MORPHOLOGICAL DISCONTINUITY OF KINETOSOMES DURING THE LIFE CYCLE OF *OXYTRICHA FALLAX*

GARY W. GRIMES. From the Department of Zoology, Indiana University, Bloomington, Indiana 47401

INTRODUCTION

The problem of morphological continuity of kinetosomes (basal bodies) and centrioles has been the subject of extensive study in the past (reviewed by Fulton [2]). The most conclusive evidence of morphological discontinuity of kinetosomes comes from a study of the soil amoeba, *Naegleria gruberi*. Fulton and Dingle (3) observed random sections through numerous amoebae and flagellates. No kinetosomes were ever observed in amoebae; sections of flagellates frequently showed kinetosomes.

The present study uses a different approach, serial sectioning of entire cells, cysts of the ciliate protozoan, *Oxytricha fallax*, but comes to the same conclusion: kinetosomes are morphologically discontinuous.

MATERIALS AND METHODS

The techniques of culture and electron microscopy were those previously described (4, 6). Cells were fixed in glutaraldehyde, postfixed in OsO_4, dehydrated, and embedded in a thin layer of Epon. Cell diameters were measured under a compound light microscope. Individual cells were selected and mounted on blank blocks.

Blocks with nonparallel faces were obtained by hand-trimming so that circular ribbons could be obtained, thus allowing more sections per grid. Sectioning was done on a Porter-Blum MT-2 ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.) equipped with a DuPont diamond knife (E. I. DuPont de Nemours and Co., Wilmington, Del.). Serial sectioning was begun as soon as the cyst was entered. When a ribbon of sufficient length was obtained, it was broken by touching with a sable hair of a paint brush. The ribbon was picked up by "patting" and dried. Sectioning was then resumed. In this manner, entire cysts were sectioned, with accurate knowledge of missing sections. Individual grids (1 mm inside hole) contained from 30 to 50 sections. Complete cysts were placed on as few as six grids.

After staining (uranyl acetate followed by lead citrate [4]), grids were examined and the number of folds and other artifacts observed. All sections of selected cells were photographed at original magnifications of between × 3,800 and × 5,600, depending upon which magnification filled the 3¼ × 4 inch acetate film.

RESULTS

16 cysts were completely sectioned. Four in which several consecutive sections were completely lost were immediately discarded. The 12 remaining cells were examined under the phase-contrast microscope for the incidence of obscuring artifacts. Folding was the most frequently observed artifact. Cells possessing several regions with folds over the same area of cytoplasm in three or more consecutive sections were discarded, because a minimum of three consecutive obscured sec-

TABLE I

Data on Cells Photographed for Study

Cysts	Sections		Three consecutive sections with folds*		
	Total	Number containing folds	Number of groups	Approx. percent of area obscured‡	Approx. percent of total cells obscured§
				%	%
1	247	111	1	10	0.1
2	265	104	1	10	0.1

No sections lost in either cyst. Both cysts had diameters of 18 μm.

* This omits all sets of sections with folds that did not obscure the same area of cytoplasm in all three sections.

‡ This includes only the area which was obscured on all three sections.

§ This represents the total percent of cell area obscured by the area of cytoplasm which was obscured on all three sections.

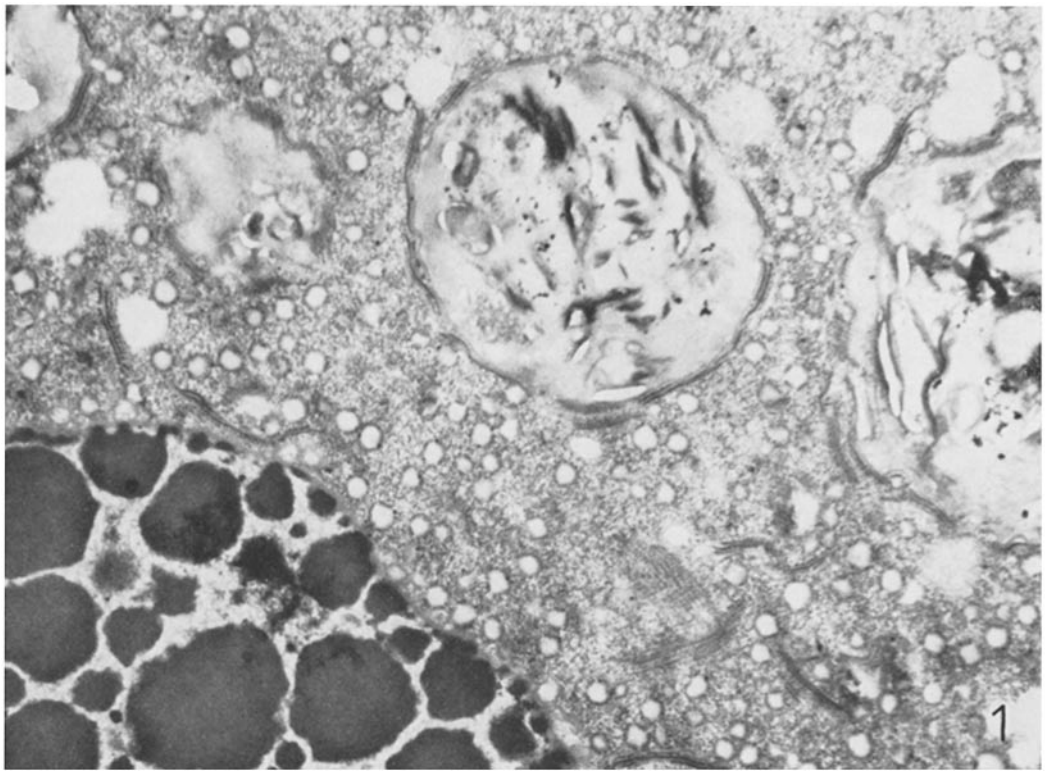

FIGURE 1 Portion of a cyst printed at the lowest magnification at which negatives were studied. × 19,000.

tions (~700 Å thick) is required to miss a mature kinetosome sectioned in its smallest dimension (diameter of 2,000 Å).

The two cells that showed the least number of artifacts were selected for photographing. The data on lost visibility in these cells are presented in Table I. No sections were completely lost. Although many sections contained folds, each cyst possessed only one set of three consecutive sections with folds over the same area of cytoplasm

in each section. The amount of cytoplasm obscured in the three sections of each cyst was estimated by tracing the fold and the cytoplasmic area on graph paper after photographing, then calculating the ratio of cytoplasmic area obscured by folds on all three sections to total cyst area in the three sections.

All sections were photographed and the negatives studied on a light box using a 5× hand lens. Fig. 1 is an area of a section printed to the comparable lowest magnification (× 19,000) at which the negatives were restudied. The resolution is certainly adequate to visualize a kinetosome; but not a single kinetosome was found in either cell.

DISCUSSION

The conclusion clearly indicated is that no mature kinetosomes are present in the resting cysts of the ciliate, *O. fallax*. This conclusion must be restricted to mature kinetosomes, as nascent kinetosomes can be confined to only one section (1, 5). Hence, early stages of development would be difficult, if not impossible, to recognize (see Fulton and Dingle [3] for an extensive discussion of these problems).

The same conclusion has been reached in other studies, most convincingly by Fulton and Dingle (3) in their exhaustive study of *Naegleria* transformation. However, the validity of the conclusion can be questioned, even in their study as they point out, because of difficulties involved in demonstrating the absence of organelles.

Likewise, the present study is subject to criticism, mostly because of technical limitations involved with serial sectioning. The most important criticisms concern section folding. As stated above, a minimum of three sections is required to section a kinetosome in its narrowest dimension. In cells possessing no folds over identical areas of cytoplasm in three consecutive sections, a mature kinetosome could not be completely obscured. However, both cells studied here contained one such set of three consecutive sections; kinetosomes could conceivably have been completely obscured by the folds in these sections. The percentage of the total area of a cell lost in each of these regions of folds is approximately 0.1% (i.e., 10% of three sections in a total of 250 sections). This percentage may be considered as the probability of obscuring kinetosomes; hence, the probability that kinetosomes were completely obscured in both cells by these folds is even less (0.1% × 0.1% = 0.01%).

Another criticism is that it is difficult to recognize a kinetosome if sectioned through its wall. Sections of kinetosome walls, even in vegetative cells, are the most difficult to identify. The likelihood of failing to recognize such a section increases in the cyst because of the increased density of cytoplasm. The most easily recognizable longitudinal section of kinetosomes (through the middle) could be obscured by a single fold. The sections with folds (225 total for the two cysts) were estimated to have an average of 10% of their area obscured by folds; hence, about 3.9–4.5% of each cell is obscured by folds (i.e., 10% of 104/265 or 111/247). Multiplying the percentage of obscured areas yields a probability of about 0.002 that longitudinal sections of kinetosomes were obscured in both cells by single folds. This type of game with numbers could be continued. For example, the probability of sectioning a kinetosome longitudinally could be calculated, assuming random sectioning and kinetosome orientations. This type of exercise would only increase the improbability of missing kinetosomes due to section folding.

Other criticisms can also be raised. For example, both focus and specimen drift can render entire sections useless. However, neither of these factors was of significant concern. In addition, other artifacts (e.g., stain precipitate, dirt) were rare.

Although the criticisms cannot be completely excluded, the conclusion derived from examination of serial sections of *O. fallax* cysts is strengthened by other evidence. Protargol staining of resting cysts reveals no kinetosomes, even though protargol stains internal nuclei, cilia, and presumably kinetosomes (6). Moreover, encysting and excysting cells contain easily detectable kinetosomes; even small numbers of kinetosomes can be detected in excysting cells (6). Electron microscope study of encystment stages indicates complete loss of structure of ciliary organelles (6). Extensive searching of numerous nonserially sectioned cysts has also yielded negative results; not a single kinetosome has been observed. In addition, kinetosomes can arise distant from existing kinetosomes during cell reproduction, indicating an optional utilization by the cell of existing kinetosomes or their micromilieu as nucleating sites for new kinetosomes (5). Hence, the cell has no "need" for maintaining kinetosomes in the cyst.

Thus, all evidence strongly agrees in supporting the conclusion of morphological discontinuity

of kinetosomes in *O. fallax*: they are present before encystment and after excystment, but not in the resting cyst.

The source of components of the ciliature formed during excystment is unknown. Microtubules could be assembled from a pool of subunits derived from the resorbing ciliature of encysting cells and stored in the cyst. However, some or all microtubule subunits may be synthesized during excystment. Experiments are in progress to determine the extent of subunit reutilization. If most subunits are reutilized, then assembly during excystment may depend upon the synthesis of recently proposed "regulator proteins" (7). Further speculation concerning the control of assembly should await experimental results.

SUMMARY

Serial sections of two entire cysts of *O. fallax* (approximately 250 sections per cyst) were photographed and studied in a search for kinetosomes. No kinetosomes were detected, even though vegetative, encysting, and excysting cells contain numerous and easily detectable kinetosomes. The probability of having missed kinetosomes by section folding, the chief technical difficulty, is estimated to be of the order of 0.0001. These observations, along with others presented elsewhere, thus strongly support the conclusion of morphological discontinuity of kinetosomes in *O. fallax*.

The author gratefully acknowledges the aid of Professors T. M. Sonneborn and R. V. Dippell throughout the course of this work and in the preparation of the manuscript.

Contribution no. 879 from the Department of Zoology, Indiana University. This research was supported by grants PHSR01 GM15410-05 to T. M. Sonneborn and by Public Health Service Genetics Training Grant no. GM 82 from Indiana University.

Received for publication 11 August 1972, and in revised form 4 December 1972.

BIBLIOGRAPHY

1. DIPPELL, R. V. 1968. The development of basal bodies in *Paramecium*. *Proc. Natl. Acad. Sci. U. S. A.* **61**:461.
2. FULTON, C. F. 1971. Centrioles. *In* Results and Problems in Cell Differentiation. Origin and Continuity of Cell Organelles. J. Reinert and H. Ursprung, editors. Springer-Verlag, Berlin. **2**:169–221.
3. FULTON, C. F., and A. DINGLE. 1971. Basal bodies, but not centrioles, in *Naelgeria*. *J. Cell Biol.* **51**:826.
4. GRIMES, G. W. 1972. Cortical structure in nondividing and cortical morphogenesis in dividing *Oxytricha fallax*. *J. Protozool.* **19**:428.
5. GRIMES, G. W. 1973. Origin and development of kinetosomes in *Oxytricha fallax*. *J. Cell Sci.* In press.
6. GRIMES, G. W. 1973. Differentiation during encystment and excystment in *Oxytricha fallax*. *J. Protozool.* In press.
7. WILLIAMS, N. E., and E. M. NELSEN. 1973. Regulation of microtubules in *Tetrahymena*. II. Relation between turnover of microtubule proteins and microtubule dissociation and assembly during oral replacement. *J. Cell Biol.* **56**:458.