THE ISOLATION OF CILIARY BASAL BODIES (KINETOSOMES) FROM *TETRAHYMENA PYRIFORMIS*

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Seaman (7, 8) has devised a method for the isolation of ciliary basal bodies (kinetosomes) from *Tetrahymena pyriformis* and has presented data on the chemical properties of his fraction. However, only light microscopy was employed as a means of identification of the kinetosomes. In the experiments described here the material obtained after various steps in the isolation procedure was examined by electron microscopy. Consequently it became necessary to modify Seaman's procedure in order to obtain a fraction which appeared to be relatively pure as viewed in the electron microscope. The kinetosomes are not, in fact, isolated free, but retain a bit of pellicular material attached to the distal region and a kinetodesmal fiber projecting at right angles from their basal end. This paper gives the present modifications of the isolation procedure in detail, as well as some preliminary nucleic acid analyses.

MATERIALS AND METHODS

Isolation Procedure

The following modifications have been introduced into the Seaman procedure (7). Points (A) through (D) represent minor changes and procedural clarifications; point (E) includes major modifications.

(A) CULTURE OF ORGANISMS: *Tetrahymena pyriformis*, strain B, are grown for 48 hours without aeration in culture medium containing 1 per cent proteose peptone (Difco Laboratories, Inc.), 0.25 per cent yeast extract (Difco), and 0.1 per cent glucose. Six to 8 liters of medium originally inoculated with 5 ml of cells in the stationary phase of growth will yield the 10 to 12 gm of wet-packed cells for which the modified procedure is designed. Organisms allowed to grow longer than 54 hours under these conditions acquire many large intracellular inclusions. Such cells solubilize either very slowly or not at all when subjected to digitonin treatment, and hence do not yield good preparations of kinetosomes.

(B) COLLECTION OF CELLS: The organisms are harvested by centrifugation at 250 *g* for 10 minutes at 0 to 4°C, and are washed once with 300 ml of cold phosphate buffer (0.015 M, pH 6.5).

(C) ETHANOL TREATMENT: The collected cells are suspended in 300 ml of 40 per cent ethanol at −15° to −20°C and are stored at the same temperature for a minimum of 3 hours. As pointed out by Watson, Hopkins, and Randall (9), the cold ethanol kills the organisms and hardens the cilia, which then readily detach when the cells are transferred to a digitonin solution. It is essential that the temperature of the alcoholic cellular suspension remain well below 0°C, for at temperatures above freezing the proteins of the cytoplasm coagulate and the cells become resistant to solubilization (2). It is best to pour the cold alcohol directly over small quantities (1 to 2 ml) of wet cells in the centrifuge tubes used for harvesting and washing, all the while stirring vigorously. Cells used within a few days seem to undergo solubilization more uniformly than those stored longer in ethanol.

(D) DIGITONIN SOLUBILIZATION: After removal of the cells from the ethanol solution, they are washed with 50 ml of cold 1 per cent digitonin solution in KCl, resuspended in 50 ml of digitonin solution and allowed to solubilize, all as described by Seaman. The initial digitonin wash is essential, since a small amount of residual alcohol will coagulate the cytoplasm and prevent proper solubilization. In well solubilized cells the nuclei and cilia disappear, the cytoplasm becomes clear (2), and only the pellicle with its rows of kinetosomes remains visible in the phase microscope (see photograph in Child and Mazia, reference 2). Since cells react at different rates, it is best to continue the process until some cells are oversolubilized, whereupon even the pellicle begins to lose its integrity, and virtually all cells are solubilized to some extent. In this manner contamination of the kinetosome fraction with cytoplasmic and nuclear particles from unsolubilized cells is minimized. Oversolubilized pellicles, from which the kinetosomes may be partially lost, remain in the supernate and are discarded after the next centrifugation.

(E) SEPARATION OF KINETOSOMES: After the ½ to 2½ hour solubilization period, the pellicles are concentrated by centrifugation at 1500 *g* for 10 minutes. The digitonin supernate, which contains a flocculent mass of pellicular fragments and released cilia, is decanted, the whole pellicles are rinsed once with 50 ml of 0.25 M sucrose and are ground vigorously for a minimum of 15 minutes in a Potter-Elvehjem tissue homogenizer with Teflon plunger. The homogenate is diluted with sucrose solution to a total volume of 60 ml, and is centrifuged at 250 *g* for

20 minutes. This centrifugation removes oral plates, intact pellicular fragments and the few remaining nuclei and unsolubilized cytoplasmic inclusions. The supernate, which contains kinetosomes, is decanted and stored in the cold, while the precipitate is resuspended in 20 ml of sucrose solution and ground for another 15 minutes to release additional kinetosomes from the remaining bits of pellicle. After dilution as above, the oral plates and other debris are again removed by low speed centrifugation and are finally discarded.

The supernates of the two preceding centrifugation steps are combined and centrifuged at 4000 *g* for 15 minutes in order to sediment the kinetosomes. At this point the supernate is discarded and the pellet is examined under the phase microscope. If oral plates or other debris are still present they are removed by another low speed centrifugation (250 *g*), and isolated kinetosomes are again concentrated at 4500 *g* for 15 minutes. The final white gelatinous pellet is then examined by electron microscopy to determine its purity.

Before chemical analysis or storing, the kinetosome fraction is rinsed at least once (preferably twice) with 40 ml of Ringer's solution without sugar, each time spinning at 4500 *g* for 15 minutes. Stored kinetosomes retain their morphological integrity for approximately 24 hours. The yield of isolated kinetosomes is about 8 mg of kinetosome protein per 10 to 12 gm of wet-packed cells.

Studies on the Isolated Kinetosomes

ELECTRON MICROSCOPY: For electron microscopy, small drops of suspended kinetosomes were placed directly on carbon-coated grids. The grids were then inverted on the surface of 2 per cent uranyl acetate for fixation and staining. Some grids were air-dried, while others were carried through the critical point procedure described by Anderson (1). Pellets were also fixed in 1 per cent OsO_4 buffered to pH 7.4 with Veronal-acetate, and embedded in Vestopal for thin sectioning.

CHEMICAL ANALYSES: The nucleic acid and protein contents of the isolated kinetosome fraction were determined and compared with those of whole cell homogenates. Chemical analyses were made directly upon material suspended in a minimal volume of Ringer's solution. Protein assays were made by the biuret method (10). RNA was determined by the orcinol procedure (6), and DNA by the diphenylamine reaction (3) using dichromatic readings to correct for the possible interference of carbohydrates.

RESULTS AND DISCUSSION

In the final fraction most kinetosomes are not free, but retain the kinetodesmal fiber and a small tab of pellicle at the basal and apical ends, respectively. Thus they have a characteristic flag-like appearance, shown in Fig. 1. The low magnification field seen in Fig. 2 is representative

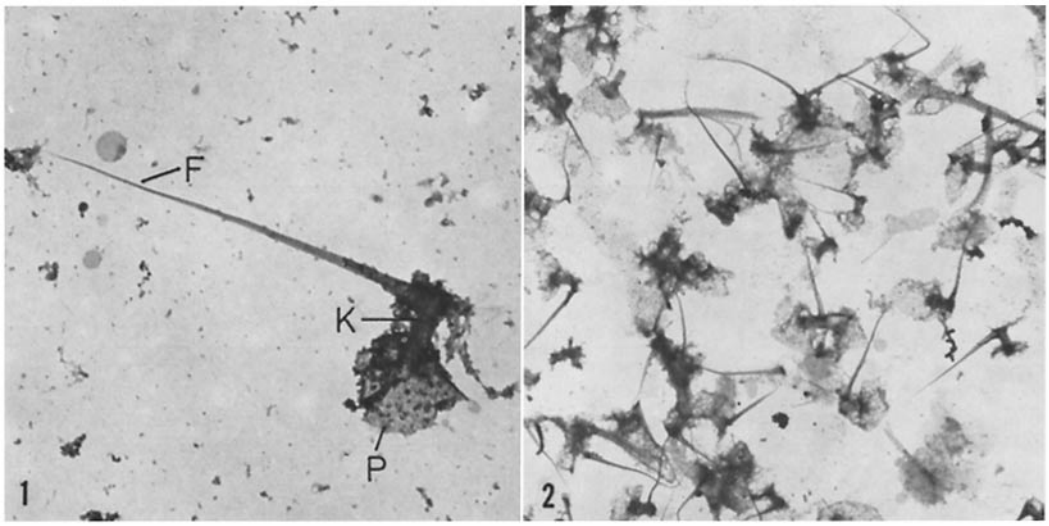

FIGURE 1 Single kinetosome (*K*) with attached kinetodesmal fiber (*F*) and pellicle fragment (*P*). Air-dried whole mount. × 21,000.

FIGURE 2 Low magnification survey view of material in the final fraction. In addition to kinetosomes, one finds fragments of pellicle and cilia as well as kinetodesmal fibers. Air-dried whole mount. × 6,700.

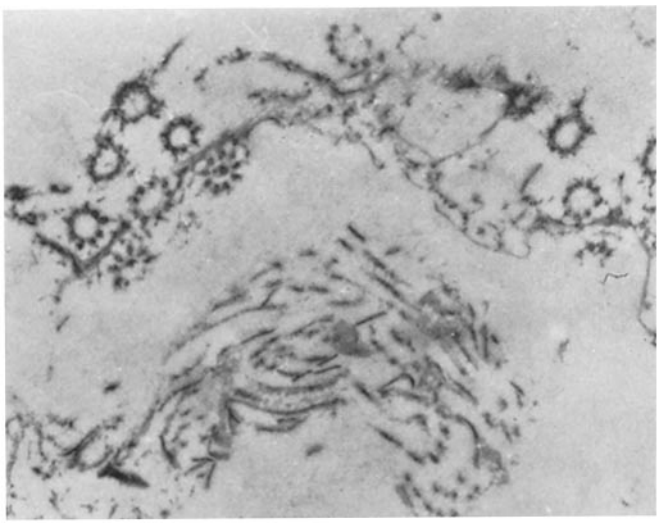

FIGURE 3 Section of a fixed pellet of the final fraction. Kinetosomes seen in both longitudinal and transverse section. OsO_4 fixation. × 25,000.

TABLE I

Nucleic Acid Content of Kinetosomes

Kinetosome preparation	DNA/protein	RNA/protein
	per cent	*per cent*
I	0.9	2.6
II	0.9	3.6
III	0.6	4.6
IV	0.0	7.9
V	0.5	0.6
Average I to V	0.6	3.9
Results of Seaman (7)	6.2	4.2

TABLE II

Nucleic Acid Contents of Whole Cells

Whole Cell Homogenate	DNA/protein	RNA/protein
	per cent	*per cent*
Average of seven preparations	1.9	22
Results of Seaman (7)	2.4	33

of the final fraction and gives an impression of the degree of purity. A few cilia are usually present in the final fraction as well as some unidentified small dense bodies (nucleoli?). Only in very impure preparations do nuclei and oral plates appear in any number. In an occasional preparation the majority of kinetosomes are detached from their kinetodesmal fibers, although both are present together in the pellet. A section through a fixed pellet of the final kinetosome fraction is shown in Fig. 3; kinetosomes, pellicle, and kinetodesmal fibers are seen in various orientations. In transverse sections of the kinetosomes the typical 9 fibers are easily identifiable (4, 5). Longitudinal sections of the kinetosomes occasionally include the point of attachment of the kinetodesmal fiber.

Table I summarizes the results of nucleic acid and protein analyses on five kinetosome preparations. Each of these was comparable in purity with that of Fig. 2. The DNA/protein and RNA/

protein ratios for kinetosomes should be compared with the similar ratios obtained for whole cells and shown in Table II. The values presented here agree reasonably well with those given by Seaman, with the exception of the DNA/protein ratio in the kinetosomes. This discrepancy may possibly be due to the omission from the modified procedure of the final ammonium sulfate fractionation described by Seaman (7). This step was believed to achieve the selective removal of pellicular fragments. It is not included in the modified procedure, however, since the bulk of the pellicular material remaining at this stage appears firmly attached to the kinetosomes.

It is impossible to decide, on the basis of available evidence, whether the small amounts of DNA and RNA in the final fraction are due to contamination from nuclear and ribosomal material. Observations by light microscopy on Feulgen-stained sections of the pellet show very little material which can be interpreted as nuclear fragments. On the other hand, ribosomes may well remain attached to the kinetosomes and their associated fibers. Extraction of the nucleic acids and further characterization by density gradient centrifugation and base ratio analyses should help to clarify their origin.

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