

PATTERN VARIABILITY IN MICROTUBULAR ARRAYS  
ASSOCIATED WITH THE TENTACLES OF *ACTINOBOLINA*  
(CILIATEA: GYMNSTOMATIDA)

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INTRODUCTION

Structural-functional roles of microtubules in cells and cell appendages are becoming well established. A "static" supportive function could be assigned to the microtubular arrays in the axonemes of Heliozoa (*Actinophrys*, 7; *Echinospaerium*, 18) and Radiolaria (*Nassellaria*, 5), in the tentacles of Suctorina (*Acineta*, 3; *Tokophrya*, 12), and in the endosprits of the ciliate *Cyathodinium* (9). Models for "dynamic" functions of microtubular arrays have been proposed for the ciliary axoneme (4, 13, 15), the coccid sperm flagellum (11), the extension of heliozoan axonemes (16), and the transport of cellular substances (2, 19).

A new pattern of microtubular arrays is revealed within the retractable tentacles of the curious gymnostome ciliate *Actinobolina*. This note briefly describes the array, reports on its variation, and suggests a functional model for tentacle extension and retraction.

MATERIALS AND METHODS

*Actinobolina* sp. was collected from a freshwater pond in central Illinois by Dr. Eugene B. Small. The ciliates were fixed at room temperature in 2.5% glutaraldehyde in 0.005 M Sörenson's phosphate buffer, pH 7.4, rinsed in the same buffer (three changes over 1 h), and postfixed in 2% osmium tetroxide in Sörenson's buffer. For comparative purposes, additional cells were fixed in one of the following: Karnovsky's fixative in 0.1 M Sörenson's phosphate buffer, pH 7.45, postfixed in 2% osmium tetroxide; 2% Dalton's chrome osmium; or 2% osmium tetroxide in Palade's acetate-Veronal buffer.

Dehydration was carried out by dropwise addition of a graded ethanol-acetone series. After dehydration,

cells were taken through two changes of 100% acetone and embedded in Epon 812. Thin sections were picked up on uncoated 75- $\times$ -300-mesh copper grids and stained for 30 min in 2% aqueous uranyl acetate and for 15 s in lead citrate. Grids were examined with a Hitachi EMU-12 electron microscope. To prevent contamination of the specimens, liquid nitrogen was used throughout the system. The Hitachi was operated at an accelerating voltage of 75 kV, with a 30- $\mu$ m objective aperture. The micrographs were initially magnified from 5,500 to 100,000 and then enlarged further photographically with the use of point source illumination.

The arrays were classified into eight categories, and counts of the microtubule number in the arrays of 12 ciliates were made. Only arrays cross-sectioned nearly perfectly were selected.

OBSERVATIONS

*Actinobolina* is a rarely observed ciliated protozoan whose oval to elongate body is armed with toxicyst-bearing retractable tentacles. The tentacles occur in the ciliary meridians, located amongst the somatic cilia. In the resting organism, the extended tentacles are long and slender; in the swimming ciliate, they are completely retracted. When touched by the tentacle, prey organisms are immobilized by the discharging toxicyst. They are then passed by the combined action of the cilia and tentacles to the cytostome located at the anterior end of the body.

An extended tentacle averages 80–100  $\mu$ m in length. It is limited by a unit cell membrane which is continuous with the somatic unit cell membrane and is subtended by pellicular alveoli. A trans-

verse section (Fig. 1 *b*) through the tentacle reveals rings of microtubules entad to the alveoli. In longitudinal view (Fig. 1 *a*) these microtubules penetrate the endoplasm, converging proximally to form a complex of microtubular arrays (Fig. 4). Frequently occupying the lumen of each array, especially in the peripheral cytoplasm or in the tentacle proper, is a single toxicyst (Figs. 1 *b*, 2, 4). After osmium fixation, toxicysts appear as extremely fine darts projecting beyond the tips of the tentacles (8).

Examination of transections of nontentacular arrays reveals a variety of array patterns (Fig. 5) which, determined by configurations of subarray rings, spirals, and crescents, may be characterized as follows: pattern A, from one to several concentric rings of microtubules; pattern B, two or three concentric rings surrounding a crescent of microtubules; pattern C, a complete spiral with three to four turns; pattern D, a complete spiral surrounding a crescent; pattern E, a complete ring surrounding a spiral of from one to three

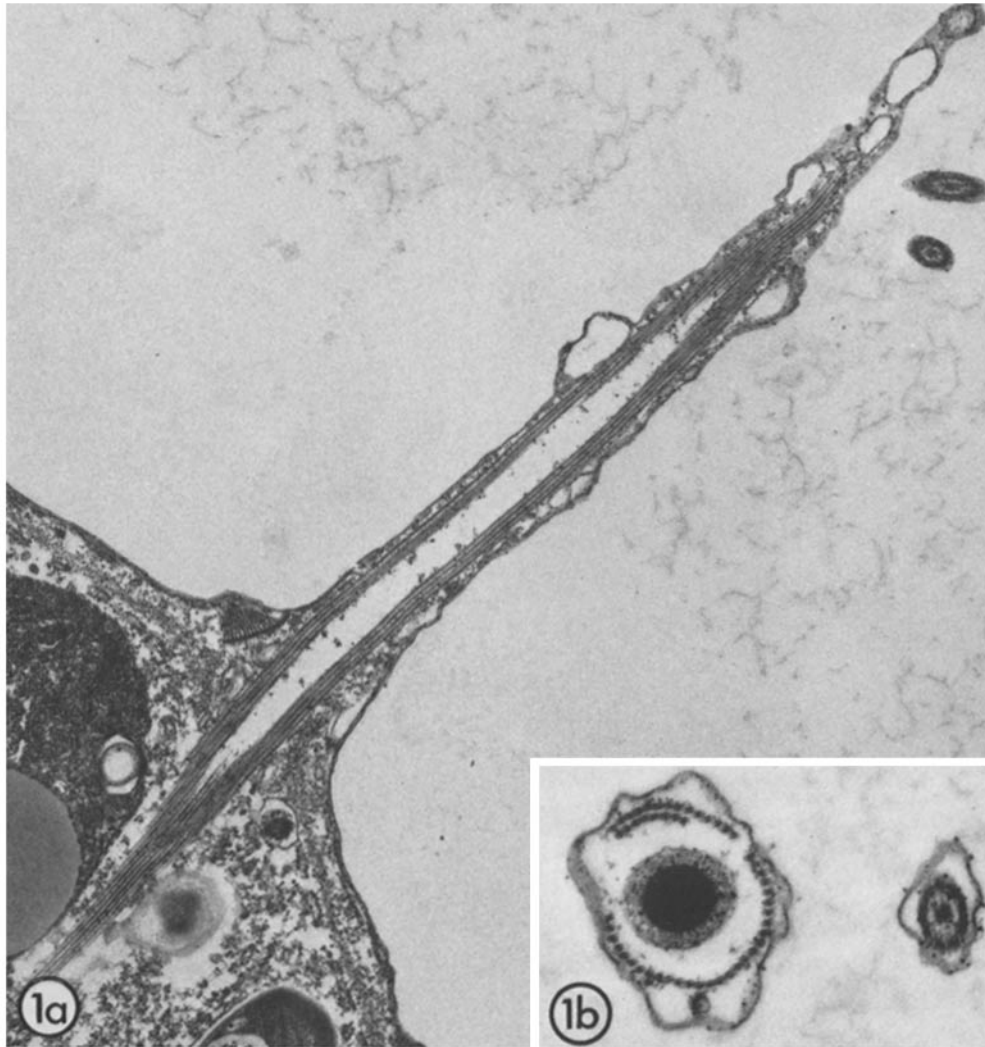
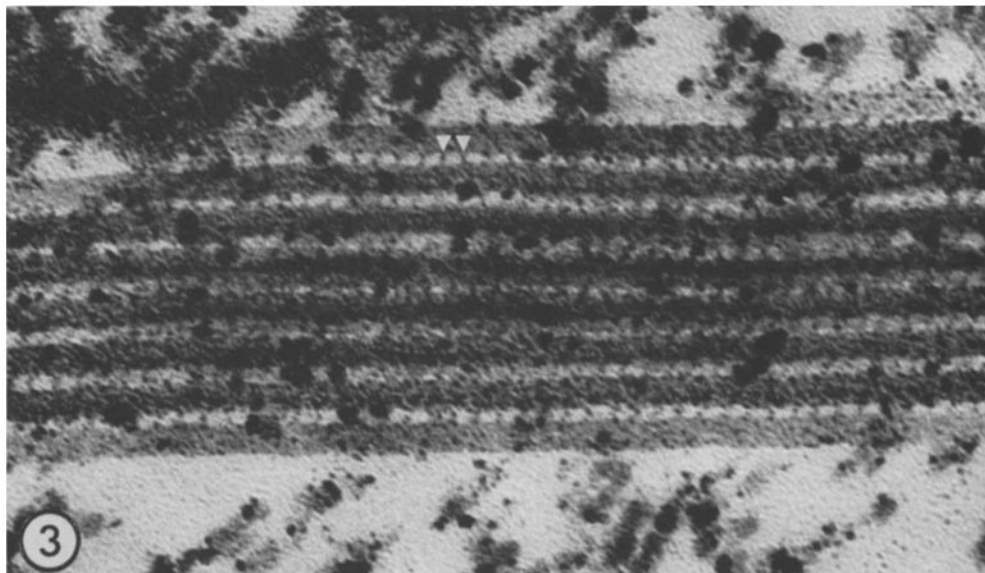
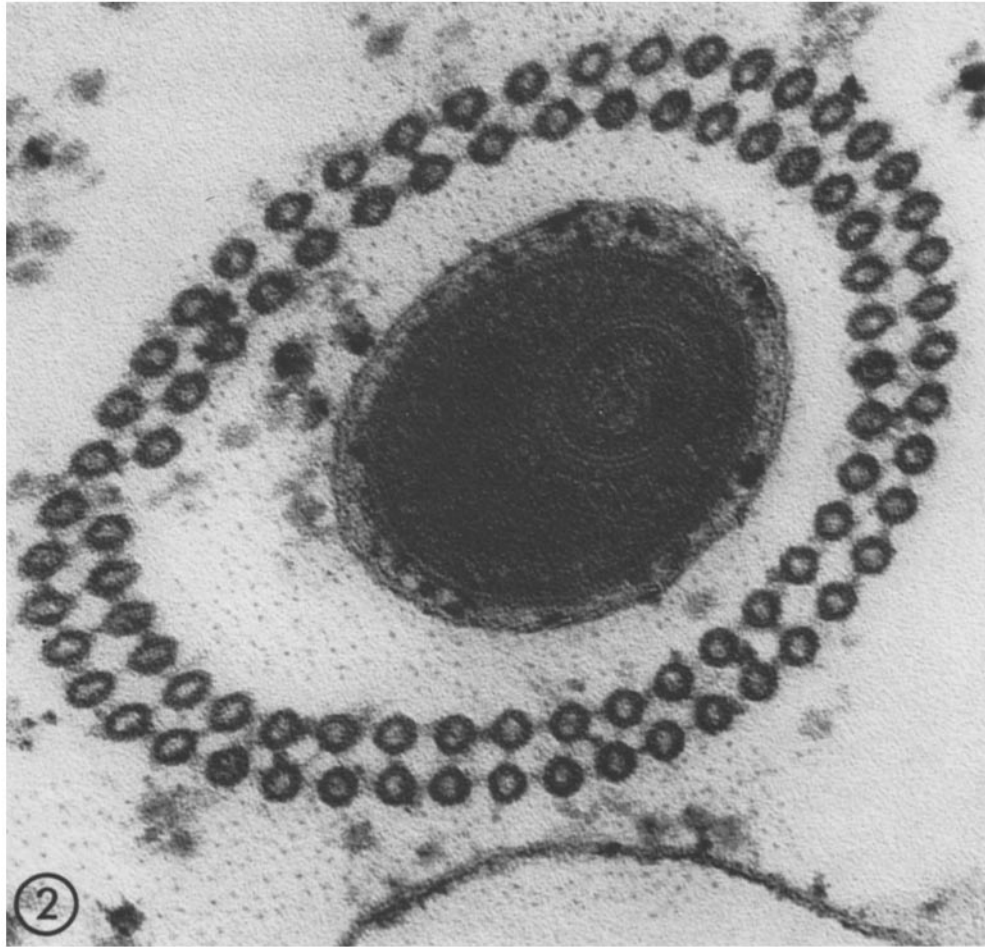


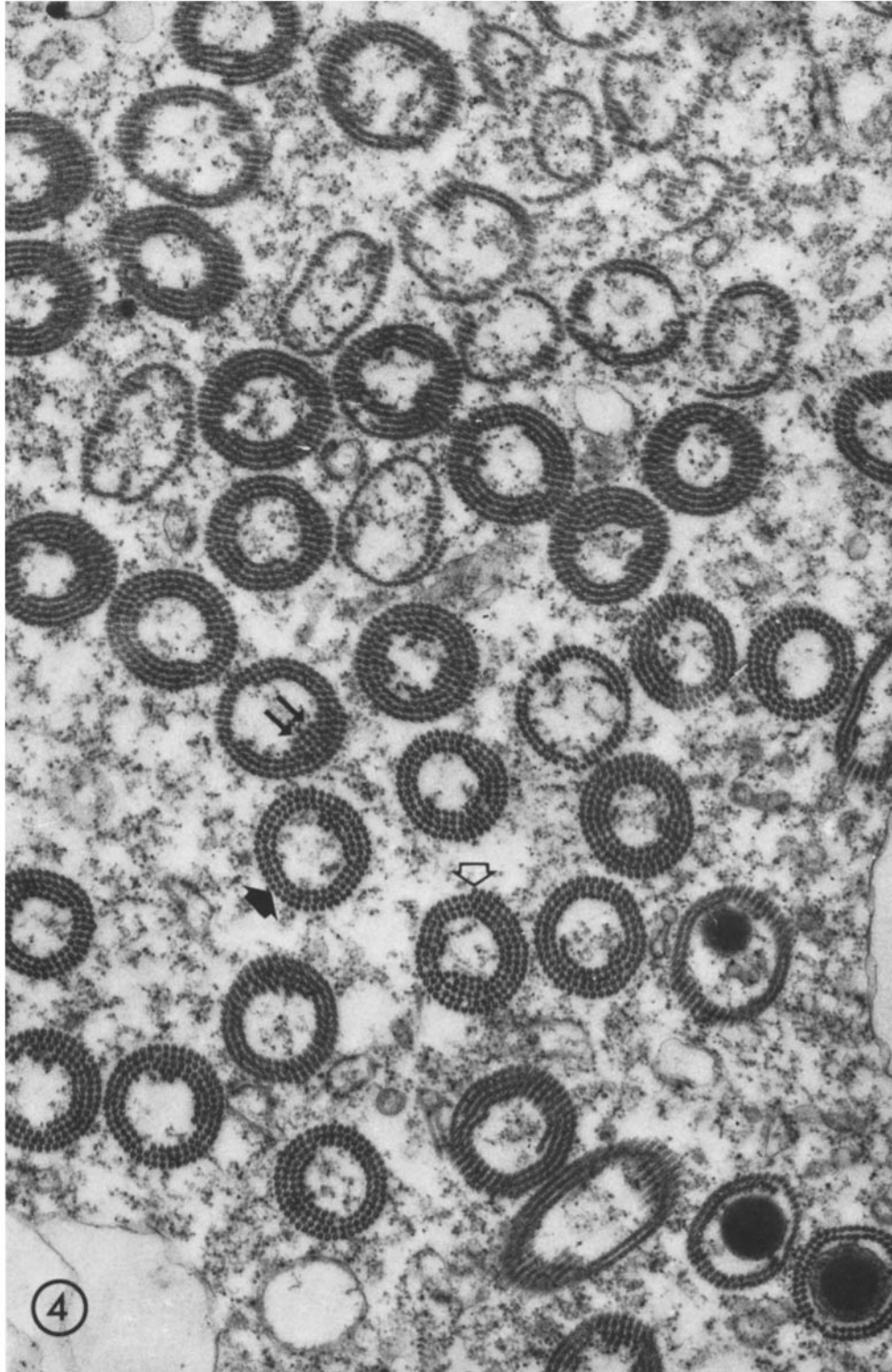
FIGURE 1 *a* A longitudinal section of a tentacle. Note alveolar pellicle limiting the tentacle. Microtubules extend throughout its length. Glutaraldehyde-osmium tetroxide.  $\times 28,000$ .

FIGURE 1 *b* A cross-section through distal end of a tentacle. In the lumen is a toxicyst surrounded by crescents of microtubules. A cilium is shown at viewer's right. Glutaraldehyde-osmium tetroxide.  $\times 28,900$ .



**FIGURE 2** A cross-section of an array in endoplasm of the cell. Note bridges linking adjacent microtubules. Toxicyst in lumen contains tubular elements. Glutaraldehyde-osmium tetroxide.  $\times 230,000$ .

**FIGURE 3** A longitudinal section of microtubular array. Note bridges (arrowheads) at intervals along the microtubules. Glutaraldehyde-osmium tetroxide.  $\times 163,200$ .



**FIGURE 4** Cross-section of microtubular arrays located in endoplasm. Many of the arrays contain rings (arrow). Others possess crescents (double arrow). Certain arrays (open arrow) are arranged in a spiral. Toxicysts are visible in some of the configurations (lower right). Glutaraldehyde-osmium tetroxide.  $\times 30,200$ .

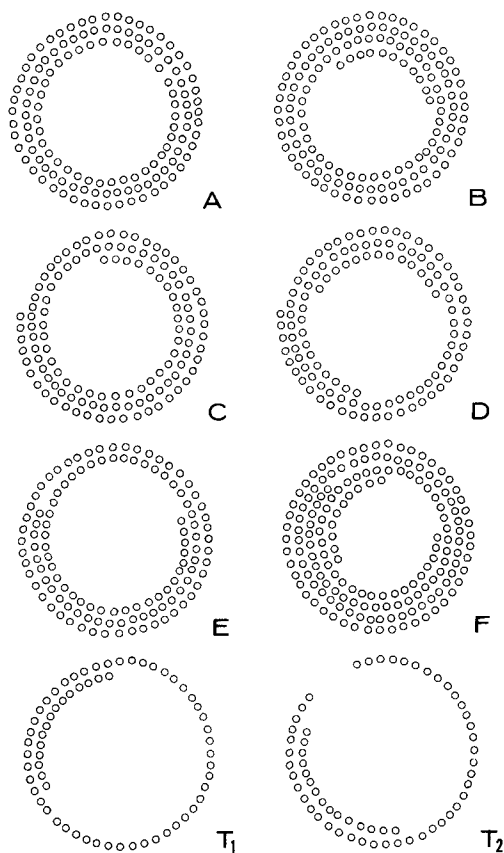


FIGURE 5 Diagram of array patterns. A-F are patterns observed in ectoplasm and endoplasm. T<sub>1</sub> and T<sub>2</sub> are observed in tentacles.

turns; pattern F, from one to three complete rings surrounding a spiral and a crescent. Patterns A, B, D, and E were found in both ectoplasmic and endoplasmic sections; C and F were observed only in the endoplasm. Two additional patterns (Fig. 5) were observed in cross sections of tentacles, characterized as follows: pattern T<sub>1</sub>, a complete ring surrounding a crescent; pattern T<sub>2</sub>, an incomplete ring surrounding a crescent.

To determine the degree of variability within the patterns described above, subarray microtubules were counted. Sample sizes were small. When distributions exhibited no mode, medians were the substitute central statistic. Relative abundance of patterns is reflected in the number of arrays counted, assuming that good transections occur randomly (Table I). All patterns were not observed in each ciliate. For a given pattern, the variation within and among ciliates appeared similar.

Within the arrays, microtubules are linked by electron-dense bridges. Intrasubarray links are ca. 90 Å long; intersubarray links range from 100 to 200 Å in length. Bridges are distributed at intervals of ca. 180 Å along the length of the microtubules (Fig. 3). Each microtubule has varying numbers of bridges. From two to seven bridges have been observed joining a microtubule to other members of the array, usually two intrasubarray bridges and from one to five intersubarray bridges.

#### DISCUSSION

Previous investigations have revealed variability in the patterning of microtubular arrays in cell appendages (see references 10, 14). These might be classified as follows: (a) an invariant array pattern, invariant subarray pattern, usually invariant number of linked subarray microtubules (cilia, 1; Suctorina, 2); (b) an invariant array pattern, invariant subarray pattern, variable number of linked subarray microtubules (*Actinosphaerium*, 18); (c) a variable array pattern, no subarray pattern, variable number of linked array microtubules (*Raphidiophrys*, 17).

In *Actinobolina* a fourth class of pattern variability is observed. In this new class, the array and subarray patterns are both variable. Moreover, the variability in the number of linked subarray microtubules is dependent upon the subarray pattern; outer, middle, or inner ring subarrays have a less variable number of microtubules than spiral or crescent subarrays (Table I). That spiral subarrays might be generated by a slip in linkage of microtubules in concentric ring arrays is a possibility which cannot be eliminated; thus, two rings and a crescent (one-half ring), as in pattern B, could yield  $2\frac{1}{2}$  spirals. Then, the addition of a variable number of crescent microtubules would account for the variability in microtubule number in spiral subarrays.

Possible functions of microtubular arrays in protozoan appendages include the production of form, support, extension and retraction, transport of materials, locomotion, and bending.

Tilney (16, 18) has demonstrated experimentally for *Actinosphaerium* that microtubules support and maintain axopod form. In *Actinobolina* this supportive role is verified, since retracted tentacles are shapeless and no microtubules are found within them.

Several models for extension and retraction have been proposed. In Heliozoa, extension and

TABLE I  
Pattern Variability in Microtubular Arrays of *Actinobolina*

Array pattern	Subarray pattern (modal number of microtubules)						Sample size		
	Outer ring	Middle ring	Inner ring	Spiral	Crescent	Total	Ar-rays	Cili-ates	
Tentacle									
T <sub>1</sub>	46 (42-47)					10 (4-24)	56 (51-81)	11	4
T <sub>2</sub> *	45† (37-58)					20 (18-36)	73 (64-87)	5	3
Ectoplasm									
A	48 (43-50)	43 (37-43)	29, 35				101 (92-122)	4	3
B	45 (42-48)	38 (25-42)	30, 33			20 (2-38)	86 (65-130)	17	5
E	47 (45-49)			84 (48-109)	8 (6-17)		141 (100-158)	10	3
Endoplasm									
A	46 (46-48)	40 (40-42)	31, 36				117 (88-126)	3	1
B	46 (43-48)	40 (37-42)	35 (30-36)			11 (3-33)	131 (96-163)	8	1
E	45 (41-49)			80 (41-100)			125 (85-148)	21	6
F	46 (42-49)	37, 39		79 (33-109)	7 (3-22)		133 (101-163)	30	7

\* Outer ring of microtubules incomplete (Fig. 5).

† All italicized numbers are medians; when array sample size is even, the medians are rounded up to the nearest whole number.

retraction result from the polymerization and depolymerization of microtubular axonemes (16, 18). In some Suctoria, extension and retraction are accompanied by changes in tentacle diameter and are presumed due to changes in the angle of climb of the helically oriented microtubular subarrays (2). For *Actinobolina*, a third model of extension and retraction is suggested. The similarity in ranges of numbers of outer ring microtubules in tentacular, ectoplasmic, and endoplasmic arrays suggests the presence of a continuous structure. We propose that this outer ring moves along the inner core structure by a sliding filament mechanism (6, 13). The lack of an outer ring in endoplasmic sections of some arrays (pattern C, Fig. 5) lends support to this idea. However, the problem could profitably be further explored with experimentally treated organisms at different stages of tentacle extension: such work is beyond the scope of the present investigation.

With respect to the role of microtubules in the transport of cellular substances, Tucker (19) suggests that arms, attached to microtubules lining the digestive tract in the ciliate *Phascolodon*, are involved in the transport of materials alongside microtubules during the ingestion of food. Arm-bearing subarray microtubules in suctorian

tentacles may attach to and pull inward prey membrane (2). In *Actinobolina*, microtubules bordering the lumen of the tentacle may transport the toxicyst to the distal end of the organelle.

Robison (11) has emphasized the functional significance of morphological asymmetry in bending microtubular arrays. The common occurrence of the crescent arrangement of microtubules in *Actinobolina* tentacles confers an asymmetry on the array pattern and suggests another application of Robison's model.

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