Journal of Cell Science 109, 57-62 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS7016

Assembly and function of *Chlamydomonas* flagellar mastigonemes as probed with a monoclonal antibody

Shogo Nakamura[1,*], Genta Tanaka[2], Tsukasa Maeda[2], Ritsu Kamiya[3], Tsukasa Matsunaga[4] and Osamu Nikaido[4]

[1]Department of Environmental Biology and Chemistry, and [2]Department of Biology, Faculty of Science, Toyama University, Toyama 930, Japan
[3]Zoological Institute, Graduate School of Science, University of Tokyo, Tokyo 113, Japan
[4]Division of Radiation Biology, Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa 920, Japan

*Author for correspondence (e-mail: nakamura@sci1.sci.toyama-u.ac.jp)

SUMMARY

Mastigonemes are hair-like projections on the flagella of various kinds of lower eukaryotes. We obtained a monoclonal antibody (mAb-MAST1) to mastigonemes of *Chlamydomonas reinhardtii,* and found that it reacts with a single flagellar glycoprotein of about 230 kDa. Interestingly, immunofluorescence microscopy demonstrated that mAb-MAST1 recognizes not only the flagellar mastigonemes but also a ring composed of 10 or more particles located in the anterior end of the cell body close to the flagellar bases. The ring structure may be the pool of the mastigoneme protein. When the flagella are amputated, they regenerate to their original length in 90-120 minutes. We found that mastigonemes appear on the new flagellar surface as early as 15 minutes after deflagellation, and that new mastigonemes are mostly assembled onto the distal region of the flagellar surface. Mastigonemes thus appear to be inserted into the membrane only in the distal region of the flagellum. Alternatively, mastigonemes may be inserted at the base and transported very rapidly to the distal portion where they are trapped. When live cells are treated with mAb-MAST1, mastigonemes disappear from the flagellar surface. In these mAb-MAST1 treated cells, the swimming velocity decreases to 70-80% of the normal value, although the flagellar beat frequency increases to ~110% of the control. These findings demonstrate vectorial transport of mastigonemes to their assembly sites, and show that mastigonemes function to increase flagellar propulsive force by increasing the effective surface of the flagellum.

Key words: Mastigoneme, *Chlamydomonas*, Monoclonal antibody

INTRODUCTION

The unicellular green alga *Chlamydomonas reinhardtii* has two flagella of equal length, and the flagellar surface has an important role in mating of gametes (see Harris, 1989) and gliding motility (Bloodgood, 1988). The flagella have fine hair-like projections termed mastigonemes on their surface (Ringo, 1967; Witman et al., 1972). Witman et al. (1972) reported that mastigonemes have a uniform size and contain a glycoprotein with an apparent molecular mass of 170 kDa. It has been suggested that mastigonemes may be involved in motility by enlarging the effective surface area of the flagellum (Bouck, 1972), or play a role in species-specific flagellar agglutination during mating (Wiese, 1969). However, Snell (1976) suggested mastigonemes are not involved in specific adhesion of flagella in the mating process, and Adair et al. (1983) showed that agglutinins, large linear glycoproteins, are specific molecules for the adhesion. The function of mastigonemes is therefore not understood.

In the course of raising monoclonal antibodies against flagellar components in *C. reinhardtii*, we found a monoclonal antibody (mAb-MAST1) which recognizes mastigonemes. Here, we characterize the mAb-MAST1, describe its pattern of assembly onto the flagellar surface, and test its functional effects on flagellar motility.

A preliminary report of some of these results appeared previously (Tanaka et al., 1994).

MATERIALS AND METHODS

Cells and cultures

Wild-type strains of *Chlamydomonas reinhardtii*, C-238 (UTEX 89, mt–) and C-239 (UTEX 90, mt+), were used. Cells were cultured in medium I (Sager and Granick, 1953), modified by addition of 1 g/l sodium acetate, trihydrate, at 23-28°C with constant aeration under continuous light.

Isolation of flagella

Cells were deflagellated by pH shock in 10 mM Tris-HCl, pH 7.8, containing 5% sucrose, and flagella were isolated by differential centrifugation (Witman et al., 1972). The isolated flagella were solubi-

lized by dialysis against TEM solution (0.1 mM EDTA, 0.01% 2-mercaptoethanol, 1 mM Tris-HCl, pH 8.0) for 12 hours at 4°C.

Production of antibody

Balb/c mice (6 weeks old) were injected intraperitonealy with solubilized flagellar components (50 μg of protein) in complete (the first injection) and incomplete (from the second to the fifth injection) Freund's adjuvant (1:1 (v/v); Difco Laboratories, Detroit, MI). The mice were given a booster from a tail vein 2 weeks after the fifth injection. Three days after the booster injection, the spleens were excised from the mice and dissociated by passage through 100-mesh steel gauze. Cell fusion was performed by the method of Mori et al. (1988). Cultures of fused cells in 24-well culture plates (Nunc, Denmark) were tested for antibody activity by means of the immunofluorescence microscopy and western blot mentioned below. Cells in promising cultures were cloned twice by limiting dilution in a 96-well culture plate (Becton Dickinson Labware, Lincoln Park, NJ).

The monoclonal antibody mAb-MAST1 was prepared in the following way. One of the clones obtained, MAST1, was cultured for 4 days and the medium was mixed with an equal volume of ice-cold saturated ammonium sulfate solution (pH 7.0). The precipitates formed were centrifuged at 12,000 ***g*** for 30 minutes, and the pellet was dissolved in a small volume of distilled water. This solution was dialyzed against phosphate buffered saline (PBS, pH 7.2) and stored at –80°C until use.

The class and subclass of mAb-MAST1 was determined with a mouse monoclonal antibody isotyping kit (Amersham International plc, Amersham, UK).

Immunofluorescence microscopy

Cells were fixed for 30 minutes at room temperature in freshly prepared 3.7% formaldehyde (Wako Pure Chemical Industries, Ltd, Osaka, Japan) or 0.2% glutaraldehyde (TAAB Laboratories Equipment Ltd, Aldermaston, Berkshire, UK) in culture medium, and were allowed to adhere to poly-L-lysine (Sigma) coated slide glasses. They were then rinsed 3 times, 10 minutes each, with PBS, and treated with 0.5% Triton X-100 in PBS for 5 minutes. After blocking with 1% BSA in PBS for 60 minutes, the cells were incubated with mAb-MAST1 diluted 1:100-1:500 in PBS for 60 minutes, and rinsed as above. The samples were then incubated in fluorescein-conjugated rabbit anti-mouse IgG (Wako; diluted 1:500 in PBS) for 60 minutes to localize the primary antibody. Coverslips were mounted in 50% glycerol/PBS containing 0.1% *p*-phenylenediamine and examined on an Olympus BH2 microscope equipped with epifluorescence optics. Micrographs were taken on Kodak EKTAR 1000 film or Tri-X Pan film.

Immunoelectron microscopy

For immunoelectron microscopy cells were fixed in 0.2% glutaraldehyde for 10 minutes and rinsed 3 times, 10 minutes each, with PBS, then blocked with 1% BSA in PBS for 60 minutes. The samples were rinsed once with PBS and incubated with mAb-MAST1 for 60 minutes at room temperature. After three rinses with PBS, the samples were incubated in 10 nm colloidal gold-conjugated goat anti mouse IgG (1:20 in PBS)(Bio Cell Research Laboratories, Cardiff, UK) for 60 minutes at room temperature. Samples were placed on Formvar-coated grids and examined in a JEOL 100SX electron microscope operating at 80 kV.

Western blot analysis

SDS-polyacrylamide gel electrophoresis of isolated flagella was performed on 5% acrylamide gels (Laemmli, 1970), and stained with Coomassie Brilliant Blue R or silver. To detect carbohydrates some gels were stained with PAS (Zacharius et al., 1969). For immunoblots, proteins on gels were electrophoretically transferred to 0.45 μm nitrocellulose membrane (Hybond-ECL, Amersham) according to the method of Towbin et al. (1979). Nitorocellulose strips were probed with alkaline phosphatase-conjugated goat anti-mouse immunoglobulins (Promega Corporation, Madison, WI, USA) as the secondary antibody. Immunoreactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (Promega Corporation).

To digest proteins on the nitrocellulose strips or in the samples for the PAGE, they were treated with 200 μg/ml or 1 mg/ml (final concentration) pronase (Boehringer Mannheim, Mannheim, Federal Republic Germany).

Flagellar regeneration

Flagella were mechanically removed from the cells with a homogenizer (Nihonseiki Co., Ltd, Ace-1) used at 16,000 rpm, for 3 minutes. Aliquots of the experimental cultures were taken at suitable time intervals and immediately fixed in 3.7% formaldehyde in culture medium. In some experiments, flagella were detached and regenerated in the presence of 20 μg/ml cycloheximide (Sigma).

Beat frequency and swimming velocity

After incubation with mAb-MAST1 or mAb-BTUB1 (both mAbs were produced at the same time, and mAb-BTUB1 recognized beta-tubulin) for 15 minutes, flagellar beat frequencies in those swimming cells were measured by a fast Fourier transform (FFT) method (Kamiya and Hasegawa, 1987). This method analyzes the light intensity fluctuation in the microscopic image of a population of swimming cells and yields an average beat frequency.

The swimming velocities of the normal cells and mAb MAST1 or

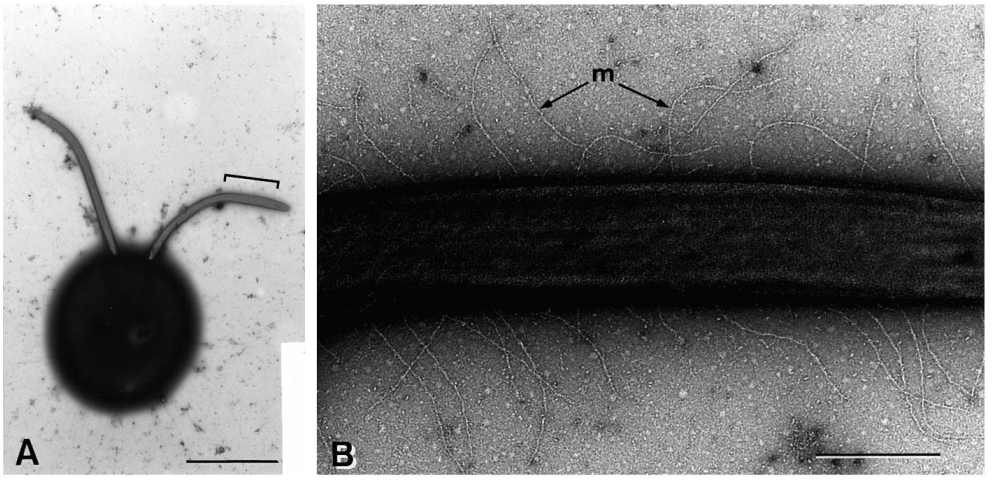

Fig. 1. Electron micrographs of negatively stained *Chlamydomonas reinhardtii* (1% uranyl acetate). (A) Whole cell; (B) high-magnification of the bracketed portion of the flagellum showing mastigonemes (m) on both sides. Bars: (A) 5 μm; (B) 0.5 μm.

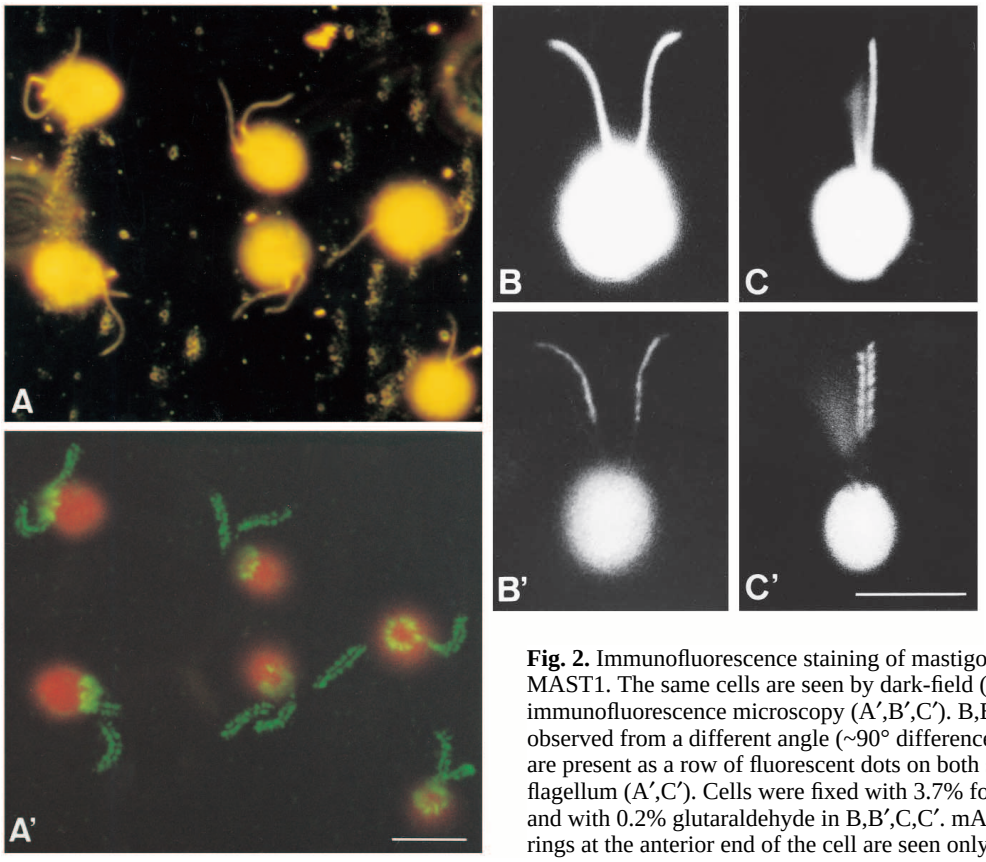

Fig. 2. Immunofluorescence staining of mastigonemes with mAb-MAST1. The same cells are seen by dark-field (A,B,C) and immunofluorescence microscopy (A′,B′,C′). B,B′ and C,C′ were observed from a different angle (~90° difference). Mastigonemes are present as a row of fluorescent dots on both sides of the flagellum (A′,C′). Cells were fixed with 3.7% formaldehyde in A,A′, and with 0.2% glutaraldehyde in B,B′,C,C′. mAb-MAST1 positive rings at the anterior end of the cell are seen only in A′. Bar, 10 μm.

mAb-BTUB1 treated cells were measured from dark-field micrographs taken with an exposure of 1 second.

RESULTS

Negatively-stained EMs of *C. reinhardtii* revealed hair-like mastigonemes projecting from either side of the flagellar surface (Fig. 1)(Ringo, 1967; Witman et al., 1972). The mastigonemes are located on the distal two-thirds of the flagellar surface, and they are uniform in length (about 1 μm) and shape as reported by Witman et al. (1972).

By raising monoclonal antibodies against flagellar components in *C. reinhardtii*, we obtained a monoclonal antibody (mAb-MAST1, IgGl) against mastigonemes. We used mAb-MAST1 to examine the distribution of the antigen in cells by immunofluorescence microscopy. Fig. 2A′ and C′ shows mAb-MAST1 staining as a pair of dotted lines along the distal two-thirds; the lines seem to locate on both sides of the flagellar beat-axis (compare Fig. 2B′ with 2C′). We confirmed that mAb-MAST1 recognized mastigonemes by immunoelectron microscopy (Fig. 3). Immunogold particles bound uniformly along the whole mastigonemes.

Surprisingly, cells fixed in 3.7% formaldehyde often displayed a mAb-MAST1 positive fluorescent ring composed of more than 10 particles at the anterior end of the cell body (Figs 2A′, 8). However, autofluorescence of chlorophyll was strong and the ring of particles was not visible in cells fixed with 0.2% glutaraldehyde (Figs 2B′,C′, 10). We suppose that glutaraldehyde stabilized the cell membrane to 0.5% Triton X-100, thereby preventing access of antibodies into the cell. From these results and through-focus microscopic observations of formaldehyde-fixed cells, we believe that the mAb-MAST1 positive ring is located near the plasmic cortex. However, we could not detect corresponding bodies in this region by transmission electron or immunoelectron microscopy.

On western blots mAb-MAST1 specifically reacted with a

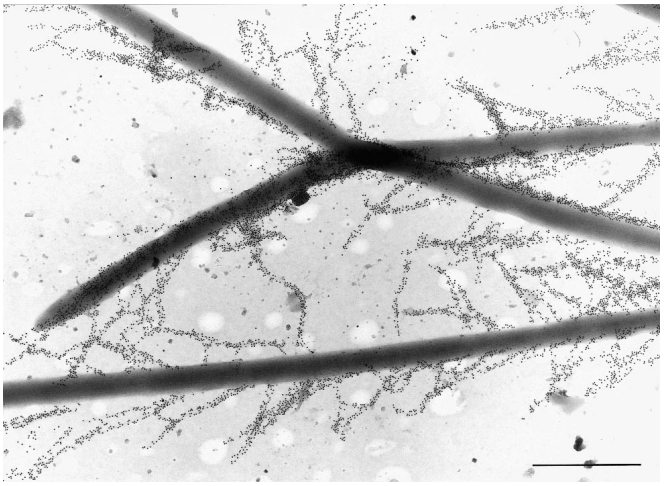

Fig. 3. Electron microscopic immunogold staining of mastigonemes with mAb-MAST1 followed by goat anti-mouse IgG coupled to 10 nm gold. Immunogold staining is evident along the entire length of the mastigonemes. Bar, 1 μm.

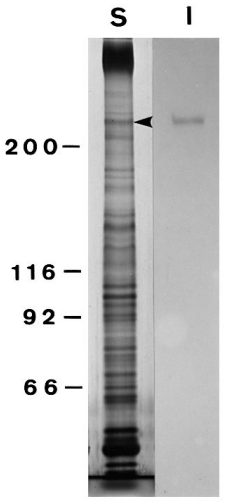

Fig. 4. Western immunoblot analysis of mAb-MAST1 antigen. S, a silver-stained gel of whole flagellar components used for immunoblot analysis; I, an immunoblot-stained strip of nitrocellulose using mAb-MAST1. Only one band (arrowhead) of ~230 kDa is recognized with mAb-MAST1. Molecular mass standards are shown on the left in kDa.

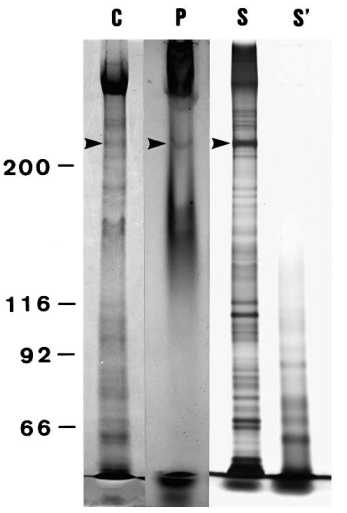

Fig. 5. Characterization of mAb-MAST1 antigen by SDS-PAGE. Whole flagellar components were electrophoresed on a slab gel. A lane in the same slab gel was stained with Coomassie Blue dye, C; stained by the periodic acid-Schiff (PAS) procedure, P; stained with silver, S. S′ shows pronase (200 μg/ml) treated whole flagellar components. The same 230 kDa band was recognized in C, P and S (arrowheads); however, this band and others are absent in S′. (The band in plane P is curved because a much larger amount of sample was loaded than in other lanes.) Molecular mass standards are shown on the left in kDa.

~230 kDa band from a wide range of other bands of flagellar components (Fig. 4). This 230 kDa-band was digested by pronase, and was PAS positive (Fig. 5). On the other hand, we were unable to detect any mAb-MAST1 positive band in a western blot of extracts from deflagellated and SDS solubilized cells. Thus the epitope that gave rise to the ring structure within the cell body remains to be studied further.

When flagella of C. *reinhardtii* are amputated, they regenerate to their original length in 90-120 minutes (Rosenbaum et al., 1969; Nakamura et al., 1987). We found that mastigonemes appeared on the surface of regenerating flagella as early as 15 minutes after amputation. Newly formed mastigonemes do not appear at the flagellar base, but are assembled on the distal 2/3 to 3/4 of regenerating flagella, the same region where mastigonemes are attached on full-grown flagella (Fig. 6). Mastigonemes also are assembled on the distal 2/3 to 3/4 of half-grown flagella regenerated in the presence of 20 μg/ml cycloheximide (data not shown).

When live cells were treated with mAb-MAST1 for 15 minutes or more, immunofluorescence revealed an absence of mastigonemes on the flagellar surface (Fig. 7). The bright ring of mAb-MAST1 positive particles at the anterior end of the cell was still present, however (Fig. 7). Western blotting also showed that mAb-MAST1 treated cells lost the ~230 kDa band (the component of mastigonemes) from their flagella (Fig. 8). This phenomenon was confirmed with negatively stained samples by electron microscopy (data not shown). When the mastigoneme-less (mAb-MAST1 treated) cells were washed and transferred to normal medium, they regenerated mastigonemes on their existing flagella (i.e. without flagellar regeneration) in about 4 hours. Fig. 9 shows new mastigonemes appeared on flagella in the same region as where they existed originally, and the regeneration around the flagellar tip occurred a little faster than the other area.

During regeneration of mastigonemes (after flagella amputation or mAb-MAST1 treatment), the mAb-MAST1 positive ring did not appear to change in fluorescence intensity, even in the presence of cycloheximide (20 μg/ml).

We found that mAb-MAST1 treated cells did not change their flagellar wave shape, but their swimming velocity decreased to about 70-80% of that of untreated cells and another mAb (mAb-BTUBl: a beta-tubulin antibody) treated cells (Table 1). On the other hand, flagellar beat frequency of mAb-MAST1 treated cells increased ~10% that of mAb-

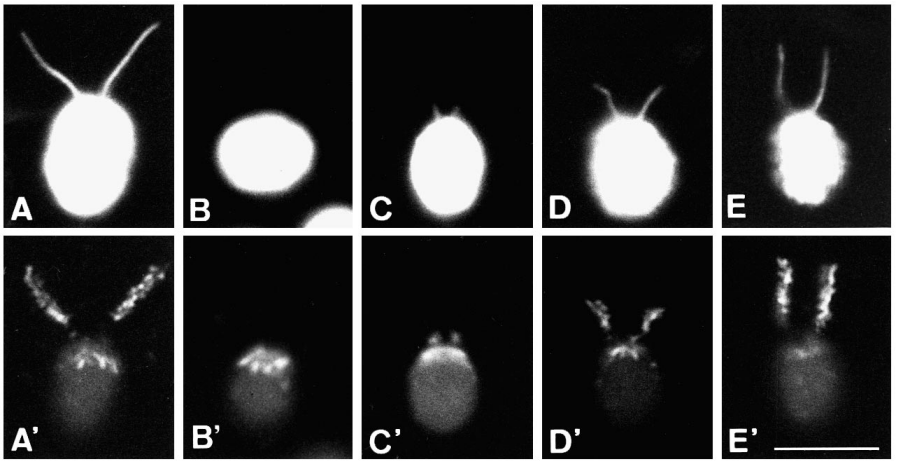

Fig. 6. Immunofluorescence staining of mastigonemes with mAb-MAST1 during flagellar regeneration. Corresponding dark-field (A-E) and immunofluorescence micrographs (A′-E′) of cells after staining with mAb-MAST1. (A,A′) Before flagellar amputation. Time after flagellar amputation in minutes: 0 (B,B′), 15 (C,C′), 30 (D,D′), 60 (E,E′). Mastigonemes regenerate with flagella, but do not appear (assemble) at the base. Bar, 10 μm.

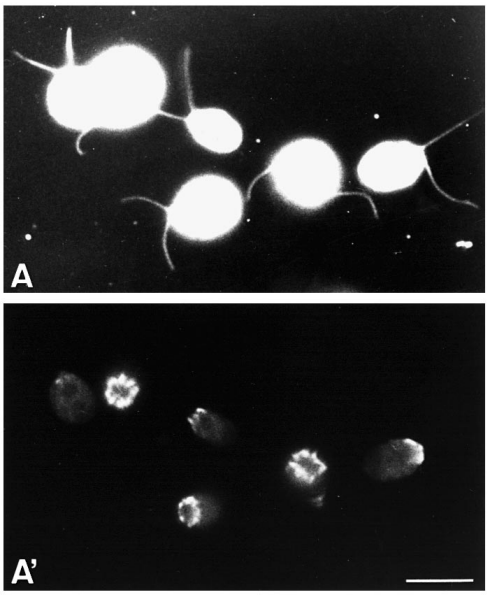

Fig. 7. mAb-MAST1 treated live cells lose mastigonemes but retain mAb-MAST1 positive particle rings. (A,A′) Cells after a treatment with mAb-MAST1 for 15 minutes. Darkfield (A) and immunofluorescence (A′) micrographs. Bar, 10 μm.

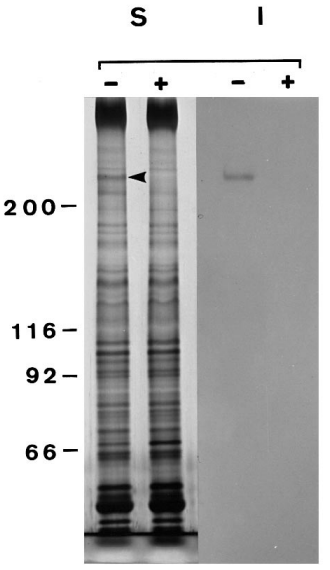

Fig. 8. Western immunoblot analysis of mAb-MAST1 treated cells showing loss of the ~230 kDa band (the component of mastigonemes). S, silver stained gels of the whole flagellar components; I, immunoblot stained strips of nitrocellulose using mAb-MAST1; –, flagellar components of untreated cells; +, flagellar components of mAb-MAST1 treated cells. Molecular mass standards are shown on the left in kDa.

BTUB1 treated and untreated cells (Table 1). However, mAb-MAST1 treated cells showed the gliding motility (Bloodgood, 1988) on a slide glass.

Table 1. Motility change in cells treated with mAb-MAST1

Antibody used	Swimming velocity* (μm/second)	Flagellar beat frequency† (Hz)
None	137.3±16.0	50.0-52.0
mAb-BTUB1‡	140.7±14.8	53.0-53.5
mAb-MAST1	108.7±15.1	57.0-59.0

Cells were mixed with mAbs for 15 minutes immediately before motility measurements. After the motility measurements, we confirmed by immunofluorescence microscopy that the flagella of the cells that had been treated with mAbMAST1 lost mastigonemes, while the above two control samples retained them.

*Average ± standard deviation in 100 cells.

†Median values of beat frequencies determined by fast Fourier transform analysis of the light intensity fluctuation in the microscope images of populations of swimming cells (Kamiya and Hasegawa, 1987). Range of data from two to four different cultures are shown. From the shape of the peak in power spectra, the standard deviation in beat frequency distribution is estimated to be 10-20% of the median value. Temperature, 23°C.

‡Monoclonal antibody against beta-tubulin, used as a negative control.

DISCUSSION

We have obtained and characterized a monoclonal antibody (mAb-MAST1) which is specific for mastigonemes. Immunofluorescence microscopy and immunoelectron microscopy show that mAb-MAST1 antigens are distributed along mastigonemes. This observation indicates that mastigonemes have a structure wherein mAb-MAST1 antigens repeat longitudinally. mAb-MAST1 recognized a band with an apparent molecular mass of 230 kDa on nitrocellulose transblots of flagellar components. The band was a glycoprotein because it was PAS positive, CBB stainable, and digestible with pronase. The antigen of mAb-MAST1 is probably the same as the 170 kDa glycoprotein reported by Witman et al. (1972). The dif-

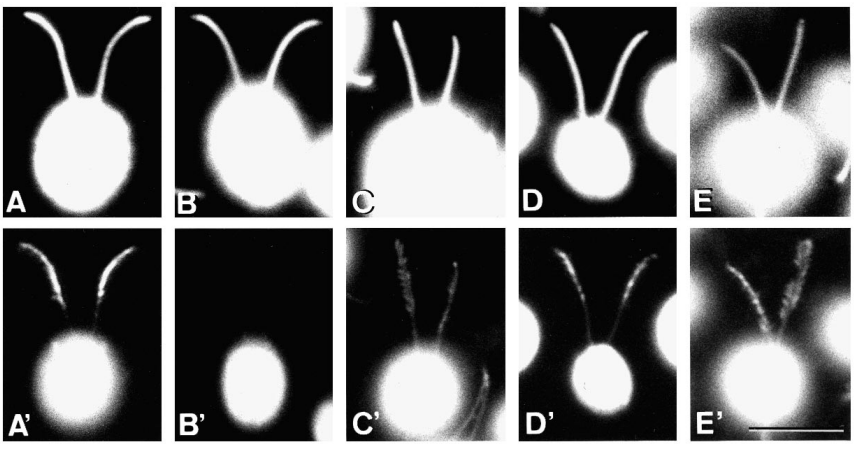

Fig. 9. Regeneration of mastigonemes in mAb-MAST1 treated cells. After 15 minutes mAb-MAST1 treatment, cells were washed with mAb-MAST1-free medium. (A-E) Dark-field, (A′-E′) immunofluorescence micrographs after fixation with 0.2% glutaraldehyde, and staining with mAb-MAST1. (A,A′), before treatment with mAb-MAST1. Time after washing with mAb-MAST1-free medium in hours: 0 (B,B′), 1 (C,C′), 2 (D-D′), 4 (E, E′). Brightness of mAb-MAST1 fluorescence gradually increases from C′ to E′. Bar, 10 μm.

ference in the apparent molecular mass between the mastigoneme glycoprotein reported by us and Witman et al. may be due to the SDS-PAGE system employed.

Fifteen minutes after the amputation, regenerating flagella already have many mastigonemes. Moreover, flagella regenerated in the presence of 20 μg/ml cycloheximide also display mastigonemes. This concentration of cycloheximide inhibits protein synthesis, and the length of regenerated flagella is about 50% that of control flagella (Rosenbaum et al., 1969; Nakamura et al., 1987). These results indicate that antigens of mAb-MAST1 are stored in a cytoplasmic pool. The particles of the mAb-MAST1 positive ring may well be the sites for such a pool. Although they did not visibly change in fluorescence intensity during flagellar regeneration, these particles may still be stores of the antigens if the particles were supplied with antigens produced during flagellar regeneration, and/or particles contain excess antigens compared with other flagellar components in the cytoplasmic pool. Therefore, during regeneration in cycloheximide, the particles also do not appear to decrease in fluorescence intensity. The ultrastructural correlate and the protein composition of the particles are not yet known.

When live cells were treated with mAb-MAST1, mastigonemes disappeared from the flagellar surface and this was correlated with an increase in flagellar beat frequency and a decrease in swimming velocity. This is the first direct evidence that mastigonemes play a role in motility, probably by increasing the effective surface area of flagella, and enhancing their propulsive force.

It is interesting that mAb-MAST1 treated cells (mastigoneme-less) assemble new mastigonemes on the same area of pre-existing flagellar surface (distal two-thirds) where mastigonemes were originally attached. Also, during flagellar regeneration new mastigonemes are mostly found assembled on the distal 2/3 to 3/4 of the flagellar surface. Hence, new mastigonemes may be inserted only in the distal region of the flagellum, or alternatively, inserted at the flagellar base and rapidly transported to the distal portion. It has been shown that radial spoke proteins and tubulin are transported to the tips of flagella before their assembly into the flagellar structure (Johnson and Rosenbaum, 1992). Mastigonemes may be transported by a similar system. We plan to use this regeneration system to examine the mechanism of mastigoneme storage, transport and polarized assembly onto the flagellum.

We thank Dr Sidney L. Tamm (BUMP, MBL, Woods Hole) for his helpful comments and critical reading of this paper. We are also grateful to Mr Kenji Taguchi and Miss Kaori Tomimatsu for their technical assistance to produce mAbs. This study was supported in part by Grants-in-Aids from the Ministry of Education, Science and Culture of Japan to S.N. (No. 06640860).

REFERENCES

Adair, W. S., Hwang, C. and Goodenough, U. W. (1983). Identification and visualization of the sexual agglutinin from the mating-type plus flagellar membrane of *Chlamydomonas. Cell* **33**, 183-193.

Bloodgood, R. A. (1988). Gliding motility and the dynamics of flagellar membrane glycoproteins in *Chlamydomonas reinhardtii*. *J. Protozool.* **35**, 552-558.

Bouck, G. B. (1972). Architecture and assembly of mastigonemes and related structures. In *Advances in Cell and Molecular Biology* (ed. E. J. Dupraw), vol. 2, pp. 237-271. Academic Press Inc., New York.

Harris, E. H. (1989). *The Chlamydomonas Sourcebook.* Academic Press, San Diego.

Johnson, K. A. and Rosenbaum, J. L. (1992). Polarity of flagellar assembly in *Chlamydomonas. J. Cell Biol.* **119**, 1605-1611.

Kamiya, R. and Hasegawa, E. (1987). Intrinsic difference in beat frequency between the two flagella of *Chlamydomonas reinhardtii*. *Exp. Cell Res.* **173**, 299-304.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Littlefield, J. W. (1964). Selection of hybrids from matings of fibroblasts in vitro and their presumed recombinants. *Science* **145**, 709-710.

Mori, T., Matsunaga, T., Hirose, T. and Nikaido, O. (1988). Establishment of a monoclonal antibody recognizing ultraviolet light-induced (6-4) photoproducts. *Mutat. Res.* **194**, 263-270.

Nakamura, S., Takino, H. and Kojima, M. K. (1987). Effect of lithium on flagellar length in *Chlamydomonas reinhardtii. Cell Struct. Funct.* **12**, 369-374.

Ringo, D. L. (1967). Flagellar motion and fine structure of the flagellar apparatus in *Chlamydomonas*. *J. Cell Biol.* **33**, 543-571.

Rosenbaum, J. L., Moulder, J. E. and Ringo, D. L. (1969). Flagellar elongation and shortening in *Chlamydomonas.* The use of cycloheximide and colchicine to study the synthesis and assembly of flagellar proteins. *J. Cell Biol.* **41**, 600-619.

Sager, R. and Granick, S. (1953). Nutritional studies with *Chlamydomonas reinhardtii. Ann. NY Acad. Sci.* **56**, 831-838.

Snell, W. J. (1976). Mating in *Chlamydomonas:* a system for the study of specific cell adhesion. I. Ultrastructural and electrophoretic analyses of flagellar surface components involved in adhesion. *J. Cell Biol.* **68**, 48-69.

Tanaka, G., Nakamura, S., Maeda, T., Matsunaga, T, Nikaido, O. and Kamiya, R. (1994). A monoclonal antibody to the mastigonemes in *Chlamydomonas reinhardtii*. *Cell Struct. Funct.* **19**, 489.

Towbin, H., Staehelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proc. Nat. Acad. Sci. USA* **76**, 4350-4354.

Wiese, L. (1969). Algae. In *Fertilization: Comparative Morphology, Biochemistry and Immunology* (ed. C. B. Metz and A. Monroy), vol. 2, pp. 135-188, Academic Press Inc., New York.

Witman, G. B., Carlson, K., Berliner, J. and Rosenbaum, J. L. (1972). *Chlamydomonas* flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* **54**, 507-539.

Zacharius, R. M., Zell, T. E., Morrison, J. H. and Woodlock, J. J. (1969). Glycoprotein staining following electrophoresis on acrylamide gels. *Anal. Biochem.* **30**, 148-152.

(Received 18 July 1995 - Accepted 27 September 1995)