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Cell Body Contraction of *Spirostomum* Does Not Involve Shortening of Inter-Kinetosomal Distance along Ciliary Lines

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ABSTRACT—A heterotrichous ciliate *Spirostomum* is known to show characteristic rapid contraction of the cell body accompanied by twisting of ciliary lines which run longitudinally on the cell surface. The ciliary lines of *Stentor*, a closely related heterotrichous ciliate, are known to become shorter by themselves when the cell contracts, resulting in sliding between adjacent longitudinal microtubular sheets (LMSs) running just beneath the ciliary lines. In *Spirostomum*, in contrast, there is controversy over whether lengths of the ciliary lines alter or not. In this study, we examined changes of the ciliary line lengths by scanning electron microscopy, to measure the distance between the proximal ends of two neighboring cilia which are lining up along each ciliary line (inter-kinetosomal distances). We found that the inter-kinetosomal distance remained constant regardless of cell contraction and elongation, indicating that the cell body contraction of *Spirostomum* results from a cellular twisting motion with constant inter-kinetosomal distance. It can thus be concluded that the contraction of *Spirostomum* does not involve shortening of the inter-kinetosomal distance along ciliary lines, which is quite different from the case in *Stentor*.

INTRODUCTION

It is well known that the heterotrichous ciliate *Spirostomum* shows a characteristic twisting contraction as its genus name indicates. In some heterotrichous ciliates including *Spirostomum*, it is generally considered that the contraction and subsequent re-elongation of the cell body result from antagonistic functions of two kinds of filamentous systems, myonemes and longitudinal microtubular sheets (LMSs), both of which are located just beneath the cell surface (Ishida *et al.*, 1988; Huang and Pitelka, 1973; Yogosawa-Ohara and Shigenaka, 1985; Yogosawa-Ohara *et al.*, 1985). Huang and Pitelka (1973) showed that active contraction of myonemes induces rapid rounding-up of the cell body in *Stentor*. In this mechanism, the myonemes running in parallel with the longitudinal axis of the cell body are thought to contract, causing passive sliding of LMSs and also shortening of the length of every ciliary line. In the re-elongation stage of the cell body, active sliding occurs between the neighboring LMSs together with relaxation of the myoneme.

In *Spirostomum*, it has been speculated that the mechanisms of contraction and re-elongation of the cell body might be the same as those in *Stentor*. Theoretically, however, the cell body of *Spirostomum* can contract either with or without shortening of ciliary lines, because the organism displays twisting contractions of the cell body. Whether the cell body

contraction is accompanied by shortening of ciliary lines themselves has not been determined (Ishida *et al.*, 1988; Lehman and Rebhun, 1971; Yogosawa-Ohara *et al.*, 1985).

In the present study, we attempted to determine the change of lengths of ciliary lines in order to elucidate the movement of intracellular fibrillar systems. Since the ciliate *Spirostomum* demonstrates a special structure wherein the ciliary lines which run spirally on the cell surface, we decided to measure the distance between the two neighboring ciliary bases (inter-kinetosomal distance) for the replacement of the ciliary lines. However, it can be rather difficult to measure the correct lengths of these distances under a light microscope. We therefore used a scanning electron microscope (SEM). Samples for the SEM must be fixed prior to observation, and the same cell cannot be used before and after contraction of the cell body. We therefore measured cells which have various lengths of cell body so that we might make statistical analyses.

In *Spirostomum*, myoneme and LMSs have been reported to be connected to each other with special fibrillar rootlet-like structures (Ishida *et al.*, 1988). The LMSs are known to have rigid connections with the corresponding ciliary bases by so-called anterior fiber sheets. In the present study, we measured the distance directly between the two neighboring ciliary bases to calculate the lengths of ciliary lines at both contraction and re-elongation stages of the cell body. For this purpose, fixed and critical point-dried specimens were examined in detail under the scanning electron microscope.

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MATERIALS AND METHODS

The species used in the present study was *Spirostomum teres* Claparède et Lachmann. The cells were cultured at about 22°C in Dryl's solution containing 1 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, 1.4 mM Na_2HPO_4 , 0.6 mM NaH_2PO_4 , and 1.5 mM CaCl_2 (pH 7.0) with a boiled wheat grain on the bottom of every test tube. The culture was routinely kept in darkness. Moreover, a small quantity of wheat infusion which was previously adjusted at pH 7.0 was added to the above culture medium every day. Subculturing was carried out at the interval of about 1 week.

For electron microscopic observation, suitable volumes of culture medium from test tubes were filtrated with four sheets of gauze to remove various kinds of contamination. The filtrated medium was centrifuged by using a hand centrifuge (about $500 \times g$). The sedimented cells were re-suspended in the culture medium. Thereafter, the cells were rinsed with fresh culture medium twice, then fixed at 4°C for 30 min with fixative containing 1% glutaraldehyde, 1% osmium tetroxide, 10 mM EGTA, and 40 mM KCl. The fixative was added directly to the cell suspension for the contracted state samples. To obtain samples in the elongated state, cells were pre-treated for 4 or 5 min with the relaxation medium containing 10 mM EGTA and 20 mM HEPES. The fixed samples were dehydrated through a graded ethanol series, transferred to isoamyl acetate and finally dried by using a critical point dryer. At that time, we considered that the critical point drying technique did not present a problem because we were going to examine the distance between two neighboring ciliary bases, particularly from the quantitative viewpoint. The dried samples were sputter-coated with Platinum-Palladium and observed with a scanning electron microscope (S-800, HITACHI, Tokyo, Japan).

The scanning electron micrographs showing the cell surface on the negative films were transferred into graphic data on computer by using an image scanner (GT-6000, EPSON, Tokyo, Japan). The distances between the two neighboring ciliary bases were measured directly on the computer display. Measured data were statistically analyzed with t-test. Probability values less than 5% were considered significant.

RESULTS

The specimens were prepared from the stationary phase of *Spirostomum* culture, which contained cells of various sizes. More exactly, the prepared cells showed various lengths ranging from 204.5 to 468.8 μm and widths ranging from 41.8 to 50.1 μm . Figure 1 shows the scanning electron micrographs of an elongated cell (Fig.1a) and a contracted one (Fig.1b). As the figures show, the ciliary lines were winding on the cell surface in both organisms, although the degree of winding differed between the cells. The number of revolutions of ciliary lines was observed to increase from 1.5 to 2.7. High magnification images of the scanning electron micrographs of these cells (Fig. 1c) were transferred into graphic data on the computer. We measured the lengths between the two neighboring ciliary bases by using these graphic data.

We speculated that the distances between the two ciliary bases might differ from each other between large cells and small ones, or at different parts of the cell body. In order to reveal the change of ciliary lengths between the contracted and elongated state of the organism, therefore, cells in the two states were measured in three regions (anterior, middle and posterior) of the cells. We chose the relatively large cells ($> 400 \mu\text{m}$ in length) so that we could measure the distance between the two neighboring ciliary bases at the contracted and elongated state.

Table 1 shows the distance between two neighboring ciliary bases in three regions of the cell body. The data indicate that the inter-kinetosomal distance measured at the contracted state is not significantly different from the distance measured at the elongated state, and also that the distances at the three parts of the cell body are about the same, suggesting that every part of the cell body can be used as the scale for measuring the inter-kinetosomal distance. As a next step, the widths between two neighboring ciliary lines were measured

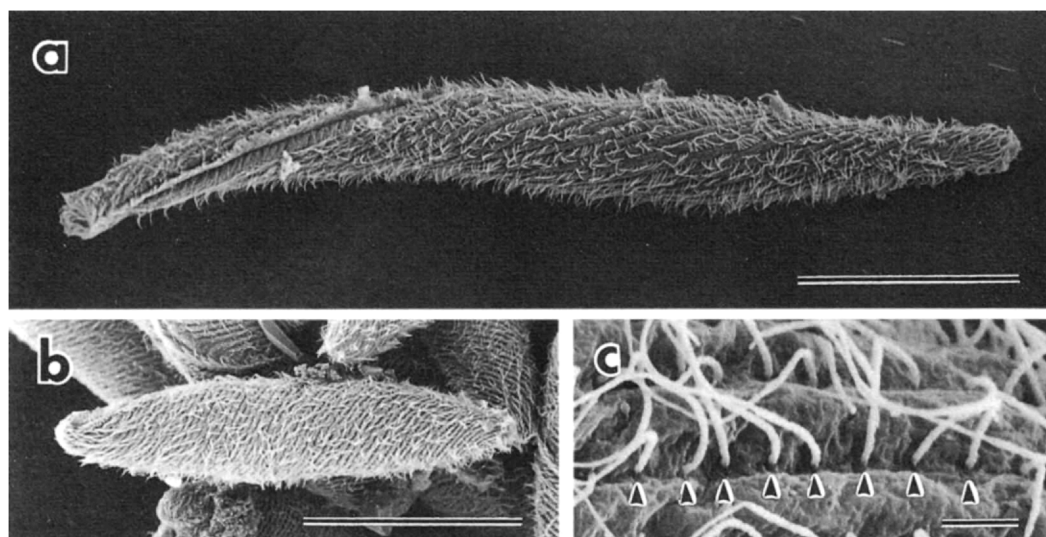


Fig.1. A scanning electron micrograph of an elongated cell (a) and a contracted cell (b). In the highly magnified electron micrograph of cell surface (c), each arrow indicates a ciliary base. Scale bar: 100 μm for a and b, and 2 μm for c.

Table 1. Distance between the two neighboring ciliary bases at the contracted and elongated states of *Spirostomum*

	Distance between ciliary bases, mean \pm S.D. (μm)		
	anterior	middle	posterior
Elongated	1.07 \pm 0.14 (101)	1.05 \pm 0.17 (96)	1.11 \pm 0.17 (101)
Contracted	0.94* \pm 0.12 (99)	1.17* \pm 0.25 (67)	1.05* \pm 0.17 (91)

*Not significantly different from elongated state ($p > 0.05$).
Numbers in parentheses indicate sample numbers.

in the middle part of larger cells ($> 400 \mu\text{m}$ in length). The widths were $2.59 \pm 0.04 \mu\text{m}$ ($n=25$) in the contracted state and $1.63 \pm 0.14 \mu\text{m}$ ($n=24$) in the elongated state. In smaller cells ($< 250 \mu\text{m}$ in length), in contrast, the widths were $1.96 \pm 0.10 \mu\text{m}$ ($n=28$) in the contracted state and $1.56 \pm 0.10 \mu\text{m}$ ($n=23$) at the elongated state. From these data, it is presumed that the widths between two neighboring ciliary lines might change considerably upon the cellular change from the contracted state to the elongated one, suggesting that cellular contraction and elongation can be attributed mainly to the twisting motion of the cell body and its relaxation.

Larger cells and smaller ones showed almost the same distance between the two neighboring ciliary bases. This distance did not change among the different parts of the cell body or change before and after contraction of the organism; as shown in Fig. 2, the main peak remained constant at around $1 \mu\text{m}$ in both cases.

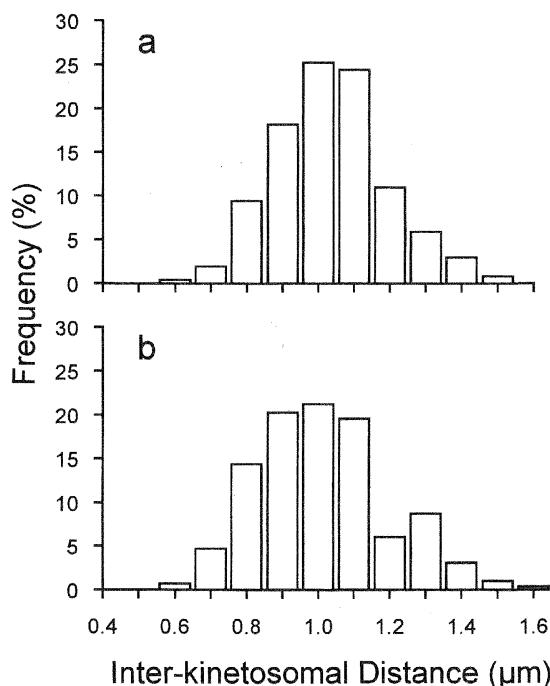


Fig. 2. A histogram showing a relationship between the inter-kinetosomal distance and its frequency at elongated (a) or contracted (b) state of the organism. The main peaks can be seen at around $1 \mu\text{m}$ in both cases, and there is not a significant difference between them.

DISCUSSION

The heterotrichous ciliate *Spirostomum* shows a characteristic twisting contraction when it receives external and/or internal stimuli. Considerable shortening and twisting motions occur simultaneously on the cell body at the period of contraction. It has been speculated that the myonemes composed of a number of fine fibrils generate active force for that kind of contraction. The vector of this force might be divided to shortening and twisting motion of the cell body. However, the longitudinal microtubular sheets (LMSs) might generate the force for elongation when the cell body recovers from contraction. This force caused by the LMSs might be split into the force of elongation and relaxation of the cell body. The movement mechanisms of intra-cellular fibrillar systems such as myoneme and LMS remain to be clarified.

The possibility that the distances between the two neighboring ciliary bases might change with cell growth should be considered. However, we found that the distance in the elongated state of the cell did not change with the size of the cell. Moreover, the distance in the anterior, middle and posterior parts of the cell did not change significantly at any state of the cell and at any part of the cell.

Yogosaawa-Ohara *et al.* (1985) made a dummy-model of *Spirostomum* to explain the number of revolutions of ciliary lines and change of ciliary lengths. In their study, the lengths of ciliary lines were observed to change to 68% of the original lengths in the contracted state. In the present study, the number of revolutions of ciliary lines was found to increase from 1.5 to 2.7, but the length did not change significantly. The difference between these findings might be caused by a technical difficulty in measurement due to a rotation of ciliary lines at the anterior and posterior end. It may be difficult to determine the alternating pitch of ciliary lines on the dummy-model at the anterior and posterior ends from light microscopic images.

According to conventional theory, it is thought that contraction of the cell body in *Spirostomum* and *Stentor* is caused by the contraction of myoneme that is located just under the cell surface (Ishida *et al.*, 1988; Huang and Pitelka, 1973), and also that the longitudinal microtubular sheets (LMSs) connecting to every ciliary base slide actively to overlap between the neighboring LMSs at re-elongation of the cell body, but slide passively to each other to the opposite direction at the period of contraction of the cell body. This force of sliding

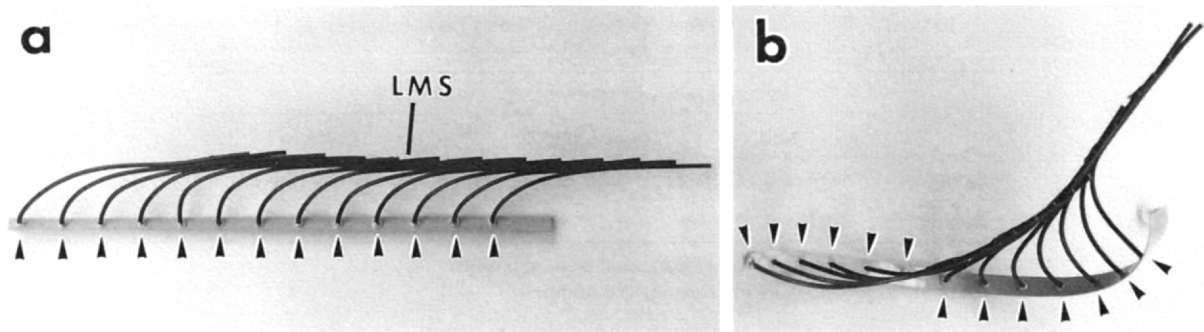


Fig. 3. Two types of plastic models of ciliary line and LMSs at elongated (a) or contracted (b) state of the organism, showing that the body contraction occurred without shortening of the inter-kinetosomal distance (distance between two neighboring arrowheads in this model) but with passive sliding of LMSs. Twisting of the contraction of the cell body was the result.

recovers the cell body to its original length. However, in the present study, our data showed that there were few changes in the length of ciliary lines which can be regarded as reflecting the inter-kinetosomal distances. These data may not necessarily rule out the traditional hypothesis, and the sliding between the two neighboring LMSs might occur without a shortening of the distance of the two neighboring ciliary bases (Fig. 2). During the period of cell contraction, so-called passive sliding action can be considered to occur between the two neighboring LMSs which are connected raggedly with the myonemal network. Even though the distance between the two neighboring ciliary bases does not change, this passive sliding action can be considered to increase the angle of each ciliary line against the longitudinal axis of the cell body, resulting in a twisting motion as indicated by plastic models in Fig. 3. At the period of cell elongation, moreover, an active sliding motion can be considered to occur between the neighboring LMSs without changing the distance of two neighboring ciliary bases, resulting in the decrease of the angles of every ciliary line to the longitudinal axis. It can be presumed that this kind of mechanism might be controlled with the sasa structure (bamboo leaf-like structure) which was proposed by

Yogosawa-Ohara *et al.* (1985) to be associated with the proximal region of every LMS.

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