ZOOLOGICAL SCIENCE 5: 973–978 (1988)

Fibrillar System and Possible Control Mechanism for the Cycle of Contraction and Elongation of *Spirostomum ambiguum*

HIDEKI ISHIDA[1], YOSHINOBU SHIGENAKA[2] and MASAKO IMADA

Laboratories of Cell Biology, Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730, Japan

ABSTRACT—A large heterotrichous ciliate, *Spirostomum ambiguum*, shows the characteristic twisting contraction when stimulated by certain mechanical or chemical factors. This contraction is attributed to a special contractile fibrillar system which is termed myoneme, while the subsequent elongation after the contraction occurred might be induced by another type of fibrillar system, longitudinal microtubular sheets (LMSs). The myoneme is composed of a great number of fine filaments which are heavily packed and lie in parallel with each other. The diameter of individual filament varies from 3 to 5 nm in the elongated state but raises up to 7 to 9 nm in the contracted state. Every nonciliated basal body has a rootlet-like structure, which is attached to the myoneme not only in the contracted state but also in the elongated state. If the myoneme and LMSs have antagonistic functions to each other, the force of each fibrillar system is assumed to be transmitted by these rootlet-like structures. On the other hand, anterior fiber sheet was found to be attached to the LMS in the extended state but detached from that in the contracted state of organism. From these observations, the switching or control mechanism was proposed and discussed for the cycle of contraction and elongation of the organism.

INTRODUCTION

Among a number of heterotrichous ciliates, there are some typical contractile ones such as *Condylostoma*, *Spirostomum* and *Stentor*. However, *Spirostomum ambiguum* is the only one showing its own characteristic twisting contraction [1–3]. Although the contraction of the cell body of these organisms is easily induced by various kinds of stimuli this is believed to be caused directly by a special contractile fibrillar system which is termed myoneme [1–6] or M-band [7]. On the other hand, the subsequent elongation of the organism after the contraction occurred has been proposed to be attributed mainly to another fibrillar system, longitudinal microtubular sheets (LMS) [2, 3].

Regarding to the twisting contraction mechanism of *Spirostomum*, Yogosawa-Ohara *et al.* [3] have proposed the following sequence: (1) Shortening along the long axis occurs due to the activity (probably sliding) of myonemal filaments, (2) at the moment when the body length decreases all of the neighboring LMSs slide with each other possibly involving the anterior fiber sheet, and (3) the curving of each LMS is derived from the sliding between the LMS and the sasa structure (SS) by means of obliquely arranged projections on the SS which is a bamboo leaf-like structure attached to the proximal region of every LMS. Coordination of these phenomena might result in the twisting contraction of the organism.

However, the ultrastructural details have not thoroughly been studied especially with respect to the switching mechanism of contraction and elongation of *Spirostomum*. Therefore, the present study aimed to examine the details of myoneme, longitudinal microtubular sheets and their associated structures for elucidating the structural changes between two states of contraction and elongation. As the results, a possible mechanism controlling the contraction-elongation cycle has been proposed here.

Accepted January 28, 1988
Received January 6, 1988

[1] Present address: Biological Institute, Shimane University, Matsue 690, Japan.

[2] To whom reprint requests should be forwarded.

MATERIALS AND METHODS

Organisms

Live samples of *Spirostomum ambiguum* Ehrenberg were originally collected and kindly supplied by Dr. T. Suzaki from the Research School of Biological Sciences, the Australian National University, Canberra, A. C. T. 2601, Australia. They were cultured in our laboratory using 0.01% Knop solution as culture medium containing some boiled wheat grains and small quantity of 0.5% hay infusion at about 24°C. Other special food sources were not supplied to the medium. Subculturing was carried out at a regular interval of about 3 weeks.

Light Microscopy

Light micrographs were obtained from the living organism in both states of contraction and elongation by using a differential interference microscope (Olympus, BH). To cause the contraction, mechanical stimuli were applied to the organisms by means of tapping the specimen-loaded glass slide or electrical stimuli (35 V, DC) were given directly to the organism. In case of electrical stimulation, the organism was placed at the center between the two platinum electrodes (5 mm in distance).

Electron Microscopy

Prior to the fixation, living organisms were rinsed twice to clean them up with fresh culture medium, 0.01% Knop solution. Preparations in the elongated state were prepared by treating the organisms primarily with the relaxation medium containing 10 mM EGTA, 3 mM $MgSO_4$ and 10 mM phosphate buffer (pH 6.8) for 10 min just before the fixation. The other preparations in the contracted state were obtained by stimulating them in the fresh culture medium (0.01% Knop solution) by means of tapping the tube containing the organisms.

The pre-fixation was performed in 1% glutaraldehyde with 33 mM phosphate buffer (pH 6.8) for 30 min at 0°C. The fixed samples were rinsed with 33 mM phosphate buffer (pH 6.8). Following that, they were post-fixed in 1% OsO_4 with 33 mM phosphate buffer (pH 6.8) for 30 min at 0°C. After the double fixation, the samples were dehydrated with a graded ethanol series and embedded into Spurr's low viscosity embedding medium [8].

Ultrathin sections were produced with a Porter-Blum ultramicrotome (MT-1) equipped with glass knives. The sections were stained with 3% aqueous uranyl acetate for 7 min and Reynolds' lead citrate stain [9] for 3 min at room temperature. Observations were carried out under a transmission electron microscope (JEOL, JEM-100S) at the accelerating voltage of 80 kV.

RESULTS

A large heterotrichous ciliate, *Spirostomum ambiguum*, shows the characteristic contraction when applied by chemical, mechanical or electrical stimuli [1–5, 10]. Figure 1 shows the two states of the same free-swimming organism; Figure 1a is in the elongated stage and Figure 1b in the electrical-

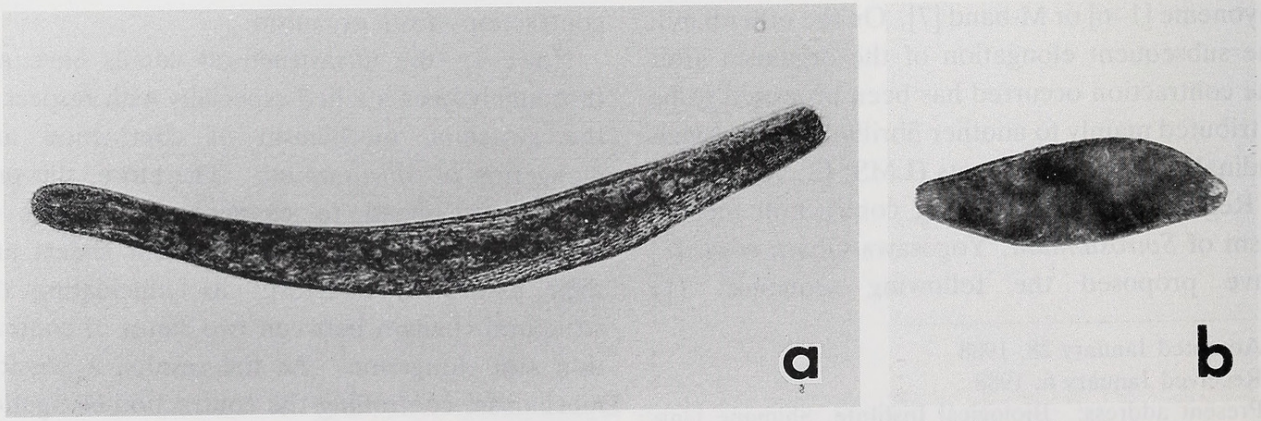

FIG. 1. Light micrographs of the same living *Spirostomum ambiguum* in the extended (Fig. 1a) and the contracted (Fig. 1b) states (×920).

ly stimulated and contracted state.

When observed under an electron microscope, the cortical region of organism is characterized by three typical types of fibrillar systems and their associated structures; sub-pellicular microtubules, longitudinal microtubular sheets and myonemes (Fig. 2a). The myoneme is located at the transitional plane between ectoplasm and endoplasm and featured by being surrounded by various sizes of vacuoles. Every myoneme is composed of a great number of filaments which are heavily packed and lie in parallel with each other inside the myoneme. The diameter of individual filament varies from 3 to 5 nm in elongated state of the organism (Fig. 3a), but raises up to 7 to 9 nm in the contracted state (Fig. 3b).

As a whole, the bundles of myonemal filaments demonstrate a mesh-like distribution throughout the cell body just beneath the arrays of longitudinal microtubular sheets as described by Yogosawa-Ohara and Shigenaka [2]. In addition to that, the present study revealed that major bundles of the myonemal meshes ran in parallel with the body axis in the elongated state of organism but altered their axes diagonally in the contracted state. In the contracted state, on the other hand, the myonemal

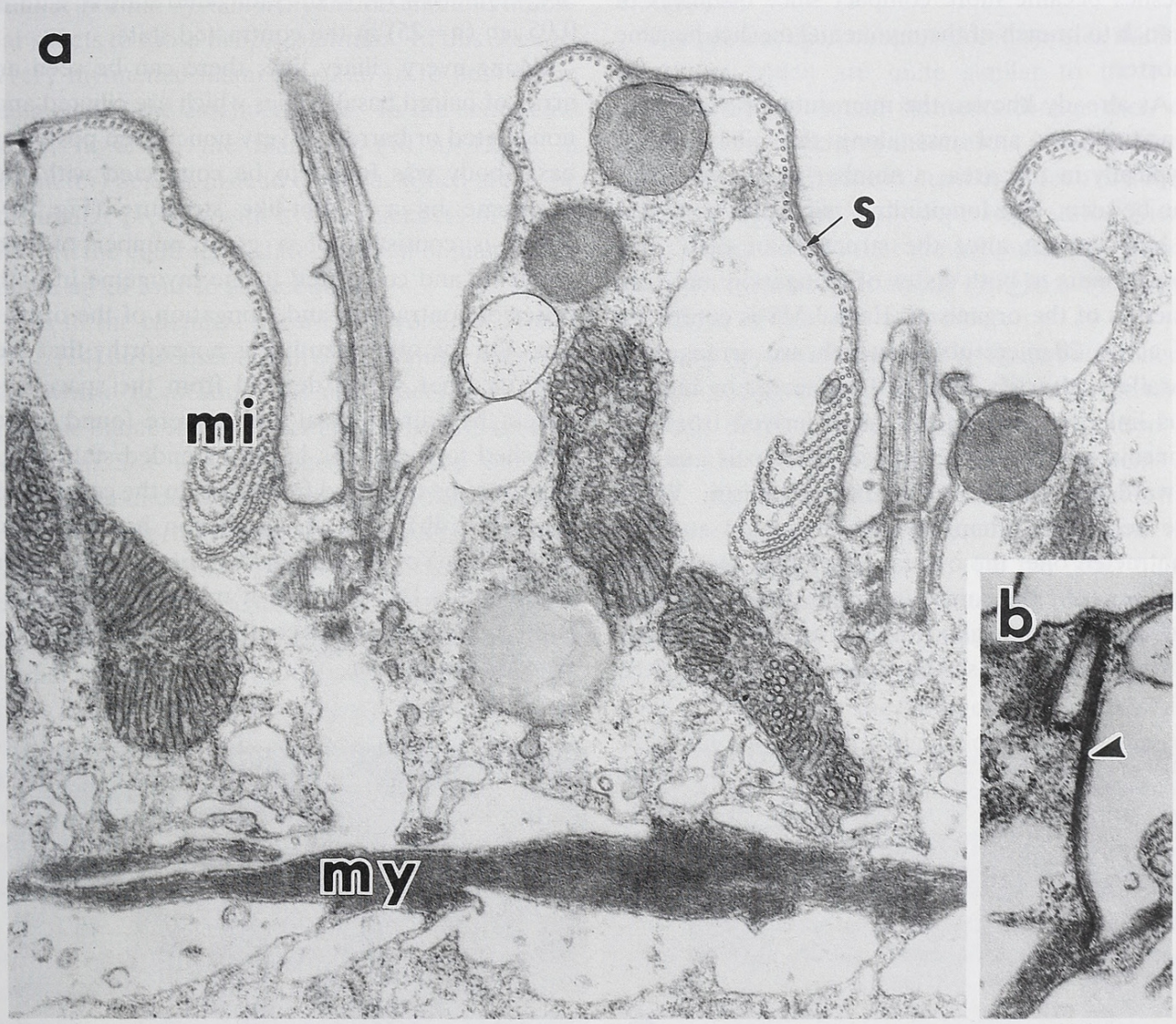

FIG. 2. Electron micrographs of vertical sections through the area of cell cortex. Microtubular sheets (mi) and myoneme (my) lie just under the cell surface (Fig. 2a). An inserted electron micrograph (Fig. 2b) is of the rootlet-like structure in the contracted state, in which a nonciliated posterior basal body was found to be connected with the myoneme by the rootlet-like structure (arrowhead). Just under the cell membrane, sub-pellicular microtubules could be seen (s). (×24,500).

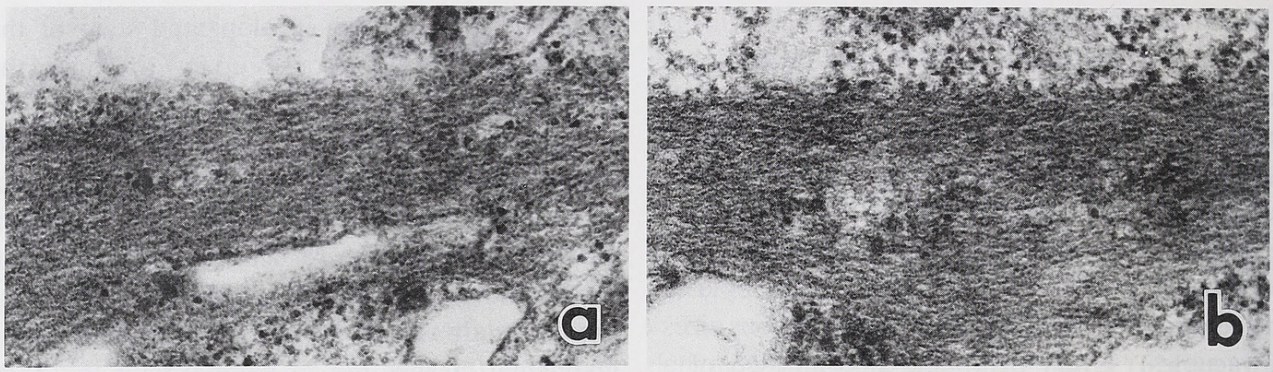

FIG. 3. Electron micrographs of longitudinal sections through the myoneme in the extended (Fig. 3a) and the contracted (Fig. 3b) states. The bundle of myoneme consists of 3 to 5 nm filaments in the extended state and 7 to 9 nm filaments in the contracted state. Myonemal filaments run in parallel with each other. (×40,700).

meshes became more compact since distances of branch to branch of the myonemal meshes became shorter.

As already known, the microtubular sheets run longitudinally and just along the ciliary lines. Typically in this area, a number of mitochondria can be seen. The longitudinal microtubular sheets (LMS) do not alter the structure of their own components in both states of elongation and contraction of the organism. Each LMS is composed of about 20 microtubules which are arranged in parallel with each other and connected by numerous links to one another, and derived from the nonciliated one of every basal body pair and run toward the posterior end of the organism. When the cell shape changes from extended state to contracted one, the overlapping LMSs increase in number. At the same time, the center-to-center distance of two neighboring and antero-posteriorly arranged ciliary bases becomes shorter from 3.05 $\pm$0.05 μm (n=14) in the elongated state to 2.25$\pm$ 0.05 μm (n=25) in the contracted state.

Along every ciliary line, there can be seen an array of paired basal bodies which are ciliated and nonciliated or barren. Every nonciliated posterior basal body was found to be connected with the myoneme by a rootlet-like structure (Fig. 2b), which is consisted of a great number of fine filaments and connected to the myoneme in both states of contraction and elongation of the organism. On the other hand, it is noteworthy that the anterior fiber sheets derived from the space between the pair of basal bodies were found to be attached to the LMSs in the extended state (Fig. 4a) but to be detached from them in the contracted state (Fig. 4b). This phenomenon has not been noticed by Yogosawa-Ohara *et al.* [3] and other investigators [1], although it might be closely related to controlling the contraction-elongation cycle of the organism.

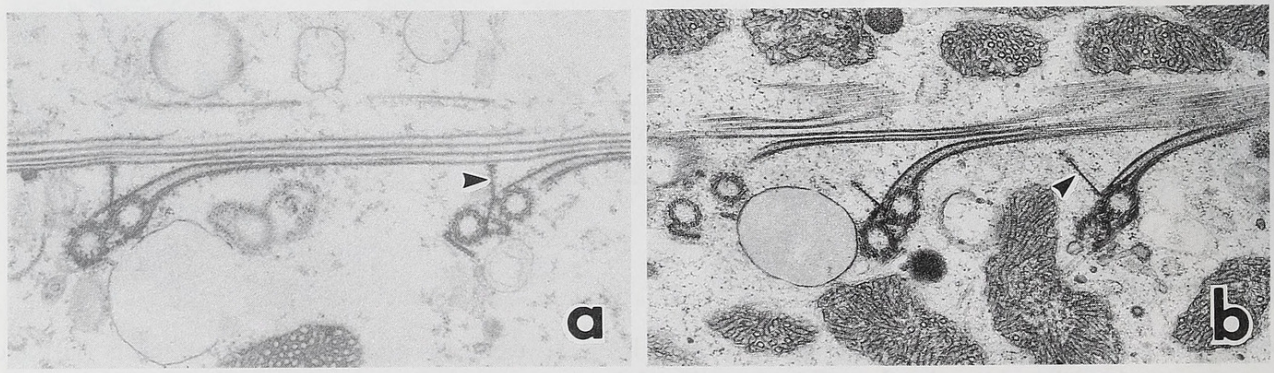

FIG. 4. Electron micrographs of the longitudinal microtubular sheets (LMSs). The anterior fiber sheets derived between the pair of basal bodies (arrow heads) are attached to the LMSs in the extended state (Fig. 4a) but become detached from them in the contracted state (Fig. 4b). (×12,000).

Just under the cell membrane, so-called subpellicular microtubules could be seen (Fig. 2a). These microtubules are closely associated to the cell membrane and run in parallel with the ciliary line, suggesting that they might have a cytoskeletal role without being related to the contraction-elongation cycle.

DISCUSSION

Just like in the present organism, another heterotrichous ciliate, *Stentor coeruleus*, also shows the rapid contraction [11–13], although the style is not twisting contraction. This organism also has the similar fibrillar systems, myoneme and microtubular sheets to those in *Spirostomum*. In this *Stentor*, every myonemal filament is known to be 4 nm in diameter in the extended state. In the contracted state, however, the filaments (10 to 12 nm in diameter) appear instead of them, which are to be of tubular profiles with a wall thickness of 4 to 5 nm. In the contracted state, the wall of filaments is made up of four to six subunits.

As to the chemical nature of myoneme, Hobbs *et al.* [14] have described in *Spirostomum teres* that arrowhead decoration was not observed in cytoplasmic filament bundles although the myosin subfragment S–1 was introduced into the cells for incubation under conditions suitable for actin decoration. Furthermore, Yogosawa-Ohara and Shigenaka [2] have published in *Spirostomum ambiguum* that cytochalasin B treatment did not cause degradation of myonemal filaments even at a higher concentration (50 μg/ml). These observations strongly suggest that the myonemal filaments may not be actin-like, but may be similar to the spasmonemal filaments of peritrichous ciliates, *Vorticella* and *Carchesium* [15, 16] or the retraction fiber filaments of a dinoflagellate, *Ceratium* [17].

On the other hand, microtubular sheets which are derived from antero-posteriorly arranged ciliary pairs are overlapping and might slide relatively with each other. When contracted, the overlapping microtubular sheets increase their number in cross setions as described by Huang and Pitelka [12]. When the cell was fixed in isometric contraction, the microtubular sheets were in the state of elongation and the overlapping microtubular sheets were at minimum in number. Moreover, the internal structure of the myoneme altered in the contracted state; the myonemal filaments became to be of tubules with the diameter of 10 to 12 nm as described above.

This observation has suggested that the myoneme generates the motive force resulting into cell shortening [11]. It is thought that the myoneme and the microtubular sheets might function as antagonistic elements to each other. That is to say, the myoneme generates the motive force for cell contraction, although the microtubular sheets slide with each other to cause only cell elongation.

The present *Spirostomum* demonstrates the fine structures which are quite similar to those in *Stentor*; the myonemal filaments change their diameters and structures themselves and the microtubular sheets increase in number as seen in a cross section of organism in the contracted state. Therefore, it may be said that the myoneme and microtubular sheets of *Spirostomum* might function just like those of *Stentor*.

If the myoneme and the microtubular sheets have a function as an antagonistic system, the force of each fibrillar system must be transmitted to induce the movement of antagonistic systems of them. As the candidate for this, the rootlet-like structures might be considered to transmit the force of antagonistic system. On the other hand, the anterior fiber sheets are attached to the microtubular ribbons in the elongated state but detached from them in the contracted state, so they may have a function as the "trigger" or "switch" for inducing contraction and/or elongation of the cell body.

REFERENCES

1 Yagiu, R. and Shigenaka, Y. (1963) Electron microscopy on the longitudinal fibrillar bundle and the contractile fibrillar system in *Spirostomum ambiguum*. J. Protozool., **10**: 364–369.

2 Yogosawa-Ohara, R. and Shigenaka, Y. (1985) Twisting contraction mechanism of a heterotrichous ciliate, *Spirostomum ambiguum*. 1. Role of the myoneme. Cytobios, **44**: 7–17.

3 Yogosawa-Ohara, R., Suzaki, T. and Shigenaka, Y. (1985) Twisting contraction mechanism of a hetero-

trichous ciliate, *Spirostomum ambiguum*. 2. Role of longitudinal microtubular sheet. Cytobios, **44**: 215–230.

4 Hamnilton, T. C. and Osborn, D. (1976) Measurements of contraction latencies to mechanical and electrical stimulation of the protozoan, *Spirostomum ambiguum*. J. Cell Physiol., **91**: 403–408.

5 Hawkes, R. B. and Holberton, D. V. (1974) Myonemal contraction of *Spirostomum*. I. Kinetics of contraction and relaxation. J. Cell Physiol., **84**: 225–236.

6 Yagiu, R. and Shigenaka, Y. (1961) Electron microscopical observation of *Condylostoma spatiosum* Ozaki & Yagiu, in ultra-thin section. VIII. Myoneme (the contractile fibrils). Zool. Mag., **70**: 110–114.

7 Randall, J. T. and Jackson, S. F. (1958) Fine structure and function in *Stentor polymorphus*. J. Biophys. Biochem. Cytol., **4**: 807–830.

8 Spurr, A. R. (1969) A low-viscosity eopxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., **26**: 31–43.

9 Reynolds, E. S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., **17**: 208–212.

10 Ettienne, E. E. (1970) Control of contractility in *Spirostomum* by dissociated calcium ions. J. Gen. Physiol., **56**: 168–179.

11 Bannister, L. H. and Tatchell, E. C. (1968) Contractility and the fiber systems of *Stentor coeruleus*. J. Cell Sci., **3**: 295–308.

12 Huang, B. and Pitelka, D. R. (1973) The contractile process in the ciliate, *Stentor coeruleus*. I. The role of microtubules and filaments. J. Cell Biol., **57**: 704–728.

13 Newman, E. (1972) Contraction in *Stentor coeruleus*: A cinematic analysis. Science, **177**: 447–449.

14 Hobbs, V. S., Jenkins, R. A. and Bamburg, J. R. (1983) Evidence for the lack of actin involvement in mitosis and in the contractile process in *Spirostomum teres*. J. Cell Sci., **60**: 169–179.

15 Amos, W. B. (1972) Strucuture and coiling of the stalk in the peritrich ciliates *Vorticella* and *Carchesium*. J. Cell Sci., **10**: 95–122.

16 Amos, W. B. (1975) Contraction and calcium binding in the vorticellid ciliates. In "Molecule and Cell Movement". Ed. by S. Inoué and R. E. Stephens, Raven Press, New York, pp. 411–436.

17 Maruyama, T. (1981) Motion of the longitudinal flagellum in *Ceratium tripos* (Dinoflagellida): a retractile flagellar motion. J. Protozool., **28**: 328–336.