ISOLATION OF PURE PELLICLES CONTAINING INTACT BASAL BODIES OF TETRAHYMENA PYRIFORMIS

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SUMMARY

A new procedure for mass isolation of pure pellicles containing intact basal bodies of Tetrahymena pyriformis is reported. The success of the procedure depends on the elimination of the sticky mucocyst contents before fractionation of the cells, which is induced by Alcian Blue 8GS. Under appropriate ionic conditions > 95% of the cells are able to form a capsule by simultaneous extrusion of all mature mucocysts. About 50% of these cells are able to escape from their capsules, which are now devoid of mature mucocysts. These cells are separated from the empty capsules and encapsulated cells by passage through layers of gauze of 10 μm pore size. The fractionation of mucocyst-free cells in homogenization buffer yields pure pellicles, which are retained when the homogenate is sieved through steel sieves of 5 μm pore size.

Electron-microscopic controls show that the isolated pellicles are not contaminated with subcellular particles. Cells homogenized in the presence of low concentrations of Triton X-100 yield pellicles consisting of the known cell-surface-associated cytoskeletal elements, together with basal bodies. The cilia are detached just above the kinetosomal plate. The basal bodies of isolated pellicles are obviously undamaged, since all the known structures of native basal bodies are preserved. Even the granular matrix, a labile structure in the lumen of the basal body that probably contains RNA, is preserved.

INTRODUCTION

Ciliary basal bodies and centrioles are homologous organelles found in all metazoan cells and in some eukaryotic protists. Centrioles or centriolar complexes function as microtubule-organizing centres in both mitotic and interphase metazoan cells. The centriole is basically a cylinder, with dimensions of 0.2 μm × 0.5 μm, composed of nine triplet microtubules surrounding some internal structures. A cartwheel arrangement is frequently seen in the core of the proximal end of the organelle (for a review, see Fulton, 1971; Peterson & Berns, 1980). The basal body found at the root of cilia and flagella has the same structure as the centriole and is functionally interchangeable with the centriole (Sorokin, 1968; Fulton, 1971; Wolfe, 1972; Heidemann, Sander & Kirschner, 1977). Basal bodies serve as templates for the growth of the doublet microtubules of axonemes of cilia and flagella (Wolfe, 1972).

The ubiquity of the centriole (or basal body) and its unusual formation, i.e. regularly in close association with a pre-existing centriole, have raised speculations

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concerning the possible autonomy of this organelle (Pickett-Heaps, 1971; Hartman, 1975; Went, 1977a,b). Several approaches have been made to determine the involvement of associated nucleic acids in the function and duplication of this organelle. The results of the measurement of DNA associated with basal bodies are conflicting. Evidence in favour of the presence of basal body DNA (Seaman, 1960; Randall & Disbrey, 1965; Smith-Sonneborn & Plaut, 1967) and evidence against such DNA (Hufnagel, 1969; Flavell & Jones, 1971; Friedländer & Salet, 1971; Hartman, Puma & Gurney, 1974) have been reported.

On the other hand, the evidence for RNA associated with basal bodies is uniformly positive (Hartman, 1975; Dippell, 1976; Went, 1977b; Peterson & Berns, 1978; Rieder, 1979). A functional role of basal-body-associated RNA in the induction of aster formation has been demonstrated by Heidemann et al. (1977). Injected isolated basal bodies induced the formation of asters in unfertilized eggs of the African frog Xenopus, while RNase-treated basal bodies failed to do so.

For direct analysis of the RNA and other macromolecular components of the basal bodies, large quantities of pure intact basal bodies have to be isolated. Ciliated cells contain thousands of these organelles anchored in surface-membrane-associated cortical structures (pellicles). A preferred organism for isolation of pellicles containing basal bodies has been the ciliated protozoan Tetrahymena, because it is easily grown in mass cultures. However, pellicle isolations in Tetrahymena are greatly hampered by masses of sticky mucocyst materials, which are extruded during the homogenization of the cells.

This paper presents a simple new procedure for mass isolation of pure pellicles containing intact basal bodies of Tetrahymena pyriformis. The contaminating mucocyst components are eliminated from the cells by using Alcian Blue to induce capsule shedding (Tiedtke, 1976) before the cells are fractionated. Part of the isolation method for pellicles has been reported in abstract form (Tiedtke, 1980).

MATERIALS AND METHODS

Cells and culture

Tetrahymena pyriformis, strain GL. was obtained from Dr R. A. Flavell, University of Hull. This strain belongs to phenoset A (Borden, Whitt & Nanney, 1973) and is now designated Tetrahymena pyriformis sensu strictu (Nanney & McCoy, 1976). Cells were grown to late log phase \((4 \times 10^6\) cells/ml) in medium containing 2% proteose peptone (Difco) supplemented with 0-2% yeast (Difco) at 27°C, using one fifth of the capacity of 2-8-l Fernbach flasks. The cells were agitated continually with a magnetic stirrer.

Tetrahymena thermophila, cell line CU 325 (Mpr/Mpr; 6 mps, V) was obtained from Dr P. J. Bruns, Cornell University, Ithaca, N.Y., and was grown to late log phase \((6 \times 10^6\) cells/ml) in 1% proteose peptone supplemented with 0-1% yeast extract and 0-003% Sequestrene (Geigy Agricultural Chemicals, Ardsley, N.Y.). Cells were cultivated in 2-8-l Fernbach flasks at 30°C and 90 rev./min on a New Brunswick incubator-shaker.

Starvation

Cells from late log phase were harvested by centrifugation for 2 min at 300 g. The sedimented cells were washed twice with warmed incubation medium (1 M) and finally resuspended in incubation medium at \(4 \times 10^6\) cells/ml. Washed cells were then incubated on an incubator-shaker at 28°C
Basal bodies of Tetrahymena (T. pyriformis) and 30 °C (T. thermophila), 95 rev./min, using one fifth of the capacity of 2-8 l Fernbach flasks.

The incubation medium (MgPB) used for T. pyriformis was 5 mm-phosphate buffer (pH 6-6) containing 1 mm concentrations of CaCl₂, MgCl₂, MgSO₄, NaCl and KNO₃.

T. thermophila was incubated in a 10-fold dilution of Wagner's solution (pH 7-0). The composition of Wagner's solution is, per litre: NaCl, 2-75 g; KCl, 1-49 mg; MgSO₄·7H₂O, 246 mg; Na₂HPO₄, 1-37 g; KH₂PO₄, 320 mg.

Induction of capsule shedding

Capsule shedding was carried out after appropriate times of incubation as follows: 200-ml samples of cells were mixed under vigorous shaking with an equal volume of capsule-shedding medium. Capsule-shedding medium was prepared just before use by diluting a 2 % (w/v) stock solution of Alcian Blue 8GS (Chroma-Gesellschaft, Stuttgart) with distilled water. Immediately after induction of capsule shedding, dissolved egg albumen (Sigma) was added to a final concentration of 0-2 %.

Separation of capsule-free cells

The capsule-shedding mixture of T. pyriformis was washed twice (300 g; 2 min) in fresh incubation medium (1 M) and finally resuspended in 1 M medium. Cells that had left their capsules within 20 min were separated from the empty capsules and from the cells remaining in their capsules by filtration through 10 μm mesh polyamide gauze (Vereinigte Seidenwebereien, Krefeld).

Isolation of pellicles

The homogenization medium (HM) consisted of 10 mm-Tris·HCl (pH 6-0), supplemented with 10 mm-sodium ethylene diamine tetraacetate (EDTA), and 0-01 % Triton X-100. Cells that escaped from their capsules were homogenized with an Ultra-Turrax rotary homogenizer (Janke & Kunkel, Staufen). Steel sieves with 5 μm sized pores for separation of pellicles were obtained from Veco-Werke, Solingen.

Electron microscopy

Pellicles washed with 10 mm-sodium phosphate (pH 7-2) were fixed with glutaraldehyde (1-25 %) for 30 min at 4 °C. The washed sedimented pellicles were postfixed with 1-5 % OsO₄ for 30 min at 20 °C, washed in 70 % (v/v) ethanol, dehydrated and embedded in Epon. Thin sections were stained with lead citrate and uranyl acetate, and were examined with a Siemens electron microscope at 60 or 80 kV.

RESULTS

Elimination of mature mucocysts by capsule shedding

The isolation of pellicles of Tetrahymena is hampered by copious gels, which enmesh pellicles and other subcellular particles. These voluminous gels originate from the contents of secretory vesicles (mucocysts), which are discharged at the moment of homogenization. In order to avoid the annoying difficulties caused by these gels, capsule shedding induced by Alcian Blue (Tiedtke, 1976) was used to eliminate the contents of mature mucocysts before cell homogenization.

As seen in Fig. 1A, capsule shedding was induced quantitatively by addition of Alcian Blue. Under the ionic conditions used (see Materials and Methods), Alcian Blue stimulated discharge of mature mucocysts and precipitated their contents in the form of a capsule encaging the cell. The immediate inactivation of unbound Alcian
Fig. 1A–B. Capsule shedding induced by treatment with Alcian Blue 8GS. Phase-contrast micrographs of fixed cells. × 210.
A. The fraction of cells capable of mucocyst extrusion and capsule formation approaches 100%.
B. A sample of the same preparation fixed 10 min later. Cells can leave their capsules (arrows) through holes (arrowheads). About 40% of the cells leave their capsules within 10 min.

Blue by ovalbumen guaranteed the survival of these cells. Cells were able to move and rotate inside their capsules and to wriggle out of them through small holes (Fig. 1B). The location of the holes suggests that the oral apparatus of the cell might have been located in this position at the moment of capsule shedding.
Basal bodies of Tetrahymena

Fig. 2A–B. Appearance of cell cortices, loaded with mucocysts (mu) before induction of capsule shedding (A), and devoid of mucocysts in slightly compressed living cells (B). × 1350.

A. Mucocysts appear as small dark dots and cylinders, mainly in secondary meridians (2°) between the ciliary meridians (1°).

B. Mature mucocysts are not detectable following capsule shedding. Cilia and basal bodies of ciliary meridians are visible.

Fig. 3. Kinetics of capsule shedding. Percentage of cells in capsules is calculated as the quotient of the cells inside capsules plus empty capsules, divided by the sum of all cells counted. The standard deviation of quadruplicates was less than 5% in all cases. (O—O) *T. thermophila*; (●—●) *T. pyriformis*. 
CULTURE
1000 ml *Tetrahymena pyriformis* culture.
late log phase ~4 x 10^5 cells/ml. Wash 2x
in IM. Resuspend in IM. 4 x 10^5 cells/ml.
Shake for 12 h (Gyrotory shaker 95 rev./min. 28°C)

STARVATION (1)
2x

CAPSULE SHEDDING (2)
200 ml
starved cells
200 ml CM (0.4% Alcian Blue 8 GS)
sediment (300g. 2 min), wash 2x in IM
resuspend in 200 ml IM
Buchner funnel, 10 μm mesh gaze
empty capsules + cells remaining
in capsules

SEPARATION OF CAPSULE LEAVING CELLS (3)
capsule leaving cells (devoid
of dischargeable mucocysts)

HOMOGENIZATION (4)
2x

SEPARATION OF PELLICLES (5)
Ultra-Turrax 10^4 rev./min
2-5 min -0°C
pellicles + small
cell debris

ISOLATION OF PELLICLES
Culture: *Tetrahymena pyriformis* axenic in
proteose peptone medium, late log phase

Incubation medium = IM: 5 mm phosphate buffer
pH 6.6 + CaCl₂, MgCl₂, MgSO₄, NaCl, KNO₃, all 1 mm

Capsule shedding medium = CM: 0.4% Alcian Blue
8 GS (Chroma)

Homogenization medium = HM, 10 mm Tris-HCl
pH 6 + 10 mm EDTA

Centrifuges: Hettich Roto Silenta III, Heraeus
Christ UJ II KS, swing-out-rotor

Fig. 4. Illustration of the procedure of pellicle isolation for *T. pyriformis*. 
I have shown previously by electron microscopy that the capsules are built up of extruded mucocysts precipitated by the Alcian Blue. Following capsule shedding, the treated cells are devoid of mature mucocysts (see pp. 240–241 in the review by Hausmann, 1978). For routine controls, the success of capsule shedding was followed by phase-contrast microscopy of living cells. While starved cells were loaded with mucocysts, mainly in rows between the ciliary rows (Fig. 2A), no mature mucocysts were detected in the cortex of cells that escaped their capsules (Fig. 2B). When photographed and counted at different focal planes, untreated cells yield rough estimates of 2000–3000 mucocysts per cell.

In order to determine the minimum time of starvation needed to achieve maximum rates of capsule shedding, both species of *Tetrahymena* were starved in their respective incubation media (1M) for increasing times. As shown in Fig. 3, the maximum rate of capsule shedding was achieved in *T. thermophila* after 2 h of starvation. *T. pyriformis* had to be incubated for at least 12 h to stimulate 95% of the cells to form a capsule.

**Isolation of pellicles**

Cells that escaped from their capsules during the 10–20 min following induction of capsule shedding (about 40%) were separated from cells that remained inside their capsules and from empty capsules. These mucocyst-depleted cells were sedimented (300 g, 2 min) and resuspended in ice-cold homogenization medium (HM) at a concentration of 10^7 to 10^8 cells/5 ml. Cells homogenized at 5000 rev./min and 4°C were observed by phase-contrast to be converted quantitatively into whole pure pellicles. Pellicles floated freely in the homogenate; no gels enmeshing the pellicles were detected. The pellicles were separated from cell debris at 4°C by use of steel sieves with 5 μm sized pores. Only the pellicles remained on the sieve plate and were washed several times with HM without Triton X–100. They were finally flushed off the sieve plates, sedimentered and stored at 4°C. The complete procedure for isolation of pellicles is illustrated in Fig. 4. Although the further experiments were performed on pellicles of *T. pyriformis*, equal yields of pure pellicles were obtained with *T. thermophila* (Tiedtke, unpublished).

Purified isolated pellicles are shown in Fig. 5. The regularly spaced dark spots represent the basal bodies of the ciliary meridians (see also Fig. 7A). Isolated pellicles were rigid and, owing to their larger size, easily separated from the cell homogenate, which passed through the pores (5 μm diameter) of the steel sieves.

Electron micrographs of thin-sectioned isolated pellicles showed no contamination with particulate cell constituents (Fig. 6). Most of the surface membranes were removed by the action of 0.01% Triton X–100, but membrane blebs are occasionally seen near the basal bodies. The isolated pellicles consisted of typical surface-membrane-associated cytoskeletal elements (Williams, Vaudaux & Skriver, 1979): a continuous layer of epiplasm, microtubular ribbons above the epiplasm, and basal bodies attached to it. The basal bodies of isolated purified pellicles were well preserved (Fig. 7A). Compared with basal bodies of whole cells, those of isolated pellicles contain essentially all the known structural elements of this organelle. Even the
Fig. 5. Whole pure pellicles. Rows of dark spots represent the ciliary basal bodies. Oral apparatuses (arrows) are visible in some pellicles. Phase-contrast. × 360.

Fig. 6. Electron micrograph of thin-sectioned isolated pellicles. Intact basal bodies (arrows) are anchored in the continuous epiplasmic layer of the pellicle. Cilia are detached above the level of the axosome. Microtubular ribbons (arrowheads) are seen above the epiplasmic layer. No contaminations with cell debris is visible. × 7500.
Fig. 7. A-B. Thin sections of ciliary basal bodies of whole cells (A), and of isolated pellicles (B). The structural elements typical of intact basal bodies, such as axosome (ax), cartwheel (cw), inner granular matrix (igm), terminal plate (tp) and connecting microtubules (cmt), are also visible in basal bodies of isolated pellicles. × 62,500.

granular matrix in the lumen of the basal body was preserved in the majority of the basal bodies of isolated pellicles. This structure in the lumen of the basal body—shown to contain RNase-sensitive material by Dippell (1976)—has not been preserved in basal body isolations reported previously.

DISCUSSION

I have exploited the advantages of the ciliate *Tetrahymena* for mass isolation of basal bodies: the generation time is short, the cell titres are high and several hundred basal bodies are present in each cell. The key for development of this simple but effective isolation procedure for pellicle-attached basal bodies is the elimination of the mucocyst contents by capsule shedding before homogenizing the cells. Mucocyst-depleted cells have permitted the use of shorter homogenization times and lower concentrations of detergents than used previously (Hartman et al., 1974; Vaudaux, 1976; Heidemann et al., 1977). This improvement reflects the higher fragility of mucocyst-depleted cells and the ready separation of pellicles from the homogenate when the sticky mucocyst contents are absent.

The pure pellicles containing intact basal bodies should permit the first attempts to isolate the postulated basal-body-associated RNA to be made, since most of the
basal bodies of pellicles isolated show preserved central matrices. A rough calculation provides an estimate of the number of cells needed to obtain microgram quantities of RNA. On the basis of an estimation of the amount of RNA per basal body (Heidemann et al. 1977) of $5 \times 10^{-16}$ g, a minimum of $5 \times 10^{10}$ intact basal bodies is needed to obtain 1 $\mu$g of RNA. Assuming that the RNA is extracted quantitatively from the isolated intact basal bodies, then $1 \times 10^8$ cells, each containing $5 \times 10^2$ basal bodies, is the minimum number needed. Since cell titres of $2 \times 10^6$ cells/ml can be obtained, 10 litres of cell culture will provide $1 \times 10^{13}$ basal bodies. Even if a more realistic yield of only 0.1% of the input is calculated, microgram quantities of basal-body-associated RNA from pellicle isolates should still be obtained.

For a direct characterization of the other macromolecules of basal bodies these must be stripped off the pellicles. Techniques for this purpose, e.g. the grinding of pellicles with sand (Seaman, 1960), result in basal bodies associated with fragments of the pellicle of various sizes and structures connected to basal bodies. Efforts to separate basal bodies from pellicles, therefore, were not promising. However, the major pellicle proteins of *Tetrahymena* have been identified and their localization has been shown by using pellicles isolated with high concentrations of detergent (Vaudaux, 1976; Williams et al. 1979; Vaudaux & Williams, 1979). These preparations regularly contained basal bodies without the central matrix material. Comparison of proteins from pellicles isolated by different methods may enable us to identify the basal body proteins. Recently, a basal-body-associated protein that is not tubulin has been identified (Conolly & Kalnins, 1978; Turksen, Aubin & Kalnins, 1982). This protein has been immunoprecipitated with antibodies present in some normal rabbit sera, it has a molecular weight of 50,000 and is a common constituent of basal bodies of species as distantly related as *Tetrahymena* and the chicken.

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REFERENCES


Basal bodies of Tetrahymena


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