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▣ ORIGINAL ARTICLE

Identification of a Novel Microtubule-Binding Protein in *Giardia lamblia*

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Abstract: *Giardia lamblia* is a protozoan that causes diarrheal diseases in humans. Cytoskeletal structures of *Giardia* trophozoites must be finely reorganized during cell division. To identify *Giardia* proteins which interact with microtubules (MTs), *Giardia* lysates were incubated with in vitro-polymerized MTs and then precipitated by ultracentifugation. A hypothetical protein (GL50803_8405) was identified in the precipitated fraction with polymerized MTs and was named GlMBP1 (*G. lamblia* microtubule-binding protein 1). Interaction of GlMBP1 with MTs was confirmed by MT binding assays using recombinant GlMBP1 (rGlMBP1). In vivo expression of GlMBP1 was shown by a real-time PCR and western blot analysis using anti-rGlMBP1 antibodies. Transgenic *G. lamblia* trophozoites were constructed by integrating a chimeric gene encoding hemagglutinin (HA)-tagged GlMBP1 into a *Giardia* chromosome. Immunofluorescence assays of this transgenic *G. lamblia*, using anti-HA antibodies, revealed that GlMBP1 mainly localized at the basal bodies, axonemes, and median bodies of *G. lamblia* trophozoites. This result indicates that GlMBP1 is a component of the *G. lamblia* cytoskeleton.

Key words: *Giardia lamblia*, microtubule-binding protein 1

INTRODUCTION

Giardia lamblia infects humans as cysts that are converted into trophozoites, which multiply by binary fission in human intestines and cause diarrheal disease. A trophozoite of *G. lamblia* has 2 nuclei and characteristic cytoskeletal structures such as a ventral disc, a median body, 4 pairs of flagella, and a funis [1]. Positioning of these structures in the dividing *Giardia* cells must be finely coordinated for successful proliferation. In eukaryotic organisms, microtubules (MTs) play an essential role in the coordinated movement of cellular structures by maintaining equilibrium between polymerization and depolymerization [2]. Growing and shortening of MTs is mediated by MT-associated proteins, including end-binding 1 (EB1), which is a plus-end tracking protein [3].

An EB1 homologous protein (GlEB1) was found in the flagellar tips, median bodies, and mitotic spindles of *G. lamblia* [4,5]. The role of GlEB1 was assessed by complementation assays using a *BIM1* mutant of *Saccharomyces cerevisiae*, in which proper positioning of the nucleus was abolished [6]. Biochemical characterization of GlEB1 was performed to define the domains responsible for MT binding and dimerization [5].

Investigations on the *Giardia* cytoskeleton have focused on its unique structures such as the ventral disc and median body. Tubulin and *Giardia*-specific giardin proteins were identified, via Triton X-100 extraction, as the main components of *G. lamblia* ventral disc [7]. Recent technical progress in proteomic analysis has led to the discovery of additional proteins associated with the ventral disc, whose function is yet to be defined [8]. In addition, shotgun proteomics along with GFP-tagging of the purified ventral disc of *G. lamblia* facilitated the identification of 18 novel disc-associated proteins [9]. One of these disc-associated proteins, DAP116343, was also found in the median body and knockdown of this protein by morpholinos resulted in aberrant disc formation in *G. lamblia* [10].

Thus, dynamic MTs are expected to mediate cell division in *G. lamblia*. Various MT-binding proteins may play roles in this process. In this study, a novel MT-binding protein was discovered in *Giardia* lysates, using in vitro-polymerized MTs.

MATERIALS AND METHODS

Giardia cell culture and preparation of *Giardia* extracts

Trophozoites of the *G. lamblia* WB strain (ATCC30957;

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American Type Culture Collection, Manassas, Virginia, USA) were grown for 72 hr in TYI-S-33 medium (2% casein digest, 1% yeast extract, 1% glucose, 0.2% NaCl, 0.2% L-cysteine, 0.02% ascorbic acid, 0.2% K_2HPO_4, 0.06% KH_2PO_4, 10% calf serum, and 0.5 mg/ml bovine bile, pH 7.1) [11]. They were then resuspended in PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, and 2 mM KH2PO4, pH 7.4), and lysed by sonication.

MT-binding assay

The binding of *Giardia* lysates to polymerized MTs was performed in vitro using the Microtubule-Binding Protein Spin-Down Assay Kit BK029 (Cytoskeleton, Denver, Colorado, USA). MTs were assembled from 100 µg of pure tubulin (isolated from bovine brain; Cytoskeleton) in 20 µl of PEM [80 mM piperazine-N,N'-bis(2-ethanesulfonic acid), pH 6.8, 1 mM EGTA, and 1 mM $MgCl_2$] in the presence of 1 mM GTP and 5% glycerol at 35˚C for 20 min, and immediately stabilized in 200 µl of warm PEM-20 µM taxol (Cytoskeleton). Twenty µmoles of the MTs were incubated with 100 µg of *Giardia* lysate in a total volume of 50 µl at 25˚C for 40 min. The reaction mixtures were then centrifuged with a 50% glycerol cushion-PEM-taxol mixture, at 100,000 g at 25˚C for 40 min in an ultracentrifuge (Hitachi Koki, Tokyo, Japan). The resulting pellet fraction was then resolved on an 8% polyacrylamide gel and visualized by silver staining. The same amount of *Giardia* extract was precipitated by ultracentrifugation, and compared side-by-side with the extracts precipitated with MTs.

Liquid chromatography mass spectrometry

The protein band present in the MT fraction was excised and digested with trypsin. The trypsin-treated proteins were analyzed by quadrupole time-of-flight (Q-TOF) mass spectrometry (MS) in addition to matrix-assisted laser desorption ionization-TOF MS (MALDI-TOF MS). Product ion spectra were collected in the information-dependent acquisition mode and were analyzed with an Agilent 6530 accurate-mass Q-TOF MS. For the Q-TOF liquid chromatography-tandem MS (LC-MS/MS) data sets, tandem mass spectra were submitted to our MASCOT in-house database search engine (NCBI NR database downloaded on 31 July 2009). For protein identification, a MASCOT ion score of > 37 was used as the criterion for a meaningful result.

Expression and purification of recombinant GlMBP1 (rGlMBP1)

A 1,338 bp DNA fragment encoding the GlMBP1 open reading frame (ORF) was amplified by PCR from the genomic DNA of *G. lamblia*, using 2 primers, 8405F and 8405R (Table 1), and cloned into pET21b (Novagen, Darmatadt, Germany),

Table 1. Strains, primers, and plasmids used in this study

Strains/primers/ plasmids	Relevant characteristics[a]	Source or reference
G. lamblia		
ATCC 30957	Clinical isolate	ATCC
E. coli		
DH5a	*supE44 DlacU169 (F80 lacZ DM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Invitrogen
BL21 (DE3)	*F', ompT, hsdSB(rB-mB-) gal, dcm (DE3)*	Invitrogen
Primers		
8405F	GCGAATTCGATGTCGATGGACGTTCCTA (*EcoR*I)	
8405R	AGTTTAGCGGCCGCGCGCTTTGAGCCACACTCCA (*Not*I)	
GlMBP-F	CGATCCATGGCCGGCTCTTGTCCAACTGCT (*Nco*I)	
GlMBP-HA-R	GTTACGCGGCCGCTTAAGCGTAATCTGGAACATCG TATGGGTAAGCGTAATCTGGAACATCGTATGGGTAA GCGTAATCTGGAACATCGTATGGGTAGCGCTTTGAGCCACACTCCA (*Nco*I)	
pac down	CGCGAATTCTCAGGCACCGGGCTT	
RT-tim_F	CGAAAGTGGTTTGCGGAGAAG	
RT-tim_R	CTATGTACGGGTCTTCGTAAGA	
RT-GlMBP-F	GATGAAGTAGATAAGGCGGCA	
RT-GlMBP-R	GAGCCACACTCCATACAGAAT	
Plasmids		
pET21b	Expression vector for a histidine-tagged protein	Novagen
pETGlMBP1	pET21b, 1,338 bp encoding GlBMP1 (*Giardia*DB; GL50803_8405)	This study
pGFP.pac	Shuttle vector, Amp^R, *pac* gene	Singer et al. [13]
pGlMBP1HA.pac	pGFP.pac, 1,538 bp encoding GlMBP1 from its own promoter	This study

[a]Underlined bases indicate a restriction enzyme site.

resulting in pETGlMBP1. The rGlMBP1 was expressed in Escherichia coli BL21 (DE3), with 0.5 mM ITPG at 30˚C for 3 hr, and purified using TALON Metal affinity chromatography, as described by the manufacturer (Clontech, Mountain View, California, USA).

Quantitative measurement of GlMBP1 transcript

The level of GlMBP1 mRNA expression was evaluated by real-time PCR. Total RNA was isolated from *G. lamblia*, using TRIzol (Invitrogen, Carlsbad, California, USA). cDNA was synthesized from 5 μg of RNA using the ImProm-II™ RT system (Promega, Madison, Wisconsin, USA) following the manufacturer's directions. cDNA was then analyzed in the Light Cycler 480 II Real-Time PCR System (Roche Applied Science, Mannheim, Germany) using LightCycler 490 DNA SYBR Green I Master (Roche Applied Science). Conditions for real-time PCR were as follows: pre-incubation at 95˚C for 5 min followed by 45 amplification cycles of 95˚C for 10 sec, 56˚C for 20 sec, and 72˚C for 10 sec. Real-time PCR was carried out in triplicate in a 96-well plate using the specific primers listed in Table 1. The *tim* gene encoding triose-1-phosphate isomerase of *G. lamblia* was used as an endogenous control for the reactions.

Formation of anti-rGlMBP1 antibodies

Histidine-tagged rGlMBP1 was expressed in *E. coli* BL21 (DE3) with the addition of 0.5 mM IPTG at 37˚C for 3 hr. The rGlMBP1 protein was excised from the acrylamide gel, and used to immunize Sprague-Dawley rats (2-week-old, female) to produce polyclonal antibodies as described previously [12].

Western blot analysis

Ten μg of purified rGlMBP1 reacted with 20 μM polymerized MTs was used for MT-binding assays. After ultracentrifugation, the resulting soluble and pellet fractions were separated on 12% acrylamide gel, and then transferred to nitrocellulose membranes (Milipore, Bedford, Massachusetts, USA). The membrane was incubated with anti-histidine antibodies (1:5,000 dilution; IG Therapy, Chunchon, Korea) in a blocking solution (Tris-buffered saline containing 5% skim milk, and 0.1% Tween 20) at 4˚C overnight. Following incubation with alkaline phosphatase-conjugated anti-mouse IgG (1:2,000 dilution; Sigma, St. Louis, Missouri, USA), the immunoreactive protein was visualized in a nitro blue tetrazolium/5′-bromo-4-chloro-3-indolyphosphate system (Promega).

Integration of HA-tagged glmbp1 into a *Giardia* chromosome

To tag a hemagglutinin (HA) epitope to the C-terminal portion of the *glmbp1* gene, a 1,538 bp DNA fragment containing the promoter (200 bp) and ORF of the *glmbp1* gene was amplified from *G. lamblia* WB genomic DNA by PCR, using 2 primers, GlMBP-F and GlMBP-HA-R (Table 1). *Nco*I and *Not*I sites, located at the ends of the resulting *glmbp1* DNA, were used for cloning into the corresponding sites of plasmid pGFP.pac [13], resulting in the plasmid pGlMBP1HA.pac. This construct was verified by DNA sequencing (Macrogen, Seoul, Korea). The plasmid was linearized using its unique *Pst*I site, and 10 μg of linear DNA was then introduced into *Giardia* trophozoites. Briefly, the trophozoites were grown for 72 hr in normal TYI-S-33 medium. Transfection of linearized DNA into *G. lamblia* trophozoites was performed by electroporation under the following conditions: 350 volts, 1000 μF, and 700 Ω (BioRad, Hercules, California, USA). Trophozoites harboring pGlMBP1HA.pac were selected by adding puromycin (AG Scientific, San Diego, California, USA) to the TYI-S-33 medium at a final concentration of 10 μg/ml. After 7 or 8 days of cultivation, resistant cells were recovered. To confirm that the construct was integrated into the *Giardia* genome in the transfected cells, PCR was performed for the isolated genomic DNA from pGlMBP1HA.pac-transfected cells, using a common 5′-primer (GlMBP-F) and two 3′-primers (GlMBP-HA-R or pac down) (Table 1). PCR conditions were as follows: pre-denaturation at 94˚C for 5 min followed by 35 cycles of 94˚C for 1 min, 56˚C for 1 min, and 72˚C for 1 min 30 sec, and post-elongation at 72˚C for 10 min using a Veriti Thermal Cycler (Life Technologies, Foster City, California, USA).

To determine whether HA-tagged GlMBP1 was expressed in the transfected *Giardia*, cell extracts were prepared from *G. lamblia* WB and puromycin-resistant *G. lamblia*, and then were analyzed by sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE). The resulting membrane was incubated with rat anti-GlMBP1 antibodies (1:1,000), followed by incubation with horseradish peroxidase-conjugated anti-rat-IgG antibodies (1:1,000). Immunoreactive proteins were visualized using an Enhanced Chemiluminescence System (Youngin Frontier Company, Seoul, Korea). The membrane was incubated in a stripping buffer (ATTO Corporation, Tokyo, Japan) at room temperature for 30 min, and reacted with polyclonal *G. lamblia* anti-α-tubulin antibodies (1:10,000) as a loading control.

Immunofluorescence assay (IFA)

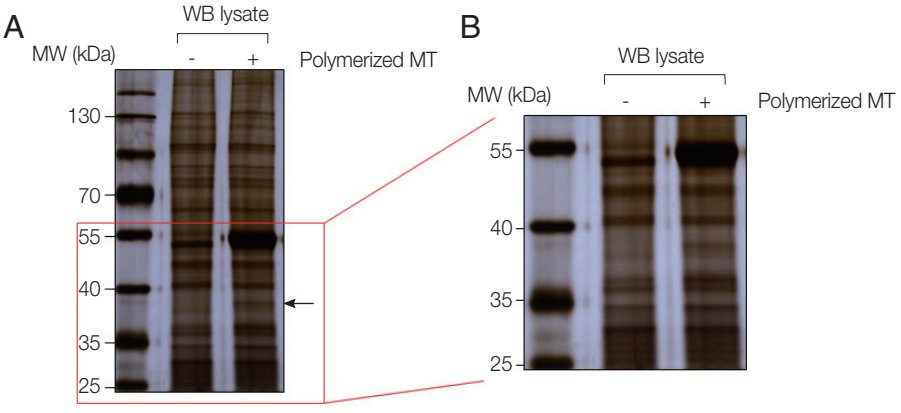
To examine the localization of GlMBP1 in *G. lamblia* expressing HA-tagged GlMBP1, the cells were attached to glass slides coated with L-lysine in a humidified chamber. The attached cells were fixed with chilled 100% methanol at -20˚C for 10 min and then permeabilized with PBS/0.5% Triton X-100 for 10 min. After 1-hr incubation in blocking buffer [PBS, 5% goat serum, and 3% bovine serum albumin (BSA)], the cells were reacted overnight with mouse anti-HA antibodies (1:50; Sigma). Following 3 times 5-min washes with PBS, the cells were incubated with AlexaFluor 488-conjugated anti-mouse IgG (1:200; Molecular Probes, Grand Island, New York, USA) at 37˚C for 1 hr. Cells were mounted on slides with VECTASHIELD Anti-fade Mounting Medium with 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, California, USA) and then were observed with an Axiovert 200 fluorescence microscope (Carl Zeiss, Oberkochen, Germany).

RESULTS

Isolation of a *G. lamblia* MT-binding protein 1, GlMBP1

The pattern seen from proteins precipitated with MTs was almost identical to that of the control, except for 2 protein bands (Fig. 1A). The larger and more abundant of these proteins was 55 kDa in size and was believed to be the tubulin monomer used in the MT-binding assay, while the smaller and less abundant protein was thought to be an MT-binding protein derived from *Giardia* extracts. An extended view of the separated proteins between 55 kDa and 25 kDa revealed a protein of <40 kDa that was among the proteins precipitated with MTs, but was absent in the control proteins as indicated with an arrow (Fig. 1B).

This protein was excised and processed for Q-TOF LC MS/MS analysis (Fig. 1C). It was identified as a hypothetical protein, annotated as GL50803_8405, and named *G. lamblia* MT-binding protein 1, GlMBP1. The putative protein had a molec-



Protein name	MW (kDa)[a]	pI[a]	Swquence Coverage[a]	Matcged peptide number[a]	Score (valid value)[b]
Hypothetucal protein GL50803_8405	48	9.65	16%	6	66(37)

[a]Matching masses from liquid chromatography-mass spectrometry when searching against all entries in NCBi using Mascot search engine.
[b]Probability value value obtained from Mascot search.

Fig. 1. Identification of GlMBP1, an MT-binding protein from *Giardia* extracts via in vitro MT-binding assays. (A) Trophozoites of *G. lamblia* WB were resuspended in PBS, and lysed by sonication. The binding of *Giardia* lysates to polymerized MTs was assessed in vitro using a Microtubule-Binding Protein Spin-Down Assay Kit BK029 (Cytoskeleton). Twenty μmoles of MTs was incubated with 100 μg of *Giardia* lysates in a total volume of 50 μl at room temperature for 40 min. The reaction mixtures were then centrifuged through a 50% glycerol cushion-PEM-taxol mixture at 100,000 g at 25˚C for 40 min using an ultracentrifuge. The pellet fraction was then resolved on 8% SDS-PAGE and visualized by silver staining. A sample with the same amount of *Giardia* extracts was precipitated by ultracentrifugation and compared side-by-side with the extracts precipitated with MTs. (B) An extended view of the SDS-PAGE gel showing proteins between 55 kDa and 25 kDa. (C) The protein band present only in the MT fraction was excised and digested with trypsin. The trypsin-treated proteins were analyzed by quadrupole time-of-flight (Q-TOF), in addition to matrix-assisted laser desorption ionization-TOF mass spectrometry (MALDI-TOF MS). For the Q-TOF liquid chromatography-tandem MS (LC-MS/MS) data sets, tandem mass spectra were submitted to our MASCOT in-house database search engine (NCBI NR database downloaded on 31 July 2009). For protein identification, a MASCOT ion score of >37 was used as the criterion for a meaningful result.

ular weight of 48 kDa and a pI value of 9.65. The Q-TOF LC-MS/MS analysis indicated 6 matched peptides, 16% coverage, and a meaningful MASCOT score of 66. Interestingly, the putative size of this annotated protein was 48 kDa whereas the excised protein was smaller (< 40 kDa). This result suggested the possibility that the excised protein was a degradation product of GlMBP1.

The open reading frame (ORF) encoding GlMBP1 was made of 445 amino acids. A BlastP search of the protein database of other metazoan organisms yielded only homologous protein except a homologous protein (SS50377_11087) in *Spironucleus salmonicida*, a protozoan pathogen in salmons. A domain search of GlMBP1, as well as the homologous protein in *S. salmonicida*, did not show any MT-binding domains such as calponin homology, cytoskeletal associated protein-glycine rich, and tumor overexpressed gene domains, which are con-

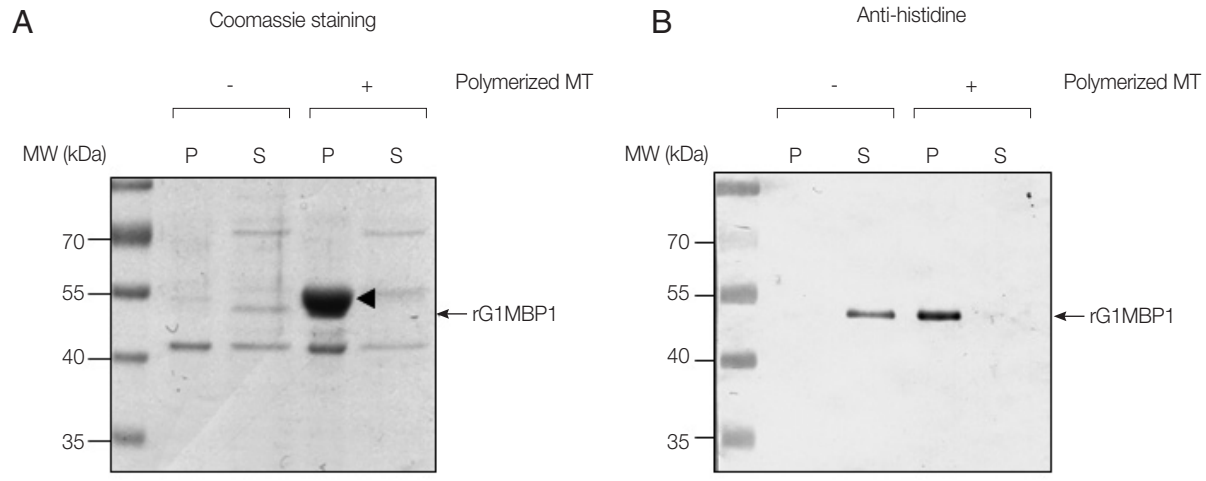


Fig. 2. In vitro MT-binding assays using rGlMBP1. Ten μg of rGlMBP1 was incubated without or with taxol-stabilized bovine MTs (20 μM), divided into pellet (P) and soluble (S) fractions by ultracentrifugation, and then separated by 12% SDS-PAGE. (A) A SDS-PAGE gel stained with Coomassie brilliant blue. (B) Western blot using anti-histidine antibodies (1:5,000 dilution). An arrowhead (about 55 kDa) indicates MTs, whereas arrows denote rGlMTBP.

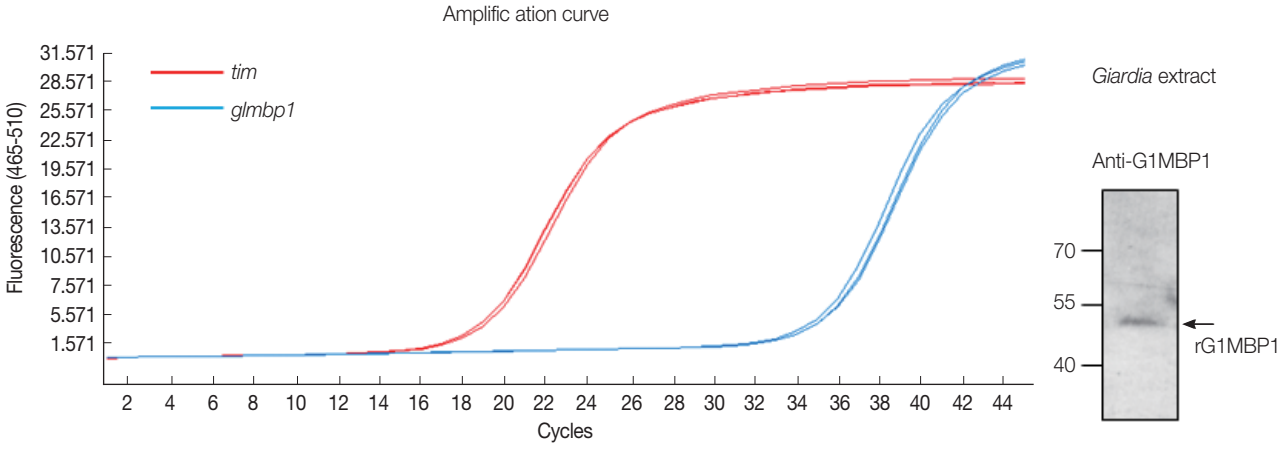


Fig. 3. In vivo expression of GlMBP1 in *G. lamblia* trophozoites. (A) Quantitative measurement of GlMBP1 transcripts. Total RNA was isolated from *G. lamblia* using TRIzol. cDNA was synthesized from 5 μg of RNA using the ImProm-II™ RT system and then analyzed with the Light Cycler 480 II Real-Time PCR System using LightCycler 490 DNA SYBR Green I Master (Roche Applied Science). Conditions for real-time PCR were as follows: pre-incubation at 95℃ for 5 min followed by 45 amplification cycles of 95℃ for 10 sec, 56℃ for 20 sec, and 72℃ for 10 sec. Real-time PCR was carried out in triplicate in a 96-well plate using the specific primers listed in Table 1. The *tim* gene encoding triose-1-phosphate isomerase of *G. lamblia* was used as an endogenous control for the reactions. (B) Western blot analysis. Ten μg of *Giardia* extracts was separated by 12% SDS-PAGE and transferred onto a nitrocellulose membrane. The membrane was incubated with anti-GlMBP1 antibodies (1:1,000 dilution), followed by secondary antibodies (1:1,000 dilution).

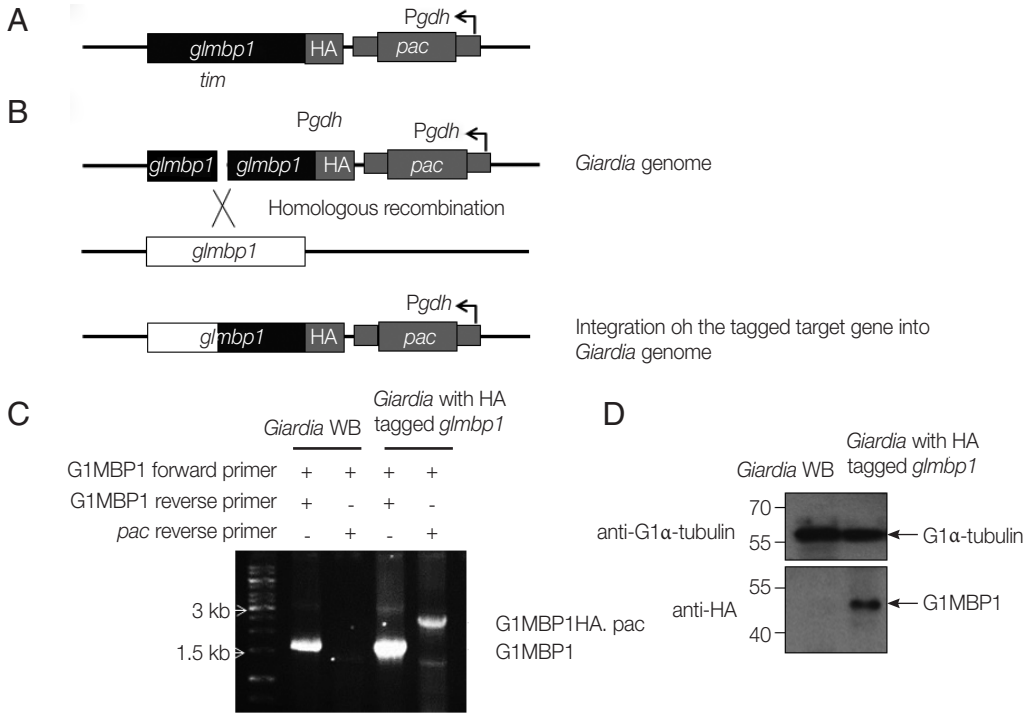


Fig. 4. Expression of HA-tagged GlMBP1 in *G. lamblia*. (A) A schematic diagram of the plasmid pGlMBP1HA.pac. This plasmid contains the in-frame N-terminal end of the *glmbp1* gene with 3 HA epitopes and the puromycin N-acetyltransferase gene (*pac*) cassette as a selective marker expressed from the glutamate dehydrogenase gene (*gdh*) promoter, P*gdh*. (B) A strategy to integrate pGlMBP1HA.pac into a *Giardia* chromosome. Closed boxes show the plasmid sequences, and open boxes indicate *Giardia* chromosomal sequences. HA-tagging sequences and the *pac* cassette are indicated by gray boxes. (C) Genomic PCR analysis. PCR was performed using the GlMBP-F and GlMBP-HA-R or *pac* down primers on genomic DNA from *G. lamblia* WB cells or pGlMBP1HA.pac-transfected cells. (D) Western blot analysis. Ten μg of *Giardia* extracts (*G. lamblia* WB cells or pGlMBP1HA.pac-transfected cells) was separated by 12% SDS-PAGE and transferred to nitrocellulose membranes. The membrane was incubated with anti-HA antibodies (1:1,000 dilution), and then secondary antibodies (1:1,000 dilution).

served in mammalian and yeasts. The only domains found in these proteins were C2HC-type zinc-finger domains.

Confirmation of the MT-binding ability of GlMBP1

To confirm the association between GlMBP1 and MTs, an MT-binding assay was performed with recombinant GlMBP1 (rGlMBP1) and in vitro polymerized MTs. In the absence of MTs, histidine-tagged rGlMBP1 was found in the soluble fraction by Coomassie-staining and western blot analysis using anti-histidine antibodies (Fig. 2A, B, respectively). Upon incubation with polymerized MTs, rGlMBP1 was found in the pellet fraction along with tubulin monomer (Fig. 2A). Because the sizes of tubulin and rGlMBP1 were similar, it was difficult to distinguish one from the other in the SDS-PAGE gel. However, western blotting using anti-histidine antibodies clearly showed that rGlMBP1 precipitated with polymerized MTs, indicating that rGlMBP1 binds to MTs (Fig. 2B).

In vivo expression of GlMBP1 in *G. lamblia*

Even though GlMBP1 was identified as an MT-binding protein in *G. lamblia* extracts, whether this protein is expressed in vivo in *G. lamblia* still needed to be determined. To assess the expression of *glmbp1* in *Giardia*, the level of *glmbp1* transcripts was monitored by real-time reverse transcriptase PCR of trophozoites using primers specific to the *glmbp1* gene (Fig. 3A). The level of *tim* transcript encoding triose 1-phosphate isomerase was used to normalize other transcripts from identical RNA samples [14]. The crossing point-PCR cycle (Cp) values were estimated as 18.8 and 35.6 for *tim* and *glmbp1*, respectively. Thus, the level of *glmbp1* transcript expression was lower than that of *tim*, but *glmbp1* transcripts were present in *G. lamblia* trophozoites.

In addition, the presence of GlMBP1 in *Giardia* trophozoites was demonstrated by western blot analysis of *Giardia* extracts with anti-GlMBP1 antibodies. An immunoreactive protein of

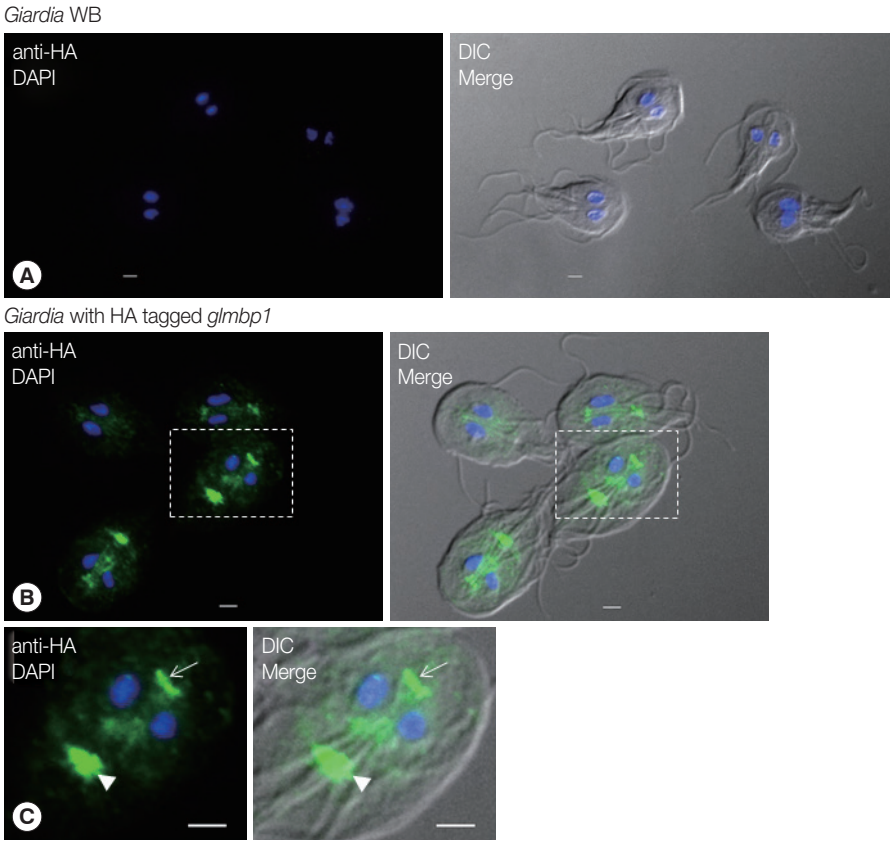


Fig. 5. Localization of GlMBP1 in *G. lamblia* trophozoites. *G. lamblia* cells expressing HA-tagged GlMBP1 were fixed with chilled 100% methanol, and then permeabilized with PBS/0.5% Triton X-100. The cells were reacted with mouse anti-HA antibodies (1:50 dilution) and then incubated with AlexaFluor 488-conugated anti-mouse IgG (1:200). The cells were mounted on slides with VECTASHIELD Antifade Mounting Medium with 4′,6-diamidino-2-phenylindole, and then observed with an Axiovert 200 fluorescence microscope (Carl Ziess). Differential interference contrast (DIC) images showed cell morphology. (A) IFA on *G. lamblia* WB using anti-HA antibodies. The bars indicate 2 μm. (B, C) IFA on *G. lamblia* with integrated HA-tagged *glmbp1* using anti-HA antibodies. The bars indicate 2 μm. Arrowheads indicate median bodies, while arrows show basal bodies.

48 kDa, the expected size of GlMBP1, was apparent (Fig. 3B).

Intracellular location of GlMBP1 in *Giardia* trophozoites

In the next experiment, we examined whether GlMBP1 was localized in the cytoskeletal structures of *G. lamblia*. First, the *glmbp1* gene tagged with HA epitopes was integrated by electroporation of linear *glmbp1*-HA DNA into the genomes of *G. lamblia* trophozoites (Fig. 4A, B) [15]. Integration of the chimeric *glmbp1*-HA gene into the *Giardia* chromosome was examined by PCR using *glmbp1*-specific primers and a primer specific for the vector plasmid, the pac reverse primer (Fig. 4C). A 1.6 kb *glmbp1* PCR product was detected in both *G. lamblia* WB as well as in pGlMBP1HA.pac-transfected cells. A 2.5 kb PCR product, the expected length of *glmbp1* and the integrated plasmid, was amplified only from the genomic DNA of *G. lamblia* transfected with pGlMBP1HA, but not from that of *G. lamblia* WB.

Giardia trophozoites with the integrated HA-tagged *glmbp1* gene were then examined for the expression of a chimeric GlMBP1-HA by western blotting with anti-HA antibodies (Fig. 4D). An immunoreactive protein band of 48 kDa was only present in the extracts of *Giardia* with the integrated HA-tagged *glmbp1* gene (Fig. 4D). *Giardia* WB extracts did not show the immunoreactive protein. These results indicated that the HA-tagged *glmbp1* and pac genes were integrated into a *G. lamblia* chromosome, and that HA-tagged GlMBP1 was expressed in *G. lamblia* transfected with linear pGlMBP1HA.pac.

IFA was performed on *G. lamblia* WB and *G. lamblia* with the integrated *glmbp1*-HA gene using anti-HA antibodies (Fig. 5). *G. lamblia* WB trophozoites did not show any fluorescence in the IFA (Fig. 5A). In contrast, transgenic *G. lamblia* with HA-tagged *glmbp1* showed green fluorescence at axonemes, medi-

an bodies, and basal bodies (Fig. 5B, C). This result indicated that GlMBP1 was located in cytoskeletal structures of *G. lamblia* trophozoites.

DISCUSSION

The cytoskeleton of *G. lamblia* is largely made of MTs, which includes a ventral disc, a median body, 8 basal bodies, and 4 pairs of flagella (anterior, posterior-lateral, ventral, and caudal). Each of the flagella is extended from an axoneme that is templated in a basal body. Basal bodies are conserved MT-organizing centers that nucleate structural apparatus such as flagella and cilia, and that function as spindle poles during cell division [16]. In *G. lamblia*, these structures were shown to be associated with MTs by electron immunocytochemistry [17] as well as IFA using anti-tubulin antibodies (Kim and Park, an unpublished result). Interestingly, γ-tubulin was found by IFA using monoclonal antibodies specific to γ-tubulin, only in the basal bodies of ventral and posterior-lateral flagella in the interphase *Giardia* cells. Further, this protein disappeared at early mitosis and reappeared in late-mitotic *G. lamblia* [18]. An extensive analysis of *G. lamblia*, based on *in silico* and proteomic methods, revealed 75 homologs of conserved basal body proteins. Thirteen of these homologs were found, by confocal microscopy, to co-localize with centrin in *Giardia* basal bodies [19]. Even though an association between GlMBP1 and basal bodies has not been reported, one of the MT-associated proteins was found to localize to cytoskeletal structures (axonemes, basal bodies, and median bodies) (Figs. 2, 5B).

In vitro interaction of GlMBP1 with MTs (Fig. 2) and its localization in the *Giardia* cytoskeleton indicates a role for GlMBP1 in cell cycle-related functions. However, the information available on GlMBP1 is too limited to allow a conclusion about its relationship with *G. lamblia* cell cycle. Monitoring the intracellular locations of GlMBP1 at various stages of *G. lamblia* will be essential to defining its involvement in cell cycle control in this organism. In addition, knockdown of *glmbp1* expression will provide insight into the function of this protein in *G. lamblia*.

In summary, a novel binding protein was isolated while screening for MT-binding protein. Association of this protein with MTs was confirmed by in vitro MT-binding assays using rGlMBP1 and polymerized MTs. The intracellular location of this protein in *G. lamblia* was observed using transgenic *G. lamblia* in which an HA-tagged *glmbp1* gene was integrated into a chromosome. IFAs using anti-HA antibodies revealed that GlMBP1 is located in cytoskeletal structures, median bodies, basal bodies, and axonemes. The physiological role of this GlMBP1-MT interaction should be defined in future investigations.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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