum. No radioactivity was immunoprecipitated with normal mouse serum (lane g).

Di5 antigen was localized by immunogold labeling of ultrathin sections of female parasites. The greatest density of
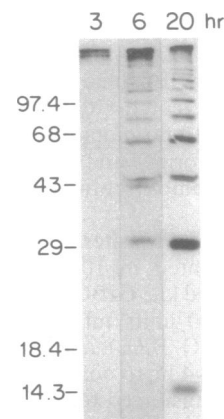
FIG. 3. Immunoprecipitation of Di5 from metabolically labeled parasites. Nine adult females were cultured *in vitro* in the presence of [³⁵S]methionine. At 3, 6, and 20 hr, three worms were removed from culture and homogenized in PBS. Di5 was detected in each homogenate by immunoprecipitation with MBP–Di5 antiserum followed by SDS/PAGE. Autoradiographic exposure, 7 days.
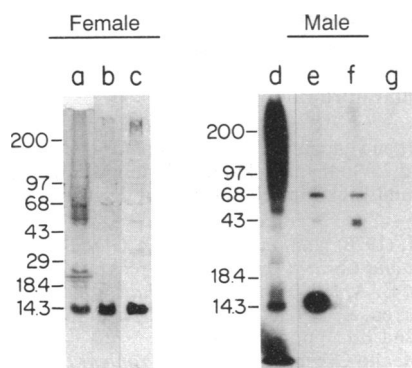
FIG. 4. Detection of Di5 in *D. immitis* E/S products. Lane a, silver-stained gel showing the protein profile of female E/S products (20 μl); lanes b and c, immunoblots of female E/S products (20 μl) and *D. immitis* protein extract, respectively, stained with anti-MBP–Di5 serum; lane d, profile of 1.5 × 10⁵ cpm of iodinated male E/S products; lanes e–g, male E/S products immunoprecipitated from 1.5 × 10⁶ cpm of material with anti-MBP–Di5, anti-MBP, and normal mouse serum, respectively. Autoradiographic exposure of lanes d–g, 2 days.

labeling with anti-MBP–Di5 was seen in the hypodermis and cuticle (Fig. 5*A*). Some staining was also apparent in the microvillar regions of the gut (data not shown). Only a background level of labeling was seen in sections of the cuticle stained with a nonspecific mouse antibody against yellow fever virus (Fig. 5*B*) or with anti-MBP (Fig. 5*C*).

A dot blot prepared with DNAs of both parasitic and nonparasitic worms was hybridized with the *Eco*RI Di5 fragment to determine whether Di5-like genes occur in other nematodes (Fig. 6). Under the conditions employed, no hybridization was detected to human (row B) *C. elegans* (C3), *T. canis* (C8), *W. bancrofti* (D1), or *O. gibsoni* (D4) DNA. A faint signal, similar in intensity to the hybridization signal generated by 6.25 ng of *D. immitis* DNA (A5), was
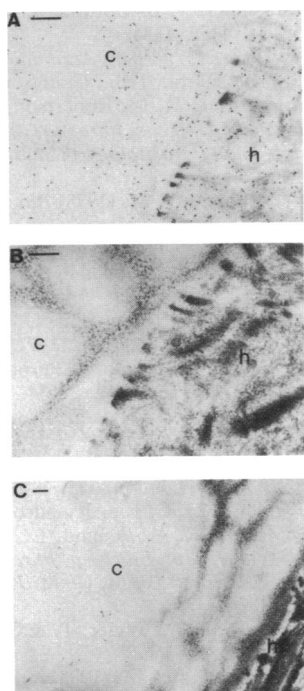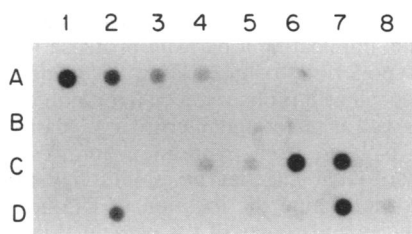
	1	2	3	4	5	6	7	8
A	D.i. (100)	D.i. (50)	D.i. (25)	D.i. (12.5)	D.i. (6.25)	D.i. (3.13)	D.i. (1.5)	D.i. (0.75)
B	H (100)	H (50)	H (25)	H (12.5)	H (6.25)	H (3.13)	H (1.5)	H (0.75)
C		A.v. (100)	C.e. (100)	B.t. (100)	B.m. (100)	B.pat. (100)	B.pah. (100)	T.c. (25)
D	W.b. (25)	S (25)	O.v. (100)	O.g. (100)	O.c. (100)	M.o. (8.8)	L.l. (100)	L.c. (100)

FIG. 6. Species specificity of Di5. A DNA dot blot was hybridized with radiolabeled Di5 fragment. The chart shows the quantity (in ng) and location of each species of DNA on the blot. D.i., *D. immitis*; H, human; A.v., *A. vitea*; C.e., *C. elegans*; B.t., *B. timori*; B.m., *B. malayi*; B.p., *B. pahangi*; T.c., *T. canis*; W.b., *W. bancrofti*; O.v., *O. volvulus*; O.g., *O. gibsoni*; O.c., *O. cervicalis*; M.o., *M. ozzardi*; L.L., *L. Loa*; L.c., *L. carinii*; S, *S. cervi*; B.pat., *B. patei*.

detected from *A. viteae* (C2), *O. volvulus* (D3), *O. cervicalis* (D5), and *M. ozzardi* (D6) DNA. Moderate to strong signals equivalent to the signal generated by 12.5 ng of *D. immitis* DNA (A4) were detected from *S. cervi* (D2), *L. loa* (D7), *L. carinii* (D8), *B. pahangi* (C7), *B. malayi* (C5), and *B. timori* (C4) DNA.

DISCUSSION

The Di5 antigen described in this study has an unusual organization; both the protein and the gene are composed of multiple copies of a nearly identical tandem repeat. The copy number of the monomer can be estimated from the number of steps in the partial Southern digest (Fig. 2*A*, lane 1), from the number of bands in an immunoblot (Fig. 2*B*, lane 1), and from a copy-number experiment (data not shown). All three methods indicate that there are 25–50 copies of the monomer per haploid genome. There appear to be no introns in the repeated body of the gene, as the spacing of the repeats in genomic DNA (Fig. 2*A*, lane 1) is 400 bp, in agreement with the size of the cDNA repeated unit (Fig. 1).

Labeling studies with [³⁵S]methionine (Fig. 3) demonstrate that Di5 protein is synthesized as a high molecular mass precursor (>200 kDa) that is cleaved to produce a ladder-like array. As cleavage continues with time, the monomer and dimer bands increase in intensity (Fig. 3). The ladder pattern might be generated by random cleavage of precursor at one proteolytic site per protein monomer, similar to the DNA pattern generated by a partial *Pst* I restriction digest of Di5 genomic DNA (Fig. 2*A*, lane 1). The ability to isolate high molecular mass Di5 protein at the early time periods demonstrates that protein cleavage is not an artifact of the extraction procedure.

Di5 antigen was located by immunoelectron microscopy in the cuticle and hypodermis of the parasite (Fig. 5). Since no protein synthesis occurs in the cuticle, Di5 is probably synthesized in the hypodermis and then exported to the

FIG. 5. Immunolocalization of Di5 antigen. Ultrathin sections of female parasites were immunostained with either anti-MBP–Di5 (*A*), anti-yellow fever virus (*B*), or anti-MBP (*C*) serum. *A–C* are sections through the body wall. c, Cuticle; h, hypodermis. (Bar = 0.25 μm.)

cuticle. Cleavage of Di5 occurs in parasites cultured outside the host, thus implicating a parasite protease located in the cuticle or hypodermis for generating the ladder. Di5 is not stage-specific, since it is observed on immunoblots of females (Fig. 2*B*), as well as males and microfilariae (data not shown). It was also isolated from expression libraries with serum raised against fourth-stage larvae. Additional studies will be necessary to determine the location of Di5 homologues in different species and stages of the parasites' life cycle.

Di5 is an E/S protein of *D. immitis*. Culture medium from females contains a major protein that migrates in gels at ≈14.3 kDa, less than the calculated 15.7 kDa. This molecule is recognized by anti-MBP–Di5 serum. The presence of Di5 in culture medium is not due to parasite death, as all parasites were viable at the end of the study. Since Di5 is found in culture media of both males and females, it is an E/S product of adult origin.

DNA probes and specific antisera were used to detect Di5 analogues in other clinically important filarial parasites. Previously, a ladder protein was described in ^{125}I-labeled *B. malayi* adults (20). Anti-MBP–Di5 immunoprecipitated this protein ladder from *Brugia*. In addition, repeats coding for this *Brugia* protein have been cloned (M.E.S., unpublished data). A similar ladder protein was seen on an immunoblot of *L. loa* probed with anti-MBP–Di5 (T. Nutman and A. Klion, personal communication).

DNA hybridization analysis with Di5 DNA confirmed that related genes exist in the human filarial parasites *B. malayi, B. timori,* and *L. loa* and in the animal filarial parasites *B. patei, B. pahangi, L. carinii,* and *S. cervi.* Although some filarial parasite DNAs did not hybridize with the probe, it is likely that a related gene exists. For example, a ladder-like protein was detected in ^{125}I-labeled *W. bancrofti* third-stage larvae (21), but no hybridization was detected to 25 ng of *W. bancrofti* DNA on the dot blot (Fig. 6). However, hybridization was detected to 25 ng of *D. immitis* DNA. Absence of hybridization to *W. bancrofti* DNA is probably a result of sequence divergence and/or fewer copies of the DNA repeat.

While this work was in progress, the partial amino acid sequence of ABA-1, the major allergen in the intestinal parasite *Ascaris suum,* was described (22). The N-terminal 41 aa of ABA-1 show 46% identity with 41 aa of recombinant Di5 beginning with NDHN (Fig. 1). ABA-1 is synthesized by all developmental stages of the worm and is released during culture of larvae *in vitro,* as is Di5. Similarities in sequence and stage specificity suggest that ABA-1 and Di5 are members of the same gene family and likely to have a similar function.

The biological function of this protein family remains a mystery. Computer searches with the UWSEQ PIR FASTP program (Genetics Computer Group, Inc., Madison, WI) have not detected any significant homologies.

Proteins with large numbers of tandem repeats, like Di5, are unusual. There are several examples of proteins with short repeats of 2–12 aa (23, 24) but only a few with long repeated sequences such as filaggrin (25) and microtubular binding protein from *Trypanosoma brucei* (26). Filaggrin aids the aggregation of mammalian keratin intermediate filaments *in vitro* and contains 20 tandem repeats of related subunits each with either 250 or 255 aa. *T. brucei* microtubular binding protein contains 50 nearly identical tandem repeats of 38 aa.

Though Di5 does not have significant amino acid homology with either of these repeated proteins, it does share a similar organization. It is possible that, like these proteins, Di5 may interact with repeated structural proteins. An alternative model is that the released monomer of Di5, not the cuticular

form, may be important. This could be analogous to epidermal growth factor, which is synthesized as a direct tandem repeat and then cleaved to a monomer before being released as a functional hormone (27).

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