Mem. Fac. Lit. & Sci., Shimane Univ.,
Nat. Sci., 11, pp. 55–62 Dec. 20, 1977

Electron Microscopy on the Myoneme of a Ciliate, *Epistylis* sp.

Akira MATSUNO
Department of Biology, Shimane Univercity, Matsue, Japan
(Received September 5, 1977)

Peritrich ciliates, such as *Vorticella* and *Carchesium*, have contractile stalks for their movements. Several papers have been dealt with the stalks as a contractile system in protozoa (1, 2, 6). The contractile structure in the stalk has been named as "Spasmoneme". Electron microscopically, it contains a myoneme which is a bundle of many thin filaments, each with a diameter of 4 to 7 nm, and some cell organelles in the cytoplasm (1, 2, 4, 7). A bundle of myoneme in a zooid (zooid myoneme) is more slender than that in a stalk (stalk myoneme), though a myoneme appears to be similar in fine structure each other. Zooid myonemes are converged into a single stalk myoneme at the region of the scopula, thus the stalk myoneme is composed of bundles of zooid myonemes. There is an another myoneme system around an oral region, which acts on contraction of the oral part (6). Zooid myonemes are always surrounded with endoplasmic reticula (E. R), the form of which, in the genus *Vorticella*, varies with the species (4). Recent investigations suggested that the E. R in *Vorticella* are sites of calsium release to trigger myonemal contraction (1). The similar structures of E. R were observed in *Zoothamnium* (5).

A peritrich ciliate *Epistylis* bears a resemblance to *Carchesium* or *Zoothamnium* in size and form under a light microscope, but the contractile movements are somewhat different among them. The stalk in *Carchesium* contracts into a coil and that of *Zoothamnium* meanders by the myonemal contraction in the stalk, whereas in *Epistylis*, the contraction and elongation take place only in the cell body. This paper is dealt with structures of the contractile system within the cell body in *Epistylis*.

MATERIAL AND METHODS

Epistylis was collected from a pond in the suburbs of Matsue City and kept for several days in a culture medium to empty out clay particles in food vacuoles. About 0.2% hay extract solution was used as a culture medium. After being cultured for several days, organisms were employed for electron microscopic observations.

The methods used for electron microscopic observations were mainly conventional. The organisms were fixed at 0–3°C in a solution containing 2.5% glutaraldehyde and 0.1 M cacodylate buffer (pH=7.3). After pre-fixing in this medium for 90 min, specimens were washed for 30 min by exchanging two times with the buffer solution

without glutaraldehyde. They were then post-fixed in 1% osmium teroxide with 0.2 M sucrose and 0.1 M cacodylate buffer (pH=7.3). Dehydration was carried out in a series of ethanol (70, 80, 90, 100%) and followed by propylene oxide. After dehydration the specimens were embedded in Epoxy resin and hardened in an oven at 45°C for 12 hrs at first and further at 60°C for several days. Sections were picked up on formvar coated grids. Thin sections were stained with saturated uranil acetate for 60 min, followed by Reynolds' lead citrate for 3 min. The sections were examined under a Hitachi HU-11A type electron microscope and photographes were taken at the magnification 2000 to 20000 times.

RESULTS AND DISCUSSION

An *Epistylis* ciliate is stalked, that is, the posterior part of the body is elongated into a thread-like stalk which is used for attachment. The ciliate is found in colonies. The cell body is about 200 μm × 100 μm in a trumpet-shape in a fully extended cell (Fig. 1). The cell body changes its form into globular by mechanical or chemical stimulations, whereas its stalk does not contract. A general contraction of the cell body results in about a 60% decrease in length. The contraction of cell body occurs noticeably at the scopula and oral regions, where the pellicle shows zig-zag form on the cell body contraction (Fig. 2, arrows).

Electron microscopically, contractile elements, myonemes, are found in the body in the form of a number of longitudinal bundles lying just under the pellicle and extending from the oral zone to the scopula region. The myonemes connect with a less electron dense layer of the pellicle at some distances (Fig. 3). The connection between myonemal filaments and the pellicle is not so clear, but sometimes binding structures are seen in a less electron dense layer of the pellicle (Fig. 5, upper arrows). The structures are shown as connections between the filaments and filamentous materials in the less electron dense layer of the pellicle. Contractions of these myoneme induce outward bends of the pellicle (Fig. 3). When the pellicle is released from the tension produced by myonemeal contraction, it recovers in an original form by the elasticity of the pellicle.

Each myoneme is composed of a bundle of thin filaments measuring about 6 nm in diameter, which are somewhat thinner than myofilaments in smooth muscle cells of mammals. These thin filaments generally arrange parallel to the myonemal axis, but sometimes connect each other at some places without junctional granules. Zooid myonemes are not surrounded with limiting membranes and do not show regular striped patterns as those observed in rootlets of cilia in many animals. Myonems always surrounded by associated E. R (a. E. R) being 0.1 μm in one direction by 0.3 μm in a perpendicular direction. Many globular E. R (g. E. R) are observed among the thin filaments (Fig. 4). Each globular E. R is about 60 nm in diameter and contains highly electron dense substances inside of the membrane (Fig. 5). As seen in Fig. 6, many

a. E. R surround a myoneme with regular arrangements. Several a. E. R are bended by the myonemal contraction (Fig. 6, arrows). The situation shows that the a. E. R do not contact with myoneme so tight that they contract in company with myoneme. The a. E. R includes the electron dense materials in its cavity. These features give suggestions that the a. E. R has the similar role of the sarcoplasmic reticulum in muscle cells. Microtubules, about 20 nm in diameter, sometimes connect with the myonemal filaments (Fig. 5, lower arrow), though they usually distribute apart from myoneme. Microtubules are not observed in the form of bundles and dispersed sporadically in the zooid.

As observed in *Carchesium* and *Zoothamnium*, there are about 100 to 140 cilia projecting into the stalk at the scopula which is the junction of a zooid and a stalk. These cilia resemble to oral ones in structure. The zooid is separated from the stalk by four plasmalemmas. The boundary structure possess pits here and there. Microtubules do not form bundles but they extend into the cytoplasm of zooid from basal bodies of cilia. Myonemes originated at an oral region extend to the scopula, where the myonemes connect with basal bodies of cilia (Fig. 8, arrows). These structures have a resemblance to connections between rootlet fibers and basal bodies of cilia in other animals.

Myonemes are not observed in the stalk. It may be the reason why the stalk in *Epistylis* do not possess an ability to contract. Annular tubes, measuring 0.15 μm in diameter and showing lateral stripes at 51 nm distances, are seen about 100 to 150 in number in the stalk (Fig. 10). Annular tubes, which originate from cilia in the scopula, run parallel to the stalk axis. Annular tubes are accompanied with networks of fine filaments in order to fix the positions in the stalk. The tubular structures of annular tubes become gradually disordered and come loose into fine filaments as the distal portion of the stalk. The fine filaments originating from annular tubes are seen randomly at the distal portion of the stalk (Fig. 9).

It was suggested that annular tubes in *Zoothamnium* serve to extend the stalk by the virtue of their elasticity (5). It is reasonable to think that the annular tubes in *Epistylis* also act a similar role. Myonemes in *Epistylis* are composed of thin filaments which are the same in size as those in *Carchesium* and *Zoothamnium*. E. R in the myoneme are somewhat different from those in *Carchesium* and *Zoothamnium*, that is, the E. R in *Epistylis* are globular, whereas in the formers they are tubular. Although functions of the globular E. R in *Epistylis* are unknown, it may be supposed that they act as calsium pools, as indicated sarcoplasmic reticula in muscle cells from their structural and distributional features. The structural connection which is observed between myonemes and cilia is suggestive of connections between rootlet fibers and cilia in mammals (3). These features impress that the myoneme originate from the rootlet fibers of the cilia.

The author wishes to express his thanks to Dr. T. Iga for his kind advice and

encouragement during the course of this work.

REFERENCES

[1] ALLEN, D. R. (1973). Structures Linking the Myonemes Endoplasmic Reticulum, and Surface Membranes in the Contractile Ciliate *Vorticella*. J. Cell Biol., **56**: 559–579.

[2] AMOS, W. B. (1972). Structure and Coiling of the Stalk in the Peritrich Ciliates *Vorticella* and *Carchesium*. J. Cell Sci., **10**: 95–122.

[3] HUAI-SAN, L. (1972). The structure and Formation of Cilia. The Cell, Tokyo, Japan, **4**: 2–16. (in Japanese)

[4] KAWAMURA, R. (1973). The Ciliary and Fibrillar Systems of the Ciliate *Vorticella*. J. Sci. Hiroshima Univ., Ser. B, Div. 1, **24**: 183–203.

[5] MATSUNO, A. (1976). Electron Microscopy on the Myoneme of the Ciliate, *Zoothamnium* sp. Mem. Fac. Lit. Sci. Shimane Univ., Nat. Sci., **10**: 77–84.

[6] SUGI, H. (1961). Volume Change During Contraction in the Stalk Muscle of *Carchesium*. J. Fac. Sci. Tokyo Univ., Sec. 4, zool., **9**: 155–170.

[7] YAGIU, R. and SHIGENAKA, Y. (1960). Electron-microscopical Studies of the Fibrillar System in the Protozoan Ciliates. Jap. J. Exp. Morph., **14**: 1–52. (in Japanese)

ABBREVIATIONS ON PLATES

a. E. R	associated endoplasmic reticulum	A. T	annular tube
B. B	basal body	C	cilium
g. E. R	globular endoplasmic reticulum	M	myoneme
M. T	microtubule	Mt	mitochondrion
P	pit	S	stalk
Sc	scopula		

Plate I

Fig. 1. The relaxed state of the *Epistylis*. Light micrograph. ×90.

Fig. 2. The contracted state by the mechanical stimulus. Many lidges of pellicle are seen (arrows). Light micrograph. × 90.

Fig. 3. A longitudinal section of the zooid. Zooid myonemes(M) are shown just under the pellicle. The pellicle is folded by the myonemal contraction in some intervals. × 12000.

Fig. 4. A cross section of the zooid. Zooid myonemes(M) are arranged not so regular but they are always distributed at the peripheral of the zooid in a cross section. Associated E. R(a. E. R) are observed around the myoneme. × 13000.

Plate II

Fig. 5. Enlarged views of myonemes. Globular E. R(g. E. R) are observed in myonemes. The myoneme is often connected to a homogeneous layer of the pellicle (upper arrow). Microtubules(M. T) sometimes have a connection with thin filaments of a myoneme (lower arrows). × 13000.

Fig. 6. Associated E. R(a. E. R) are folded by a myonemal contraction (arrows). ×28000.

Fig. 7. A longitudinal section of a scopula. Many cilia (C) are projected from the zooid to the stalk (S) at the scopula (Sc). Microtubules are connected to cilia in a central region (arrows). × 9000.

Plate III

Fig. 8. Enlarged views of the scopula. Myonemes (M) are observed to originate from the basal bodies of cilia (arrows). They resemble to rootlet fibers in ordinary cilia. × 21000.

Fig. 9. A cross section of a stalk at a distal area. Any tubular structures are not recognized in the stalk. × 9900.

Fig. 10. A longitudinal section of a stalk at the near area of the scopula. About 100 to 150 annular tubes (A. T) are arranged at the same direction to the stalk axis. × 7700.

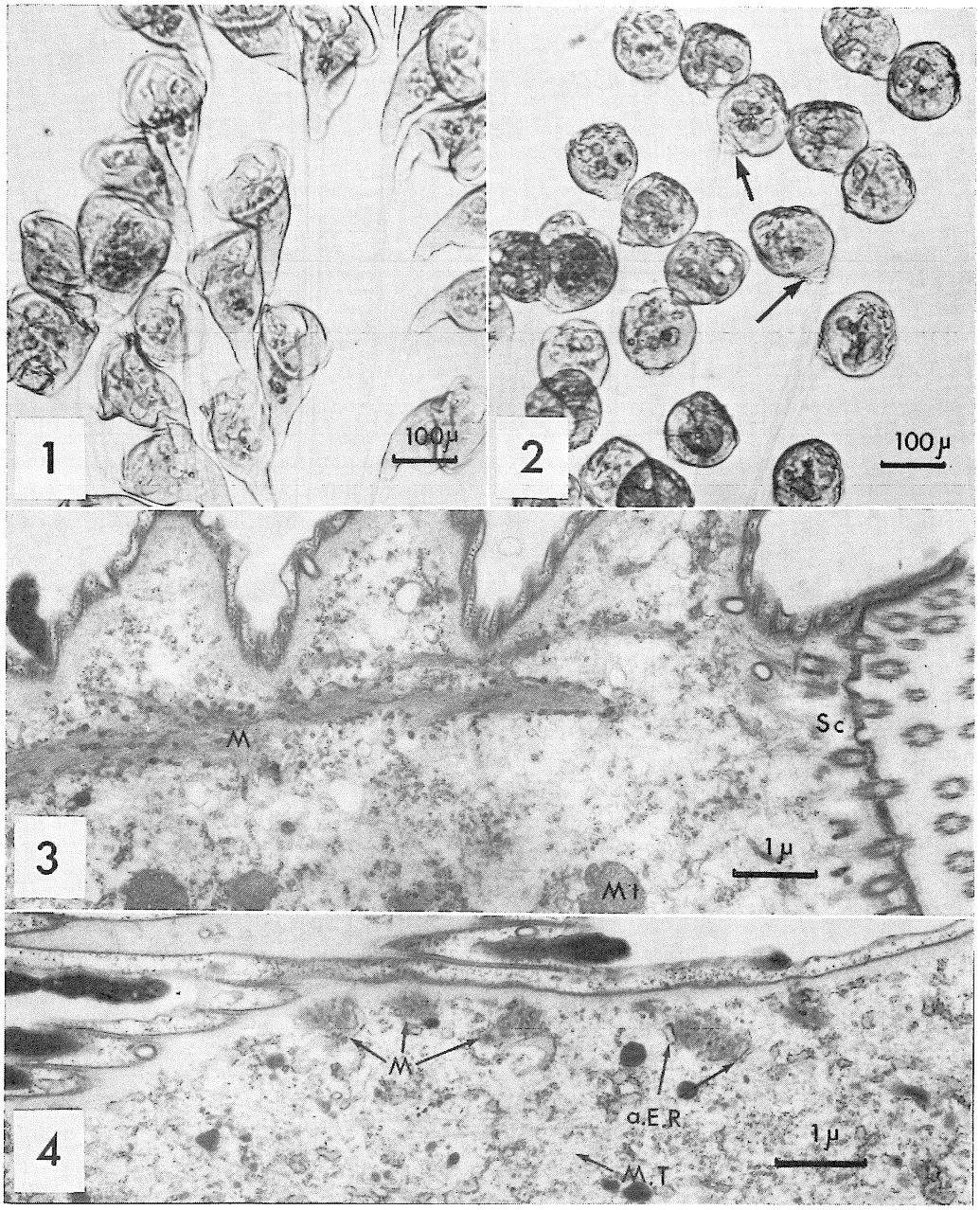

Plate I

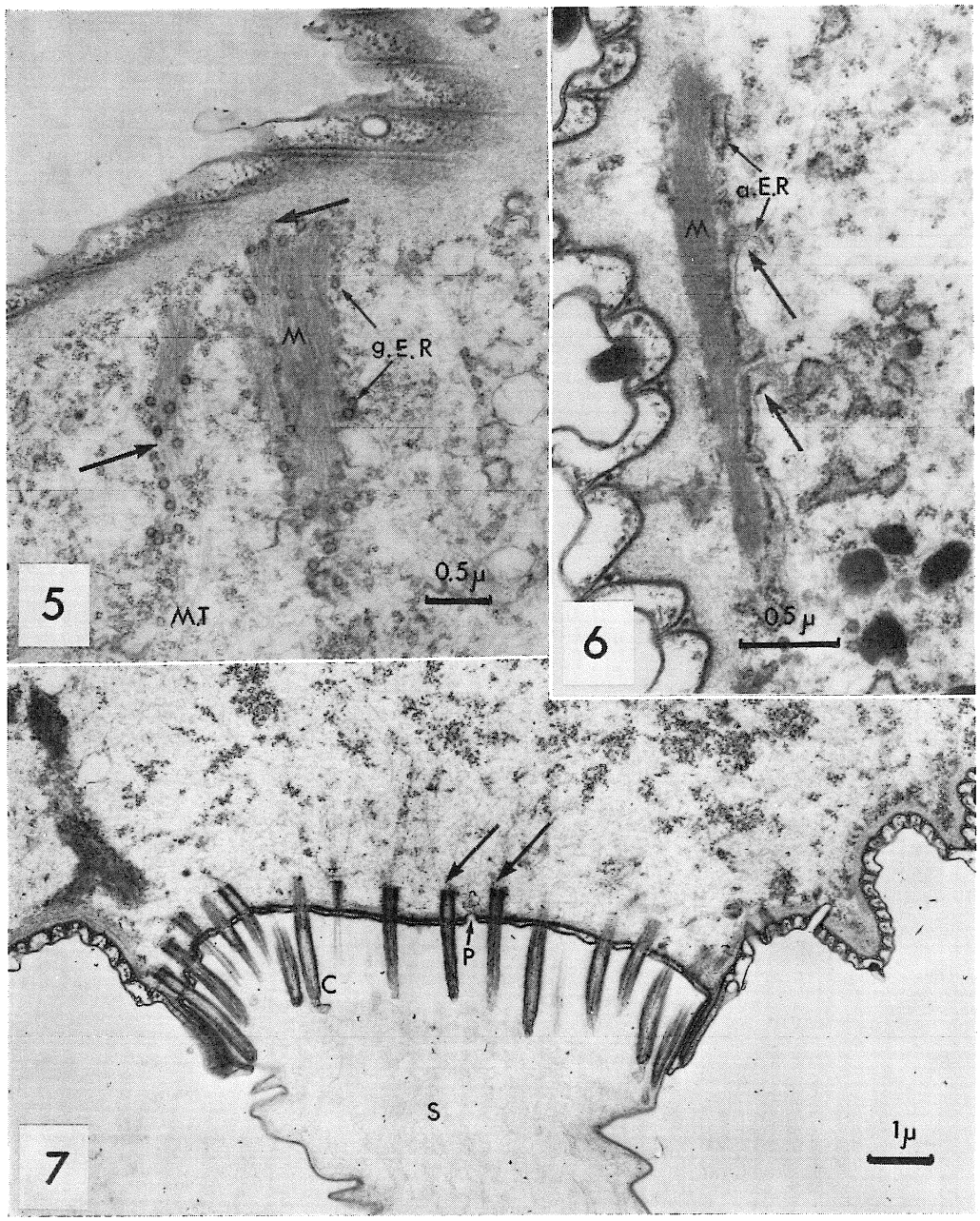

Plate II

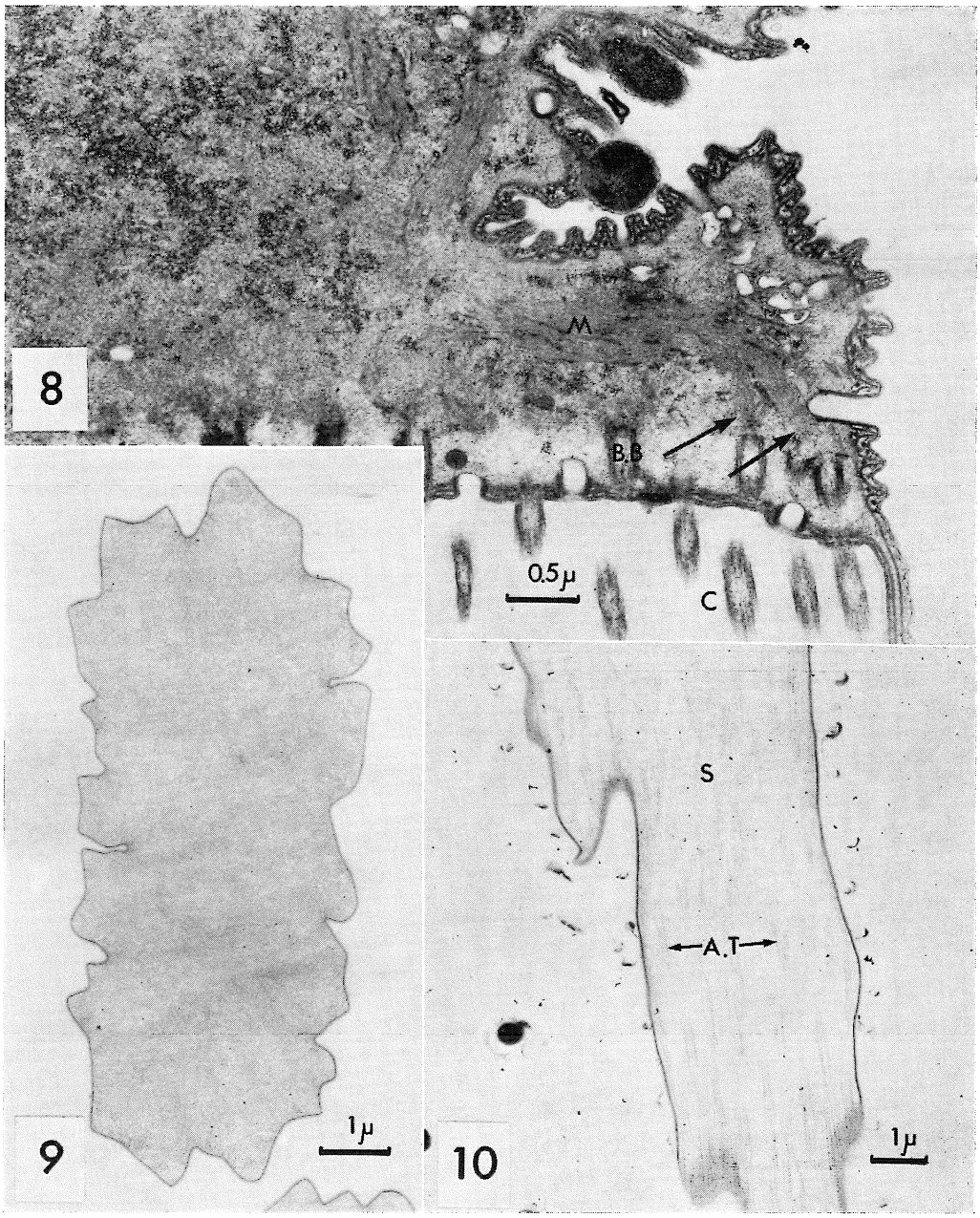

Plate III