

# Ultrastructural Localization of Giardins to the Edges of Disk Microribbons of *Giardia lamblia* and the Nucleotide and Deduced Protein Sequence of Alpha Giardin

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**Abstract.** The giardins are a group of 29–38-kD proteins in the ventral disk of the protozoan parasite *Giardia lamblia*. The disk attaches the parasite to the host's intestinal epithelium and is composed of parallel, coiled microtubules that are adjacent to the ventral plasma membrane and from which processes called microribbons extend into the cytoplasm; the microribbons are connected by crossbridges. *G. lamblia* cytoskeletons, consisting of disks and attached flagella, were isolated and used to show that the 29–38-kD proteins separate into five bands by one-dimensional electrophoresis and into 23 species by two-dimensional analysis. Rabbit antibodies raised against a 33-kD protein band, purified by one-dimensional gel electrophoresis and shown to contain three proteins by two-dimensional electrophoresis, recognized 17 proteins by two-dimensional immunoblot analysis. By immunofluorescence these antibodies reacted with the ventral disk but not with the flagella in isolated cytoskeletons. Electron microscopy revealed that the anti-giardin antibodies bound to the edges of the

microribbons but not to the microtubules, crossbridges, or other, nondisk structures. Antibodies to tubulin reacted with both the disk and flagella in isolated cytoskeletons but bound only to the microtubules in these structures. The amino-terminal sequence of the 33-kD immunogen was determined and used to construct a DNA oligomer, and the oligomer was used to isolate the alpha giardin gene. The gene was used to hybrid select RNA, and the in vitro translation product from this RNA was precipitated by the antibodies against the 33-kD immunogen. The gene sequence was a single open reading frame of 885 nucleotides that predicted a protein of 33.8 kD. The protein sequence is unique, having no significant homology to two other giardin sequences or to any sequences within the Protein Identification Resource. It is predicted to be 82% alpha helical. The downstream sequence of the gene indicates that the sequence AGT-PuAA is located six to nine nucleotides beyond the stop codon in all protein-encoding genes of *G. lamblia* that have been sequenced and reported to date.

**T**HE ventral disk of *Giardia lamblia*, an anaerobic, flagellated protozoan, maintains the parasite in the small intestine of vertebrates, attaching the organism to the intestinal mucosa and preventing peristaltic clearing (Holberton, 1973, 1974). The disk is comprised of coiled microtubules that are adjacent to the ventral plasma membrane and from which processes known as microribbons extend into the cytoplasm. Adjacent microribbons are connected by electron-dense structures termed crossbridges (Holberton, 1973, 1981; Holberton and Ward, 1981; Crossley and Holberton, 1983a,b, 1985). After detergent extraction of whole *Giardia*, the cytoskeletons consist of the disk with flagella attached (Holberton and Ward, 1981). A pair of proteins ~30 kD in size was extracted from isolated cy-

toskeletons and named the giardins (Crossley and Holberton, 1983a). Based on their mass in the cytoskeleton preparation, the giardins were regarded as components of the microribbons.

Since the original description of the giardins, multiple new cytoskeletal proteins have been identified with approximate molecular masses of 30 kD and have been shown to derive from both the flagella and the disk (Clark and Holberton, 1988). Also, the genes of two giardins have been cloned and sequenced. One of these giardins, termed beta giardin (Baker et al., 1988; Holberton et al., 1988), migrates at 29.4 kD as the lower band of the originally described doublet (Holberton and Ward, 1981). Another giardin, reported recently but not named (Aggarwahl and Nash, 1989), is similar to beta giardin. This second giardin is referred to here as beta-1 giardin because it has the same molecular mass as beta giardin but differs by 144 internal amino acid residues.

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At this point, the giardins should be considered the 29–38-kD proteins found in the ventral disk, the structure unique to *G. lamblia*. Thus, flagellar proteins in the same molecular mass range should not be considered giardins. The nomenclature of the cytoskeletal proteins separated by two-dimensional PAGE needs to be standardized, and we have done this here following the convention used for HeLa cells (Bravo et al., 1981; Bravo and Celis, 1982). Finally, since the one-dimensional gel pattern of *G. lamblia* cytoskeletal proteins is oversimplified in terms of the number of peptides, it seems inappropriate to base the names of giardin gene products on these gels. For the time being it is probably best to provide names for giardin genes with Greek letters in alphabetical order and to relate them to the two-dimensional electrophoretic pattern.

Here we describe our work on a 33-kD protein within the upper band of the original doublet described by Holberton. We have called this protein alpha giardin. There were three goals of this work: (a) to prepare rabbit antibodies against the 33-kD giardins and characterize systematically the immunogen as well as the antigens recognized by the antibodies in two dimensions; (b) to use these antibodies to localize giardins in frozen, thin-sectioned *Giardia*; and (c) to isolate the gene encoding a 33-kD product (alpha giardin) and determine its nucleotide sequence and deduced protein sequence. We show that the original 33-kD immunogen is comprised of three proteins and that the antibodies recognize 17 giardins and react specifically with the edges of the microribbons within the sucking disk. We also demonstrate that the gene for alpha giardin encodes a protein of 33.8 kD, predicted to be largely alpha helical, that has no significant sequence homology to the beta (Baker et al., 1988) and beta-1 (Aggarwahl and Nash, 1989) giardin sequences reported recently or to any protein sequence within version 18 of the Protein Identification Resource (George et al., 1986). Finally, we find that the sequence AGTGAA, six nucleotides downstream from the alpha giardin stop codon, corresponds well to the sequence AGTAAA that is found seven to nine nucleotides beyond the stop codons of three other *Giardia* genes that encode proteins. Thus, the sequence AGTPuAA is located six to nine nucleotides beyond the stop codon of the four *G. lamblia* protein-encoding genes that have been sequenced and reported to date.

## Materials and Methods

### *Giardia lamblia* Culture

Organisms of the Portland 1 strain (American Type Culture Collection, Rockville, MD) were grown to middle log phase ( $\sim 0.5 \times 10^6$  organisms/ml) at 37°C in TY1-S-33 medium supplemented with bile (Keister, 1983) in filled 25-cm<sup>2</sup> tissue culture flasks or in filled 2.5-liter culture bottles. The culture flasks were maintained in a horizontal, stationary position while the culture bottles were rotated at six revolutions per hour (Farthing et al., 1982).

### Metabolic Labeling

[<sup>35</sup>S]Methionine (New England Nuclear, Boston, MA) was added directly to the growth medium of early log phase ( $\sim 0.1 \times 10^6$  organisms/ml) *Giardia* at a final concentration of 45  $\mu$ Ci/ml. After 2 d more of growth at 37°C, the organisms were harvested by centrifugation and cytoskeletons were prepared as described.

## Preparation of Cytoskeletons

*G. lamblia* cytoskeletons were isolated as described by Crossley and Holberton (1983a). Briefly, the organisms were harvested at 1,600 g for 20 min at 4°C, resuspended in a small volume (<1 ml) of either 10 mM Tris-HCl, pH 8.3, or morpholino propane sulfonic acid pH 8.3, containing 2 mM EDTA, 2 mM DTT, 1 mM ATP, 2 mM MgSO<sub>4</sub>, and 150 mM KCl (TEDAMP or MEDAMP buffer), counted with a hemacytometer, and then extracted for 5 min at room temperature in TEDAMP (or MEDAMP) buffer plus 0.5% Triton X-100 at a concentration of 10<sup>7</sup> *Giardia*/ml. The cytoskeletons were pelleted at 15,000 g for 15 min at 4°C and were used immediately or stored at -70°C. Freshly prepared cytoskeletons were used for immunofluorescence and transmission electron microscopy.

## Protein Gels

One-dimensional slab gels were run as described by Laemmli (1970) and two-dimensional gels were run as described by O'Farrell (1975) and Jones (1980). Gels with <sup>35</sup>S-labeled proteins were fixed with acetic acid/methanol/water (10:50:40) for 60 min, soaked in EN<sup>3</sup>HANCE (New England Nuclear) for 30 min, soaked in water for 30 min, and dried before being exposed to X-OMAT x-ray film (Eastman Kodak Co., Rochester, NY) with intensifying screens (Cronex Lightning Plus; DuPont Co., Wilmington, DE). Nonradioactive proteins were visualized by staining with 0.25% Coomassie brilliant blue in acetic acid/methanol/water (10:40:50) or by silver staining (Wray et al., 1981).

## Preparation of Antiserum to Giardin

*G. lamblia* cytoskeletons (1–2 mg total protein) were resuspended in Laemmli (1970) buffer, boiled for 5 min, and loaded along the entire 11 cm width of a 15% polyacrylamide slab gel (30:1 acrylamide/bisacrylamide) 24 cm in length. After electrophoresis at 400–500 V  $\times$  h, the edges of the gel were excised and stained in 0.25% Coomassie brilliant blue as above. The 33-kD giardin band was identified using the stained edges as reference, excised from the gel, frozen with liquid nitrogen, pulverized, and resuspended in PBS (10 mM sodium phosphate, pH 7.4, 150 mM NaCl). The protein concentration was determined by the method of Lowry et al. (1951), and purity was assessed by rechromatography in one and two dimensions on polyacrylamide followed by silver staining (Wray, 1981). Three rabbits (Millbrook Farms, Amherst, MA) were bled to obtain the preimmunization serum, and then each was immunized subcutaneously with 100  $\mu$ g of 33-kD giardin emulsified in 0.5 ml Freund's complete adjuvant (Gibco Laboratories, Grand Island, NY). After 6 wk, each rabbit was boosted three times at 1-wk intervals with 50  $\mu$ g of the giardin emulsified in 0.25 ml Freund's incomplete adjuvant (Gibco Laboratories). Blood was collected by ear vein puncture before immunization and at weekly intervals after the first boost. Sera were purified by centrifugation and stored at -70°C.

## Immunoblot Analysis

Cytoskeletal proteins (amounts ranging from 10–100  $\mu$ g) separated by one- or two-dimensional PAGE were examined for reactivity with the immune serum after transfer to nitrocellulose (Towbin et al., 1979). In all experiments, the proteins were blocked overnight at 4°C with 20% calf serum in PBS, washed, incubated at 4°C overnight with serum diluted in PBS, washed, incubated at 4°C overnight with peroxidase-conjugated goat anti-rabbit IgG (Tago Inc., Burlingame, CA) diluted 1:1,000 in PBS, and developed with 0.03% 4-chloro-1-naphthol (Sigma Chemical Co., St. Louis, MO) and 0.006% H<sub>2</sub>O<sub>2</sub> (Sigma Chemical Co.) in methanol/PBS (10:90). All washes were performed at room temperature and consisted of three 10-min rinses with gentle agitation in PBS containing 0.05% Tween.

## Immunofluorescence

Glass slides were washed consecutively in Chromerge, water, 95% ethanol, and acetone and were air dried. Small drops (20  $\mu$ l) of cytoskeletons suspended in TEDAMP buffer were placed on the slides that then were incubated in a humid chamber at room temperature. Each slide was washed three times, incubated with BSA (40  $\mu$ l at 1 mg/ml in PBS) for 15 min, washed, and incubated with preimmune or immune serum (each diluted 1:40 or 1:80) in PBS for 15 min. After washing, the slides were incubated in rhodamine-conjugated goat anti-rabbit IgG (Boehringer Mannheim Biochemicals, Indianapolis, IN; diluted 1:100) for 15 min, washed, and cover-

slipped with 10% glycerol in PBS as the mounting medium. To detect tubulin, sheep anti-tubulin (6S bovine brain tubulin), and fluorescein-conjugated rabbit anti-sheep IgG (both from Southern Biotechnology Associates, Birmingham, AL) were used as above at dilutions recommended by the suppliers.

Sections were examined with an Orthoplan microscope (E. Leitz, Inc., Wetzlar, FRG) equipped with epifluorescence, an H<sub>2</sub> cube for fluorescein, and an N<sub>2</sub> cube for rhodamine. Photographs were taken with a Vario orthomat camera (E. Leitz, Inc.) on Tri-X film (Eastman Kodak Co.) that was exposed with an ASA setting of 1,600 and developed in Diafine.

### Transmission Electron Microscopy

For routine transmission microscopy, intact *Giardia*, minimally  $1.4 \times 10^5$  organisms, were fixed in mixed aldehydes consisting of 1.5% formaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at a fixative-to-medium ratio of 2:1 for 1–2 h at 4°C. Organisms were pelleted in a centrifuge (Microfuge B; Beckman Instruments, Inc., Palo Alto, CA), and the pellets were postfixed for 1.5 h in OsO<sub>4</sub> buffered in acetate veronal, stained in block for 2 h in uranyl acetate, dehydrated, and embedded in Epon 812.

Cytoskeleton preparations were fixed by a similar technique. In some experiments, however, the cytoskeletons were pelleted at 15,000 g, and the pellet was fixed in the tube in mixed aldehydes for 1 h at 4°C, postfixed, and processed as above. Cytoskeletons also were fixed in tannic acid by the method of Simionescu and Simionescu (1976).

For frozen thin section immunocytochemistry, the organisms were fixed in 4% formaldehyde in 0.1 M phosphate, pH 7.4, for 1 h at 4°C and stored in 2% formaldehyde and 1 M sucrose in phosphate buffer. The *Giardia* then were embedded in gelatin, refixed, infiltrated with sucrose, frozen, cut on glass knives with an ultramicrotome (Cryonova; LKB Instruments, Inc., Gaithersburg, MD), picked up on formvar-coated grids, and immunostained. The cryoimmunocytochemistry was performed initially in the laboratory of Drs. J. Slot and H. Geuze at the University of Utrecht (Utrecht, The Netherlands) and subsequently in Boston on a microtome provided by LKB Instruments, Inc. Sections from all types of preparations were viewed in a transmission electron microscope (100C/ASID; JEOL USA, Peabody, MA) operating at 80 kV.

Sections from gelatin-embedded parasites were stained by the methods of Geuze et al. (1981) and Griffiths et al. (1984). Serum from the rabbit immunized with giardin was used at a 1:1,000 dilution, while preimmune serum from the same animal and rabbit anti-tubulin (sea urchin; Polysciences, Inc., Warrington, PA) were used at a 1:100 dilution. All antibodies were detected with a 1:30 dilution of protein A-gold conjugated with gold of 5 or 8 nm diameter (Janssen Life Sciences Products, Piscataway, NJ).

### DNA Libraries

*G. lamblia* DNA was eluted from hydroxylapatite. Pelleted, frozen trophozoites lysed in 8 M urea, 0.16 M sodium phosphate, pH 6.8, 0.01 M EDTA, 1% SDS were extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (24:1). The aqueous layer was heated at 65°C for 15 min and loaded onto hydroxylapatite (Bio-Rad Laboratories, Richmond, CA) equilibrated with 8 M urea, 0.16 M sodium phosphate, pH 6.8. The DNA was eluted after RNA elution (see RNA Selection, In Vitro Translation, and Immunoprecipitation) using 0.48 M sodium phosphate, pH 6.8, digested under limiting conditions with the restriction endonuclease Sau 3A, and fractionated by size on a 15–40% sucrose gradient. The gradients, made in 1 M NaCl, 20 mM Tris-HCl, pH 8, 5 mM EDTA, were spun in a rotor (SW 41; Beckman Instruments, Inc.) at 125,000 g for 24 h at 20°C. The fractions containing DNA 14–20 kb in length were identified by agarose gel electrophoresis, pooled, and precipitated. The DNA was ligated into the Bam HI site of the polylinker within the lambda vector EMBL3 (Frischauf et al., 1983) and then was packaged (Gigapack; Stratagene, La Jolla, CA) into phage particles (Hohn and Murray, 1977). The phage were plated on *Escherichia coli* strain LE392 to assess the total number of phage and on *E. coli* strain P2392 to assess the total number of recombinant phage (Hohn and Murray, 1977).

### Isolation of a Clone for Alpha Giardin

The 33-kD giardins were purified from total *G. lamblia* cytoskeletal proteins using a one-dimensional polyacrylamide gel as described by Hunkapiller et al. (1983). 29 amino acids were determined from the amino terminus of the 33-kD proteins by Edman degradation on a 470-A gas phase sequencer with an on-line 120-A PTH analyzer (Applied Biosystems). An oligonucleotide

47 nucleotides in length and  $2.4 \times 10^6$ -fold degenerate, 5' AA[C,T]-GA[A,G]-[C,T]T[A,C,G,T]-AA[A,G]-CA[A,G]-GC[A,C,G,T]-AT[A,C,T]-GA[C,T]-GC[A,C,G,T]-AA[A,G]-GA[C,T]-GA[A,G]-GT[A,C,G,T]-CA[A,G]-AT[A,C,T]-GC 3', was constructed on a DNA synthesizer (8700; Biosearch, San Rafael, CA). A total of 20,000 recombinant phage were plated on *E. coli* strain LE392, at ~5,000 per plate, and the plaques were amplified in size on nitrocellulose filters (Woo et al., 1978) before denaturing, neutralizing, and baking (Benton and Davis, 1977). The filters were washed for 2 h at 42°C in 50 mM Tris-Cl, pH 8, 1 M NaCl, 1 mM EDTA, 0.1% SDS and then prehybridized for 4 h at 42°C in 0.9 M NaCl, 0.9 M sodium citrate (6× SSC), 0.1% each of Ficoll, polyvinylpyrrolidone, and BSA (5× Denhardt's solution [Denhardt, 1966]), 50 mM sodium pyrophosphate, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA. The filters were hybridized at 42°C overnight with  $4 \times 10^6$  cpm/ml of <sup>32</sup>P-labeled oligonucleotide. The hybridization solution consisted of 6× SSC, 5× Denhardt's solution, 50 mM sodium pyrophosphate, and 10% dextran sulfate (Sigma Chemical Co.). The oligonucleotide was labeled with gamma-<sup>32</sup>P-ATP using T4 polynucleotide kinase (Maniatis et al., 1982). After washing them extensively with 6× SSC at 50°C, the filters were exposed to X-OMAT x-ray film (Eastman Kodak Co.) with intensifying screens (Cronex Lightning Plus; DuPont Co.) 14 strongly hybridizing plaques were selected and purified by replating three times (Maniatis et al., 1982).

### DNA Sequencing and Database Comparison

A 1.3-kb fragment, generated with Hind III and Sal I, that came from a selected clone and that hybridized (Southern, 1975) with the synthetic DNA oligomer was subcloned into M13mpl8 and M13mpl9 (Messing, 1983) and sequenced in its entirety. The 1.3-kb fragment terminated at a Sal I site within the giardin gene (GTCGAC at nucleotide 766; Fig. 7), so another DNA oligomer (5' ATA-CCT-GAT-CAA-CTG-CGC-CT 3'; nucleotides 696–715; Fig. 7) was synthesized and used as a hybridization probe on Sph I-generated DNA fragments from the genomic clone. A 1.1-kb fragment that hybridized to this second oligomer was subcloned into M13mpl8 and M13mpl9 for sequencing. All DNA sequence determination was accomplished by the dideoxy chain termination method (Sanger et al., 1977) using <sup>35</sup>S-alpha-dATP and 60-cm buffer gradient polyacrylamide gels (Biggin et al., 1983). Deoxy-7-deazaguanosine triphosphate was used to resolve compressions due to high G and C content (Mizusawa et al., 1986), and DNA oligomers were synthesized as necessary to act as primers. Both DNA strands were sequenced completely, between two and ten times each. All sequences were compiled using the Staden-Plus DNA software (Amersham Corp., Arlington Heights, IL). The nucleotide sequence of alpha giardin was compared with version 58 of the GenBank (Bilofsky and Burks, 1988) database, and the deduced protein sequence was compared with version 18 of the Protein Identification Resource (George et al., 1986). All computer searches were performed at the facilities of the Molecular Biology Computer Research Resource Center (Dana-Farber Cancer Institute and Harvard School of Public Health, Boston, MA) and used a high-speed similarity search program (DASHER; D. V. Faulkner and T. F. Smith, Molecular Biology Computer Resource Center [Dana-Farber Cancer Institute and Harvard School of Public Health], 1987) based on a modified Wilbur-Lipman algorithm (Wilbur and Lipman, 1983).

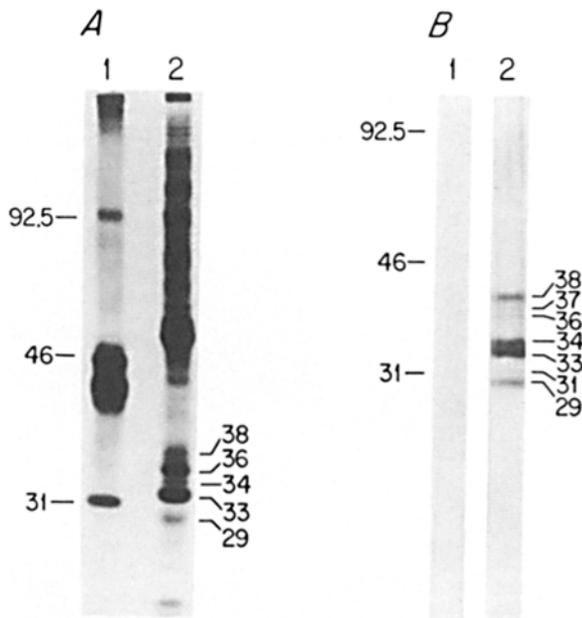
### RNA Selection, In Vitro Translation, and Immunoprecipitation

Total *G. lamblia* RNA was eluted off hydroxylapatite with 0.19 M sodium phosphate, pH 6.8, before DNA elution. Hybrid selection was performed as described by Kafatos et al. (1981) with the 1.3-kb alpha giardin gene subclone using 1 mg/ml of total RNA. In vitro translations of the selected RNA were performed in rabbit reticulocyte lysates (Promega Biotec, Madison, WI) using [<sup>35</sup>S]methionine, and immune serum was used to precipitate immunoreactive proteins (Anderson and Blobel, 1983).

### Results

#### Total Cytoskeletal Proteins and Immune Serum to Alpha Giardin

Cytoskeletons prepared from *G. lamblia* labeled in vivo with [<sup>35</sup>S]methionine and solubilized under reducing conditions



**Figure 1.** One-dimensional separation and immunoblot analysis of *G. lamblia* cytoskeletal proteins. (A) One-dimensional reducing SDS-PAGE separation (10% acrylamide) of *G. lamblia* cytoskeletal proteins labeled in vivo with [<sup>35</sup>S]methionine reveals five giardin bands. (Lane 1) <sup>14</sup>C-Labeled marker proteins; (lane 2) total cytoskeletal proteins. (B) One-dimensional immunoblot analysis of *G. lamblia* cytoskeletal proteins shows that polyclonal antibodies to giardins 8, 8a, and 8b (see Fig. 2, A and B) react with the original antigen as well as with other giardins. The cytoskeletons were separated as in A before immunoblotting and were visualized with peroxidase-conjugated goat anti-rabbit IgG developed with H<sub>2</sub>O<sub>2</sub> and 4-chloro-1-naphthol. (Lane 1) Preimmune serum diluted 1:20,000 in PBS; (lane 2) immune serum diluted 1:20,000 in PBS. Molecular masses are shown in kilodaltons.

contained proteins ranging from >200 kD to ~20 kD when examined by one-dimensional SDS-PAGE. The most prominent proteins were the tubulins around 50 kD and the proteins grouped around 30 kD that have been termed the giardins (Crossley and Holberton, 1983a; Fig. 1 A). Five giardin bands were detected and assigned relative molecular mass values of 38, 36, 34, 33, and 29 kD (Fig. 1 A) based on migration relative to molecular mass standards. The most abundant giardin, as detected by Coomassie brilliant blue (data not shown) and silver staining (data not shown) as well as by autoradiography, was the 33-kD giardin (Fig. 1 A).

[<sup>35</sup>S]Methionine-labeled total cytoskeletal proteins were separated further by charge and molecular mass using two-dimensional PAGE (O'Farrell, 1975; Jones, 1980). There were three major groups of proteins: those >70 kD, those in the tubulin size range of 50 kD, and those in the giardin cluster between 29 and 38 kD (Fig. 2 A). The majority of cytoskeletal proteins were acidic, with isoelectric points between pH 5 and 6. Table I details the molecular mass and isoelectric point for each of these proteins. Sixteen proteins had an isoelectric point between 5 and 5.9, while the remaining seven had isoelectric points between 6 and 6.5.

The 33-kD giardin band was removed from a one-dimensional gel and used to immunize rabbits. The immunogen contained proteins 8, 8a, and 8b, of which 8 was the most abundant (Fig. 2 B). The resulting antibodies reacted with

proteins in the size range of the giardins but not with higher relative molecular mass species. Diluted as high as 1:20,000 in PBS, the immune serum reacted with seven proteins on one-dimensional immunoblots with relative molecular mass values of 38, 37, 36, 34, 33, 31, and 29 kD (Fig. 1 B). Five of these bands, at 38, 36, 34, 33, and 29 kD, corresponded to the discrete giardins visible by autoradiography in Fig. 1 A. The antibodies reacted with 17 proteins separated by two-dimensional PAGE (Fig. 2 C). Fourteen of these (proteins 1a, 2, 3, 4, 5b, 8, 8a, 8b, 8c, 8d, 8e, 9, 9a, and 9b) also were visible by autoradiography (Fig. 2 A), while three (proteins 2a, 2b, and 9c) were seen only by immunoblot analysis. Of the seventeen immunoreactive proteins, all except two (1a and 5b) had isoelectric points between 5 and 6 (Fig. 2 C).

### Localization of Giardins and Tubulin in the Sucking Disk: Immunofluorescence and Electron Microscopy

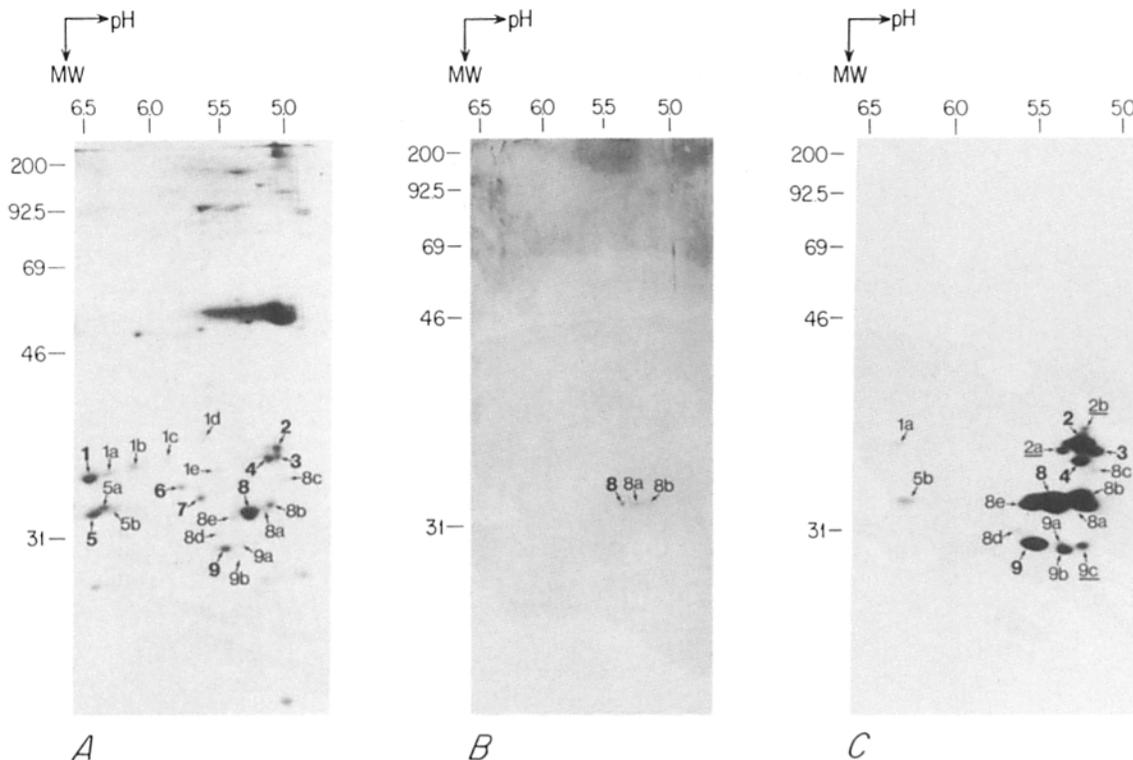
As described by others (Holberton, 1973; Holberton and Ward, 1981), the disk cytoskeleton consists of three basic structures: parallel, roughly concentric microtubules located adjacent to the ventral plasma membrane; microribbons extending from the microtubules into the cytoplasm; and electron-dense crossbridges that connect adjacent microribbons (Fig. 3, A and inset). In cross section by transmission electron microscopy, the disk cytoskeleton resembled a picket fence along the ventral plasma membrane of the disk (Fig. 3 A). Isolated cytoskeletons consisted of disks and flagella (Fig. 3 B).

By immunofluorescence, the immune serum reacted with the ventral disk but not with the flagella in isolated cytoskele-

**Table I. Molecular Masses and Isoelectric Points of 23 Giardins Separated in Two Dimensions**

Protein*	Molecular mass	Isoelectric point
	<i>kD</i>	
1	35.5	6.52
1a	36	6.42
1b	36.5	6.25
1c	36.5	6.02
1d	39	5.65
1e	36	5.58
2	38	5.18
3	37	5.16
4	36.5	5.20
5	33	6.53
5a	34	6.43
5b	34	6.20
6	34.5	5.91
7	33.5	5.76
8	33	5.37
8a	33	5.24
8b	33.5	5.22
8c	34	5.05
8d	31	5.58
8e	32.5	5.53
9	29	5.55
9a	29.5	5.38
9b	28	5.33

\* These numbers correspond to the proteins identified by two-dimensional PAGE (see Fig. 2 A) and follow the convention for HeLa cells (Bravo et al., 1981; Bravo and Celis, 1982).



**Figure 2.** Two-dimensional separations and immunoblot analysis of *G. lamblia* cytoskeletal proteins. (A) The giardins separate into 23 distinct proteins between 38 and 29 kD using two-dimensional PAGE to separate *G. lamblia* cytoskeletal proteins labeled in vivo with [<sup>35</sup>S]methionine. The proteins were numbered following the convention used for HeLa cells (Bravo et al., 1981; Bravo and Celis, 1982). (B) The immunogen contained proteins 8, 8a, and 8b when separated in two dimensions and visualized by silver staining. Protein 8 was the most abundant. (C) 17 *G. lamblia* cytoskeletal proteins react with antibodies against giardins 8, 8a, and 8b when cytoskeletal proteins are separated in two dimensions, immunoblotted with immune serum (diluted 1:20,000 in PBS), and localized with peroxidase-conjugated goat anti-rabbit IgG. The numbered proteins correspond to those in A; the numbers with underlines denote those identified by immunoblot but not by autoradiography, silver stain, or Coomassie staining. The second dimension gels were 15% polyacrylamide in A and B and 12.5% in C. All molecular masses are shown in kilodaltons.

tons (Fig. 4 A). Immune serum did not react with intact *Giardia*, and preimmune serum did not bind to the *Giardia* cytoskeleton. Anti-tubulin antibodies reacted with both the disk and the flagella in isolated cytoskeletons (Fig. 4 B).

By electron microscopy, frozen thin sections of cross sectioned disks stained with the immune serum and colloidal gold revealed gold binding throughout the full extent of the microribbons—i.e., from the basal microtubules to the tip facing the parasite cytoplasm (Fig. 5 A). There was no binding to the microtubules themselves, and most gold particles bound to the ribbons rather than in the interspace traversed by the crossbridges. Tangentially sectioned disks stained with the immune serum showed gold bound primarily to microribbons across the full width of the sectioned disk (Fig. 5 B). Due to the curvature of the disk (the lesser curvature faces the inside or center of the organism, while the greater curvature faces the outside edge), a sidedness could be established and used to localize the structures containing giardin more precisely. A total of 805 gold particles falling on the disk were counted; they were scored as falling on the outer edge of the microribbons, the center of the microribbons, the inner edge of the microribbons, or the crossbridges. These structures held 34, 18, 38, and 10% of the gold particles, respectively. Thus, 90% of the gold falling on disks fell on the microribbons, with equal numbers (totaling 72%) bound to the inner and outer edges.

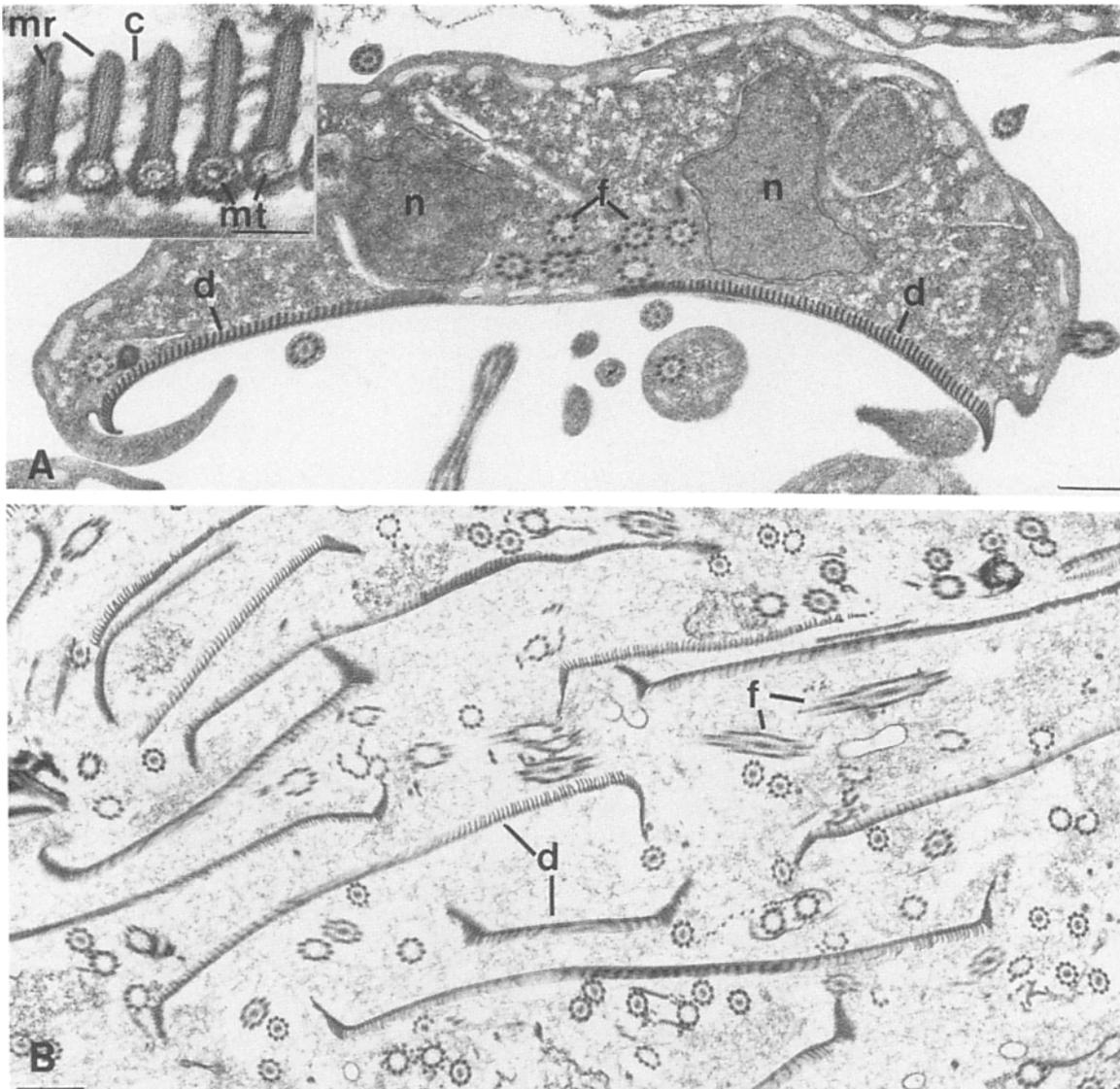
Anti-tubulin antibodies bound to the disks with a different distribution than anti-giardin antibodies. Gold bound to the microtubular end of the microribbons but not to the microribbons or crossbridges (Fig. 5 C). Control preimmune serum did not localize to the disk (Fig. 5 D) or to other giardial structures.

#### *Amino-terminal Sequence of Alpha Giardin and Isolation of an Alpha Giardin Clone*

A stretch of 29 amino acids, KVTDIANELKQAIKAKDEV-QIAFXA(S,W)EYS, was identified by Edman degradation and gas phase chromatography from the amino terminus of the antigen. 27 positions were unambiguous while 2 were indefinite (X was unidentifiable and [S,W] was either S or W). A DNA oligomer, 47 nucleotides in length and  $2.4 \times 10^6$ -fold degenerate, was synthesized based on 16 contiguous amino acids in the sequence and used to select 14 clones from a *G. lamblia* genomic DNA library. One of these clones was used to determine the complete nucleotide sequence of the alpha giardin gene.

#### *RNA Selection, In Vitro Translation, and Immunoprecipitation*

In vitro translations of *G. lamblia* RNA selected with a 1.3-kb subclone (see DNA Sequencing and Database Compari-



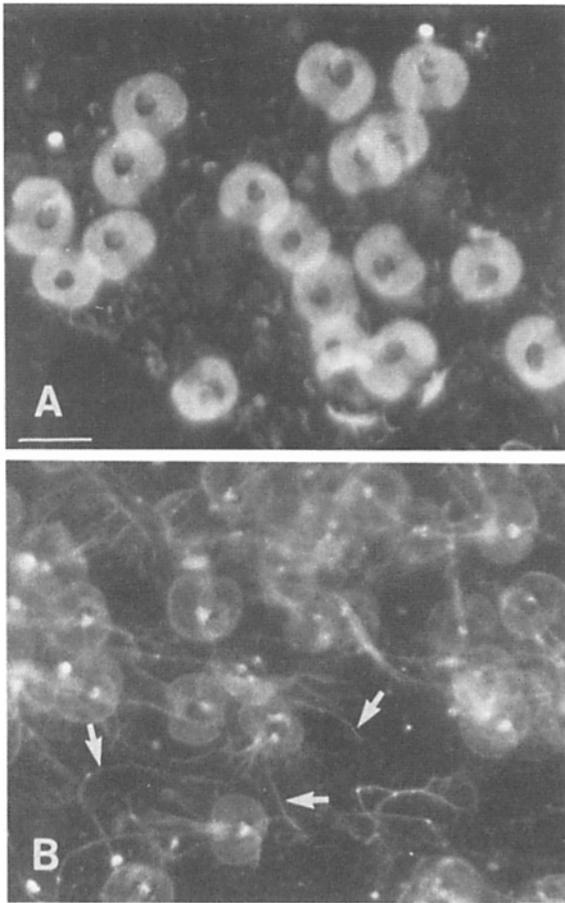
**Figure 3.** Transmission electron micrographs of *G. lamblia*. (A) An intact parasite contains two nuclear profiles (*n*) and cross sections of the flagella (*f*) and the ventral disk (*d*) along the ventral edge. The inset shows a ventral disk fixed in tannic acid at higher magnification. Note the microtubules (*mt*), microribbons (*mr*), and crossbridges (*c*). (B) A cytoskeleton preparation contains only disks and flagella. Bars: (A) 0.64  $\mu\text{m}$ ; (inset) 48 nm; (B) 0.74  $\mu\text{m}$ .

son in Materials and Methods) of the alpha giardin gene produced major protein products at 33 and 29 kD as well as endogenous reticulocyte proteins (Fig. 6, compare lane 3 with lane 9). The 33-kD product, but not the 29-kD product, was precipitated by the immune serum (Fig. 6, lane 4). A 41-kD product, apparently specific to translation of *G. lamblia* RNA but obscured by endogenous reticulocyte protein(s) of the same molecular mass, also was precipitated by the immune serum (Fig. 6, compare lanes 3, 4, and 9). Preimmune serum precipitated no in vitro translation products (Fig. 6, lane 6), and postimmune serum did not precipitate endogenous rabbit reticulocyte in vitro translation products (Fig. 6, lane 7). Immune serum precipitated the 33-kD giardin and, to a lesser degree, products at  $\sim 38$  and 200 kD directly from *G. lamblia* cytoskeletons labeled in vivo with [ $^{35}\text{S}$ ]methionine (Fig. 6, lane 5). Preimmune serum precipitated a protein  $>200$  kD from total cytoskeletons (Fig. 6, lane 8). Thus,

the alpha giardin subclone selects RNA that can be translated into a 33-kD protein that immunoprecipitates with antibodies raised against giardins 8, 8a, and 8b.

#### **The Alpha Giardin Gene Sequence and Deduced Protein Sequence**

The open reading frame of the alpha giardin gene continues for 882 nucleotides beyond the initial methionine (Fig. 7). Then there are two TAG stop codons separated by 18 nucleotides. The predicted protein is 295 amino acids (33,800 D) in size and contains many helix-forming residues such as alanine, glutamic acid, and leucine (Chou and Fasman, 1978; Table II). The 29 amino acids originally identified by Edman degradation are encoded by nucleotides 7–93 and correspond to amino acid residues 3–31 (Fig. 7, **bold characters**). The deduced protein sequence indicates that the unidentified



**Figure 4.** Fluorescent antibody staining of *G. lamblia* cytoskeletons. (A) Isolated *Giardia* cytoskeletons stained with antibodies against the 33-kD giardins. (B) Cytoskeletons stained with sheep anti-tubulin IgG. Note that the anti-giardin antibodies in A stain the ventral disks, which appear as doughnut-shaped structures, but not the flagella (arrows), while the anti-tubulin antibodies in B react both with disks and flagella. Bar, 8.6  $\mu$ m.

amino acid (X) in the original Edman degradation is isoleucine (I) and that the residue originally identified as S or W is serine (S). The degenerate DNA oligomer originally used to select the alpha giardin clone is homologous to nucleotides 25-71 (Fig. 7). Based on secondary structure algorithms (Chou and Fasman, 1978; Garnier et al., 1978), alpha giardin is 82% alpha helix, 2.7% beta sheet, 6.4% reverse turn, and 8.8% random coil. Although two of the predicted alpha helices (residues 48-59 and 275-294) have high alpha moments (Eisenberg et al., 1982, 1984), they are not amphipathic on helical wheels (Schiffer and Edmundson, 1967), indicating that the hydrophobic and hydrophilic residues are not localized strictly to opposite sides of the predicted helix.

The alpha giardin gene was scanned against version 58 (21,248 sequences and 24,690,876 bases) of the GenBank (Bilofsky and Burks, 1988) database, but no significant homology was found. Similarly, the deduced protein sequence of alpha giardin was compared with all sequences in version 18 (9,138 sequences and 2,377,999 residues) of the Protein Identification Resource (George et al., 1986), but no significant homology was identified. There was no significant homology to the deduced protein sequences of beta (Baker et

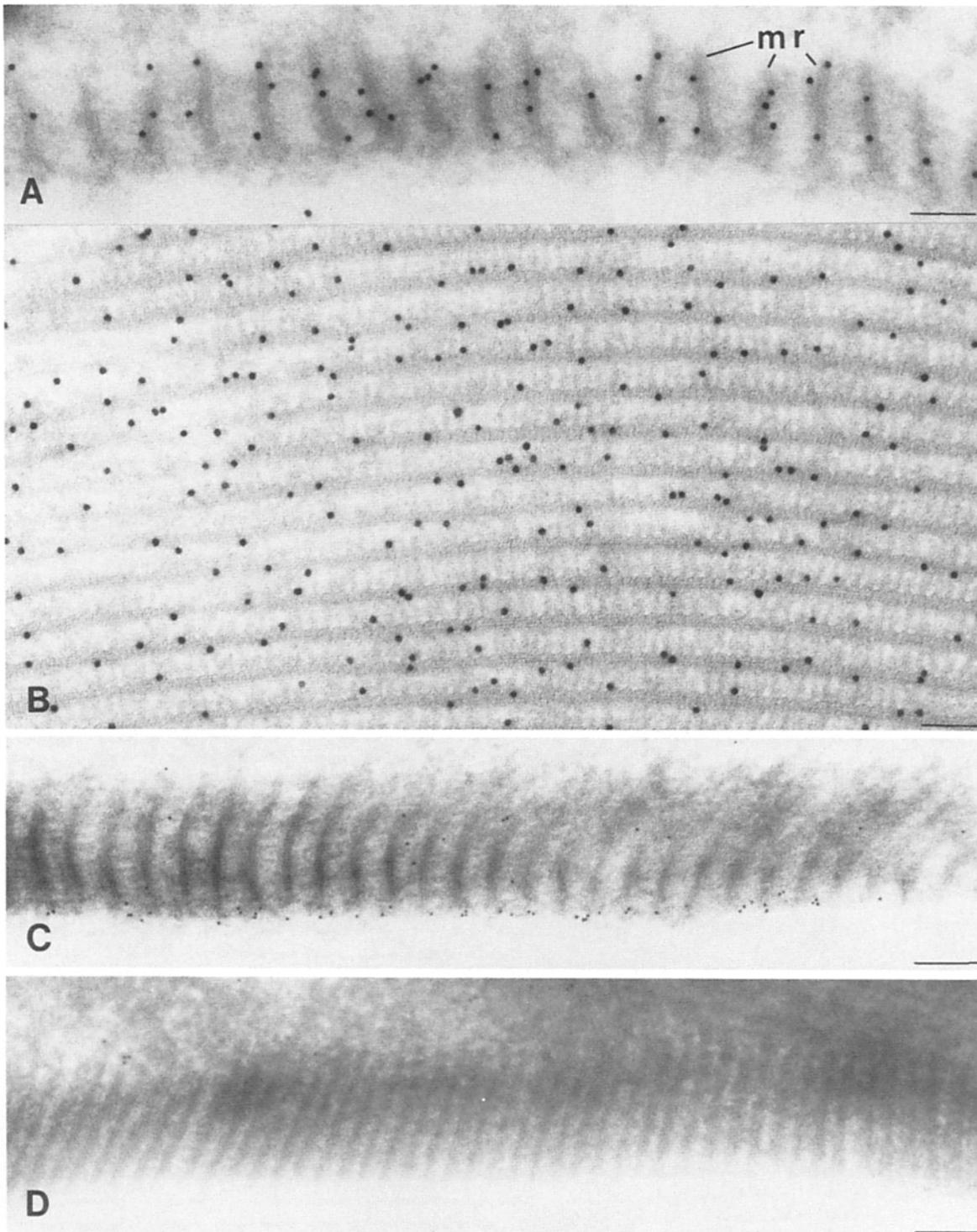
al., 1988) and beta-1 (Aggarwahl and Nash, 1989) giardin, but the nucleotide sequence AGTGAA (positions 895-900; Fig. 7) correlated well with the sequence AGTAAA that is found downstream of these other giardin genes and downstream of the *G. lamblia* beta tubulin gene (Kirk-Mason et al., 1988).

## Discussion

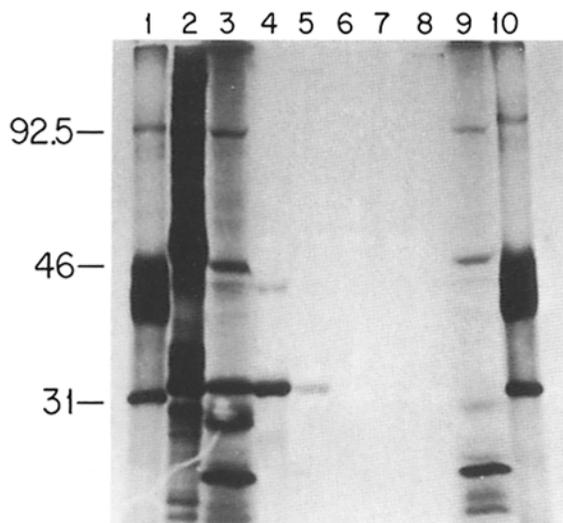
Giardins were localized to the microribbons within the ventral disk of *G. lamblia* using polyclonal antibodies to a 33-kD protein band. The band was isolated from a one-dimensional gel and contained three peptides by two-dimensional analysis. The antibodies reacted with 17 proteins in the giardin cluster, indicating the presence of shared epitope(s) on the different proteins, and bound to the inner and outer edges of the microribbons within the sucking disk (Fig. 8). They did not bind to disk crossbridges or microtubules or to the flagella. The alpha giardin gene was isolated using a synthetic DNA oligomer (derived from the amino-terminal sequence of the immunogen), and its nucleotide sequence was determined in its entirety. Alpha giardin, as deduced from the gene sequence, is predicted to be 295 amino acids in length, to have a relative molecular mass of 33.8 kD, and to be 82% alpha helical. Comparison of the three published sequences of *G. lamblia* protein-encoding genes with that of the alpha giardin gene indicates that the sequence AGTPuAA is located six to nine nucleotides beyond the stop codon in each gene. Finally, alpha giardin shows no significant homology to beta giardin (Baker et al., 1988), to beta-1 giardin (Aggarwahl and Nash, 1989), or to any sequence within version 18 of the Protein Identification Resource (George et al., 1986).

The initial description of giardin defined it as a doublet of  $\sim 30$  kD by one-dimensional SDS-PAGE (Holberton and Ward, 1981; Crossley and Holberton, 1983). More recently, five bands have been described (Clark and Holberton, 1988) with molecular masses similar to those shown in Fig. 1 B. Two-dimensional gels have resolved these five bands into 11 (Clark and Holberton, 1988) to 23 (Fig. 2 A) species. Furthermore, two-dimensional analysis of isolated *Giardia* flagella (Clark and Holberton, 1988) has revealed proteins of similar molecular mass and charge to those in the 31-37-kD region between isoelectric points 6 and 6.5 (Fig. 2 A). Thus, the protein pattern in the 30-kD region is complex, containing proteins derived from the disk, flagella, or, perhaps, both. Despite this, there must be a common epitope among some of these proteins because antibodies raised against three of them recognize 17 proteins by immunoblotting. The number of epitopes shared by these proteins is unknown, although the major antigens all must be located in the disk microribbons and not the flagella. Based on this information, we consider the giardins to be those cytoskeletal proteins of *G. lamblia* that are found in the ventral disk and that have a relative molecular mass in the range of 29-38 kD.

Immunocytochemical localization of anti-giardin and anti-tubulin antibodies indicates that the giardins are located along the edges of the microribbons in equal amounts on the inside and outside edges, while tubulin is present only in the microtubule between the microribbon and the plasma membrane (Fig. 8). Further, there is a region in the center of the microribbon that contains less giardin than the edges, and giardins apparently are absent from the basal microtubule



**Figure 5.** Immunostained frozen thin sections. (A) A disk stained with the immune serum and gold-labeled protein A shows gold bound to the microribbons (*mr*) but not to the base of the ribbon where the microtubules are located. Here, as in C and D, the disks are oriented so the cytoplasmic side is up and the microtubules are down. (B) A cross section through a disk stained as in A shows most of the gold is bound to the microribbons and not to the cross bridges. (C) A disk stained with anti-tubulin and protein A-gold demonstrates that gold binds to the base of the microribbons where the disk microtubules are located. The few gold particles over the microribbons and cross-bridges are nonspecific. (D) A control stained with preimmune serum and colloidal gold as in A and B has no gold over the disk. Bars: (A) 87 nm; (B) 85 nm; (C) 122 nm; (D) 122 nm.



**Figure 6.** In vitro translation and immunoprecipitation of selected *G. lamblia* RNA. A subclone of the alpha giardin gene selects RNA from *G. lamblia* that produces a 33-kD protein product in vitro that immunoprecipitates with serum antibodies to the 33-kD giardins. (Lane 1)  $^{14}\text{C}$ -Labeled protein markers; (lane 2) cytoskeletal proteins from *G. lamblia* labeled in vivo with  $^{35}\text{S}$ methionine; (lane 3) in vitro translation products of RNA selected with the 1.3-kb alpha giardin gene subclone; (lane 4) immunoprecipitation of the translation products using immune rabbit serum; (lane 5) immunoprecipitation of total cytoskeletal proteins using immune rabbit serum; (lane 6) immunoprecipitation of the translation products using preimmune rabbit serum; (lane 7) immunoprecipitation of the endogenous reticulocyte translation products (no *G. lamblia* RNA) using immune serum; (lane 8) immunoprecipitation from total cytoskeletal proteins from *G. lamblia* labeled in vivo with  $^{35}\text{S}$ methionine using preimmune rabbit serum; (lane 9) endogenous reticulocyte translation products; (lane 10)  $^{14}\text{C}$ -labeled protein markers.

and the crossbridges. These findings agree in general with previous work that showed that rabbit antibodies raised against the originally described giardin doublet bind to the free cytoplasmic surface of isolated disks, whereas monoclonal antibodies against tubulin bind to the basal microtubule (Crossley et al., 1986). Portions of the model proposed by Holberton and coworkers (Holberton, 1981; Holberton and Ward, 1981; Crossley et al., 1986), namely that each microribbon is composed of giardin and tubulin with the giardin central and the tubulin lateral, are questionable, however. The immunochemical technique used here—i.e., the on-grid staining of frozen thin sections of *G. lamblia*—provides access to the entire length of the microribbons because the intact organisms were fixed, embedded, and sectioned before immunostaining. The anti-giardin antibodies clearly bind preferentially to the edges of the microribbons, not to the center, and extend from the cytoplasmic surface to the basal microtubule. Conversely, the anti-tubulin antibodies bind only to the basal microtubule and not to the microribbon, the same result obtained by Crossley et al. (1986). Thus, the simplest explanation of the current immunocytochemical data is that tubulin is not present in the microribbons and that giardin is on the edges, not central.

RNA hybrid selected by a subclone of the alpha giardin gene and translated in vitro produces a product of the correct

size (33 kD) for alpha giardin that immunoprecipitates with antibodies to the 33-kD protein band (Fig. 6, lane 4). In addition, the sequence of the cloned gene predicts the 29 amino acids originally identified at the amino terminus of the immunogen (Fig. 7, *bold characters*). Thus, the isolated gene encodes alpha giardin and contains the information for one or more of its antigenic determinants. The cloned gene most likely encodes protein 8 since this is the most abundant species of the original 33-kD protein band (Fig. 2 B) and is, therefore, the one most likely to produce residues detectable by Edman degradation and gas chromatography. The integrity of the chromatographic signal that was obtained following Edman degradation in the sequence analysis of the amino terminus of the 33-kD proteins and preliminary evidence indicating that proteins 8, 8a, and 8b produce identical tryptic digests (Peattie, D., unpublished observations) suggest that the three proteins might be species of alpha giardin with different isoelectric points due to posttranslational modification such as phosphorylation.

The sequence of the alpha giardin gene has 59.5% G and C residues. This value correlates well with the G and C content of two other giardin sequences, beta giardin (59.5%; Baker et al., 1988) and beta-1 giardin (58.9%; Aggarwala and Nash, 1989), but is markedly lower than that of the *G. lamblia* genes encoding the 5.8S ribosomal RNA (76%; Boothroyd et al., 1987), the 16S-like ribosomal RNA (75%; Sogin et al., 1989), and beta tubulin (66%; Kirk-Mason et al., 1988). The 885 bp of open reading frame contain no introns relative to cDNA sequence (Peattie, D. A., and R. A. Alonso, unpublished observations). The alpha giardin gene sequence has no significant homology to DNA sequences within the GenBank database (Bilofsky and Burks, 1988) or to those encoding the proteins beta (Baker et al., 1988) and beta-1 (Aggarwala and Nash, 1989) giardin. It does, however, have a short region homologous to a sequence that is present just beyond the genes encoding beta giardin, beta-1 giardin,

**Table II. Predicted Amino Acid Composition of Alpha Giardin**

Amino acid	Occurrences	Percent of total
Ala, A	33	11.2
Arg, R	19	6.4
Asn, N	6	2.0
Asp, D	25	8.5
Cys, C	7	2.4
Gln, Q	8	2.7
Glu, E	22	7.5
Gly, G	14	4.8
His, H	8	2.7
Ile, I	18	6.1
Leu, L	20	6.8
Lys, K	25	8.5
Met, M	13	4.4
Phe, F	13	4.4
Pro, P	9	3.1
Ser, S	12	4.1
Thr, T	14	4.8
Trp, W	4	1.4
Tyr, Y	13	4.4
Val, V	12	4.1

M P K V T D I A N E L K Q A I D A K D E V Q I A F I A S E Y  
 ATG CCG AAG GTC ACC GAC ATT GCG AAC GAG CTC AAG CAG GCC ATC GAT GCC AAG GAC GAG GTC CAG ATC GCG TTC ATA GCG TCG GAA TAC  
 AAY GAR YTN AAR CAR GCH ATX GAY GCH AAR GAY GAR GTN CAR ATX GC

30  
90

S A E S R E K I A K A Y V A S Y G K E L P D D I K K A L K  
 TCC GCA GAG TCG CGT GAA AAG ATT GCG AAG GCC TAC GTT GCC TCC TAC GGC AAG GAG CTC CCC GAC GAC ATC AAG AAG GCG CTC AAG GGC  
 180

60  
180

G S E E S L L M D L F S D R H E V R A Q H I R D A L S G R N  
 GCC TCC GAG GAG AGC CTC CTG ATG GAT CTC TTC AGC GAT CGT CAC GAG GTC CGC GCC CAG CAC ATC CGC GAC GCG CTT TCC GGC AGG AAC  
 270

90  
270

D H M A F F D T V I L C T P E D W H E T V A A Y T R M F K K  
 GAC CAC ATG GCC TTC TTC GAC ACA GTC ATT CTG TGC ACC CCC GAA GAC TGG CAC GAG ACC GTC GCC GCC TAC ACG AGG ATG TTC AAG AAG  
 360

120  
360

P L V E D F M K D V G R K E D W C L L M E K W M A H E R V S  
 CCC CTC GTC GAG GAC TTC ATG AAG GAT GTC GGC CGC AAG GAG GAC TGG TGC CTG CTT ATG GAG AAG TGG ATG GCT CAC GAG CGC GTC TCC  
 450

150  
450

R P G S P E D E A Q R L D Q A F D Q K N T A Y L I D F F G T  
 CGC CCC GGC TCT CCG GAG GAC GAG GCC CAG CGT CTG GAT CAG GCC TTC GAC CAG AAG AAT ACT GCT TAT CTC ATC GAC TTC TTC GGC ACC  
 540

180  
540

V P S A E Y R P I A E A F K A Q N G K S I E Q A I A T I Y T  
 GTC CCG AGT GCG GAG TAC AGG CCC ATC GCC GAG GCT TTC AAG GCC CAG AAC GGC AAG AGC ATC GAG CAG GCT ATC GCC ACC ATC TAC ACG  
 630

210  
630

K T D Y Y T F Y C A H F A L L G M H R L A A Y L I N C A C N  
 AAG ACG GAC TAC TAT ACT TTC TAC TGC GCC CAC TTC GCC CTC CTC GGC ATG CAC AGG CTT GCG GCA TAC CTG ATC AAC TGC GCC TGC AAT  
 720

240  
720

D K G D E K R M R R I T G M M V D K C L G A K H A Y K I Y G  
 GAC AAG GGC GAC GAG AAG CGC ATG AGG AGG ATC ACG GGC ATG ATG GTC GAC AAG TGC CTC GGG GCG AAG CAC GCC TAC AAG ATC TAC GGT  
 810

270  
810

D M G T D I E R C F D K R M A P I L R T L W R V K \* G F S E  
 GAC ATG GGC ACA GAC ATC GAG AGA TGC TTC GAC AAG CGC ATG GCC CCA ATC CTT CGC ACC CTC TGG CGC GTG AAG TAG GGG TTT AGT GAA  
 900

295  
300  
900

303  
 R L \*  
 CGT CTT TAG  
 909

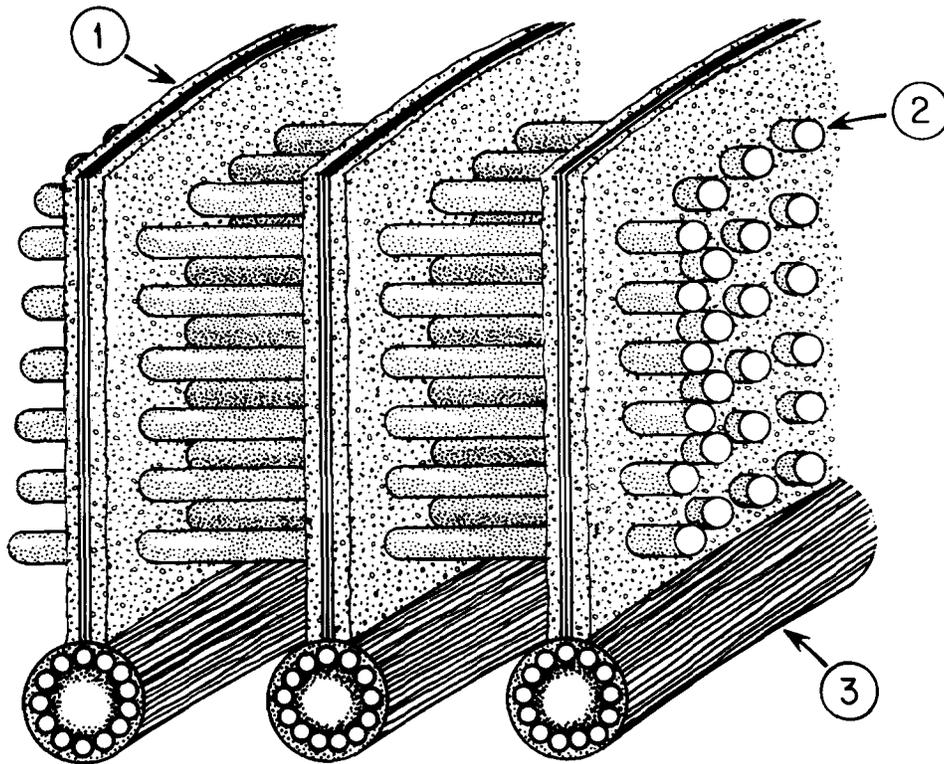


Figure 8. Schematic drawing of a portion of the ventral disk of *G. lamblia*. There are three basic structures: microribbons (1), crossbridges (2; fine stipple), and microtubules (3). The microribbons extend into the cytoplasm, and the microtubules are positioned ventrally. The giardins form thick sheets (coarse stipple) on the edges of the microribbons. The crossbridges connect the microribbons. Tubulin is present only in the microtubules.

and beta tubulin. The sequence AGTGAA is located six nucleotides downstream from the first TAG stop codon in alpha giardin (nucleotides 895-900; see Fig. 7); the sequence AGTAAA occurs 8 bp downstream from the TAA stop codon (and 8 bp before the poly(A) tail) in the beta giardin gene (Baker et al., 1988), seven nucleotides downstream from the TAA stop codon in the beta-1 giardin gene (Aggarwahl and Nash, 1989), and nine nucleotides downstream from the TAG stop codon in the beta tubulin gene (Kirk-Mason et al., 1988). (Holberton et al. [1988] identified AGTAAA as an unusual polyadenylation site but did not demonstrate this experimentally.) Thus, the sequence AGTPuAA is present six to nine nucleotides downstream from the stop codon in each of the four protein-encoding *G. lamblia* genes that have been sequenced and reported to date.

The deduced protein sequence of alpha giardin bears no significant homology to the 29.4- and 29.1-kD proteins predicted for beta (Baker et al., 1988) and beta-1 (Aggarwahl and Nash, 1989) giardin. There also is no similarity to the amino acid composition of a 30-kD giardin reported earlier (Crossley and Holberton, 1983a). Beta and beta-1 giardin probably correspond to the 29-kD proteins 9, 9a, and/or 9b

(Fig. 2 A) that react with the antibodies directed against alpha giardin (Fig. 2 C). If so, then the epitope(s) shared between the 33- and 29-kD proteins do not have a common primary sequence.

The deduced protein sequence of alpha giardin has 295 residues and a relative molecular mass of 33,800 D, corresponding well with the 33-kD size determined by gel mobility. Secondary structure algorithms (Chou and Fasman, 1978; Garnier et al., 1978) estimate that alpha giardin is largely (82%) alpha helical (Fig. 7, underlined characters), but they give no indication that it has an alpha-helical coiled coil structure that is common to several cytoskeletal proteins (see Bershady and Vasiliev, 1988) and predicted for beta giardin (Holberton et al., 1988).

The cytoskeleton is both a hallmark of eukaryotes and a measure of evolutionary maturity (see Cavalier-Smith, 1987). Additionally, *G. lamblia* is the earliest diverging lineage in the eukaryotic line of descent (Sogin et al., 1989), implying that elucidation of its cytoskeleton could reveal new details of eukaryotic evolution. *Giardia* contain actin, alpha actinin, myosin, and tropomyosin as well as the giardins and tubulin. The first four of these proteins have been localized at the edges

Figure 7. Nucleotide sequence of the alpha giardin gene and deduced amino acid sequence of alpha giardin. The nucleotide sequence of the alpha giardin gene is numbered in the 5'-to-3' direction (below the sequence) starting with the ATG triplet that encodes the initial methionine. The protein-encoding region spans 885 nucleotides, predicting a protein product of 295 amino acids (numbered above the sequence) in length and 33,800 D in molecular mass. The 29 amino acids of the original antigen determined by Edman degradation and gas phase chromatography are in bold, and the predicted alpha helices (Chou and Fasman, 1978; Garnier et al., 1978) are underlined. The location and sequence of the synthetic DNA oligomer used to isolate the giardin gene is shown beneath the gene sequence (N=A,C,G,T; R=A,G; X=A,C,T; and Y=C,T). The oligomer was constructed as a mixture of all possible sequences and had a final degeneracy value of  $2.4 \times 10^6$ .

of the ventral disk by immunocytochemistry (Feely et al., 1982), but not in the disk structure proper. The giardins are the major proteins of the disk microribbons, and tubulin is present in the disk and flagellar microtubules. The function of the disk and, hence, of the giardins is unclear. The disk apparently does not contract during adherence of the parasite to the gut wall, and adhesion may be maintained by flagellar beating and fluid flow beneath the disk (Holberton, 1973, 1974) or by grasping of the gut microvilli by the edges of the disk where the contractile proteins are located (Feely et al., 1982). The giardins could maintain disk structure, and, furthermore, since the disk must disassemble and reassemble as the parasite encysts and excysts, the giardins may be regulated and mobilized in response to specific signals. Alpha giardin is likely involved in the structural integrity of the disk through its interactions with other proteins in the microribbons or crossbridges.

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