MEMBRANE RECYCLING AT THE CYTOPROCT OF TETRAHYMENA

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SUMMARY

Exocytosis and membrane recycling at the cytoproct (cell anus) of Tetrahymena pyriformis were studied using thin-section electron microscopy. Single cells were fixed at specific times relative to the elimination of the vacuole's contents - before elimination, at elimination, 3–5 s and 10–15 s following elimination. The closed cytoproct is distinguished from other pellicular regions by a single membrane at the cell surface which is circumscribed by an electron-opaque flange that links or welds the plasma membrane to the underlying alveolar margins. Microtubules originating in the flange pass inward where they lie over, and possibly guide, the approaching food vacuoles to the cytoproct. Food vacuoles near the cytoproct are also accompanied by coats of microfilaments. These microfilaments appear to be active in the channelling and endocytosis of food vacuole membrane. Upon cytoproct opening the plasma membrane and food vacuole membrane fuse. Elimination seems to be essentially passive and is accomplished by re-engulfment of the old food vacuole membrane which is constantly associated with microfilaments. Re-engulfment of all the food vacuole membrane requires 10–15 s and results in a closed cytoproct. The membrane remnants embedded in microfilaments form a cluster under the closed cytoproct. At the periphery of this cluster remnants take the shape of 70–130-nm spherical vesicles or 2-μm-long flattened vesicles.

INTRODUCTION

In a previous study on the ciliate Paramecium caudatum we described the process of defecation and discussed the fate of membranes of expelled food vacuoles (Allen & Wolf, 1974). Food vacuoles are always expelled at one site in this organism; this site is the cytoproct. The food vacuole membrane in Paramecium first fuses with the plasma membrane at the cytoproct and, subsequently, is quickly resorbed into the cytoplasm through the process of endocytosis. Endocytosis is apparently mediated by masses of microfilaments that lie close to the old food vacuole just before defecation. Microtubules appear to be involved in directing the food vacuoles to the cytoproct.

Since egestion has been studied in only one ciliate at the ultrastructural level, we wanted to see if the expulsion process of the digestive vacuole and the fate of food vacuole membranes in other ciliates followed a similar pattern to that in Paramecium. Thus we undertook a study of digestive vacuole defecation in the extensively studied ciliate Tetrahymena pyriformis. Our results show these 2 ciliates have similarities in the processes of membrane fusion between the food vacuole and the plasma membrane and the subsequent rapid endocytosis of this membrane back into the cell. However, differences in the cytoproct architecture and in the microtubular distribution are observed.

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MATERIALS AND METHODS

*Tetrahymena pyriformis*, strain GL, was grown in an axenic medium consisting of 1 % proteose peptone and 0.1 % yeast extract. Single cells from 24 h cultures were picked up with a small-bore pipette and placed in a tiny drop of water, with or without methyl cellulose, on a microscope slide. The cell was observed until a food vacuole could be seen pushing against the pellicle at the cell's posterior end where the cytoproct is located. Some cells were fixed at this stage by flooding the slide with fixative while other cells were fixed by the same method at known times following the release of the food vacuole contents. These times were at the moment of defecation, 3-5 s after defecation and 10-15 s after defecation.

Fixation was initially in 1 % glutaraldehyde in 0.1 M cacodylate for 1 min followed by 15 min in 1 % glutaraldehyde in 0.05 M collidine. Individual cells were then washed, postfixed in 1 % OsO₄, washed again and stained before dehydration with 0.5 % aqueous uranyl acetate. After dehydration in ethyl alcohol and propylene oxide the cells were flat embedded in Epon 812. The single cells were then cut out of the polymerized Epon and glued to blocks so they could be cross-sectioned beginning at the posterior end of the cell. Serial sections of this end were made and picked up on Formvar-supported grids. Sections were stained with uranyl acetate and lead citrate and observed in a Hitachi HU-11A electron microscope. Thus all figures are from cross-sectioned cells and not from randomly oriented cells.

RESULTS

The closed cytoproct

Light microscopy has demonstrated that the cytoproct of *Tetrahymena* is located near the posterior end of the cell on its ventral surface (e.g. see Corliss, 1952, 1973). This is confirmed in our electron micrographs. In fact, the cytoproct and contractile vacuole pores are situated close enough together to allow them to be seen sometimes within the same transverse section (Fig. 1). The cytoproct is characterized by the absence of the alveolus so that the cytoplasm is here separated from the exterior by only a single membrane, the plasma membrane. The width of the closed cytoproct varies from 0.3 to 0.8 μm in those cells we have observed. A small flange of electron-opaque material follows the terminal margin of each alveolus bordering the cytoproct.

The only other differentiation of the cytoproct region at the cell surface are microtubules (Fig. 2) which pass into the cytoplasm from their apparent origins in the electron-opaque material. If a food vacuole is located near the cytoproct, the microtubules are seen to drape over the membrane of this food vacuole. Frequently several

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Fig. 1. A closed cytoproct (cp) and contractile vacuole pore (p) appear in the same transverse section near the posterior end of *Tetrahymena pyriformis*. Segments of 3 food vacuoles (fv) lie close to the cytoproct, the lateral 2 are grazing sections and show profiles of fingerlike invaginations embedded in microfilaments characteristic of food vacuoles near the cytoproct. Ribbons of microtubules (bracket) border the distal hemisphere of the contractile vacuole (cv) and connections to the nephridial tubes (arrowheads) are also evident. × 15,000.

Fig. 2. Microtubules from the closed cytoproct (cp) drape over the food vacuole membrane. Microfilaments (mf) coat the membrane's surface. a, alveolus. × 40,000.

Fig. 3. Microtubules passing from the closed cytoproct (cp) to the food vacuole may be connected to the food vacuole membrane by bridges (bracket). Channels form within a mass of microfilaments from a second food vacuole lying close by (arrows). From the same cell as Fig. 1. × 60,000.
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Food vacuoles cluster near the cytoproct of *Tetrahymena*, and microtubules will pass to each of the clustered vacuoles. We have seen no indication that these microtubules are ever linked together to form ribbons as they are at the surface of the contractile vacuole in *Tetrahymena* (bracket, Fig. 1), or in the pellicular and oral regions of this and other ciliates. However, they may be linked to the food vacuole membrane by bridges (bracket, Fig. 3).

The food vacuoles near the cytoproct, in these axenically-grown cells, contain only wisps of flocculent material which, however, is more than the nearby contractile vacuole contains. This latter vacuole appears to be free of any electron-opaque particulate material (Fig. 1). The topography of the food vacuole membrane also differs from that of the contractile vacuole. Food vacuole membrane profiles are slightly wrinkled, at least after fixation, and bear some long slender, 40–50-nm-diameter, fingerlike invaginations passing into the cytoplasm (arrows, Fig. 3). These invaginations are always encased in a mat of microfilaments. Microfilaments surround much of the old food vacuole membrane and thereby distinguish those food vacuoles near the cytoproct from others in the cell. Such invaginations and coats of microfilaments surround food vacuoles lying near the cytoproct even though they have not yet opened to the outside. Tangential sections through the surface of these food vacuoles dramatically illustrate both the microfilaments and tubular invaginations (Fig. 4). At the cytoproct the food vacuole membrane comes to lie as close as 10 nm from the plasma membrane, a distance which is apparently maintained for a period of a few seconds.

On the other hand the 'contractile vacuole' membrane (Fig. 1), although slightly wrinkled also, is not coated with any special differentiation of the cytoplasm such as the microfilaments, even though the distance separating the margins of food and contractile vacuoles can be as little as 0.3 μm. Ribbons of microtubules, not found at the cytoproct, arise from the edge of the contractile vacuole pore and lie next to the contractile vacuole membrane on its distal hemisphere. Invaginations also protrude from the contractile vacuole membrane but these are larger in diameter (90–110 nm) than those protruding from the food vacuole. These are, no doubt, extensions of the network of smooth-membraned tubules, which have been referred to as the nephridial tubules (Elliott & Bak, 1964), that are a constant feature of ciliate contractile vacuoles.

Our electron micrographs also illustrate the differences in thickness of the membranes of the contractile vacuole (being an example of the thinner, ER-like membrane) and food vacuole (which is of a similar width to the plasma membrane) (see Allen, 1978).

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**Fig. 4.** Channels and microfilaments next to a tangentially sectioned food vacuole. x 50,000.

**Fig. 5.** The open cytoproct fixed immediately at defecation. The lips (arrows) are 2 μm apart. The food vacuole membrane shows extensive channelling even though the undigested contents have not yet been completely eliminated. Microtubules are evident particularly along the portion of food vacuole at the bottom of the figure, x 40,000.

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Open cytoproct

Cells that have been fixed just at the moment of egestion show a gap of nearly 2 μm in the pellicle and a deep cleft, also of 2 μm, into the cytoplasm (Fig. 5). Undigested material can be seen within the cleft or, if this material forms a faecal ball, it can occa-

Fig. 6. A faecal ball (fb) still lies next to this closing cytoproct fixed 3–5 s after defecation. No membrane surrounds the faecal ball. Cell stained with ruthenium red. x 20000.

Fig. 7. The space occupied by the food vacuole has been eliminated although the cytoproct lips, bordered by the alveoli (a), are still far apart. Channelling of the excess membrane mediated by microfilaments continues. x 30000.
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sionally be seen external to the open cytoproct (Fig. 6). It is obvious that the food vacuole membrane is not egested (there is no membrane around the faecal ball in Fig. 6) but fuses with the plasma membrane and thereby the membrane barrier between the cytoplasm and environment is never broken. The membrane, even at this early phase, has a highly irregular profile, showing deep indentations into the cytosol. Each indentation is embedded in a mass of microfilaments.

Fig. 8. A closed cytoproct fixed 10–15 s after defecation. A ball of membrane remnants embedded in microfilaments is all that remains of the defecated food vacuole. Several small electron-opaque vesicles (arrowheads) and flattened vesicles (arrow) are found at the periphery of this ball. ×24,000.

Fig. 9. A cytoproct fixed 10–15 s after defecation. The continuity of the membrane being engulfed with the plasma membrane is clear. Short fragments of microtubules remain near the cytoproct lips. Blebbing of the membrane fragments (arrows) may give rise to electron-opaque vesicles. ×40,000.

At 3–5 s after egesation the deep cleft has disappeared and, although the cytoproct lips are still far apart, there is within the space between the lips a shallow concave depression in the cytoplasm which is bounded by highly convoluted membrane embedded in microfilaments, all of which forms a 1-μm-thick border separating the endoplasm from the cell’s exterior (Fig. 7).
Closing cytoproct

After 10–15 s the cytoproct is either closed or almost closed (Figs. 8, 9). At the cell surface the lips of the cytoproct are again separated by a distance of 0.3 μm. The food vacuole membrane has been engulfed and now consists of an assortment of multi-shaped tubules and sacs still embedded in the microfilaments which, together, take roughly the shape of a ball under the closed cytoproct. Surrounding the ‘ball’ are a number of small vesicles ranging from 70 to 130 nm that contain an electron-opaque material. Vesicles of a similar size and staining characteristic appear to be in the process of pinching away from the tubules and sacs within the ball (arrows, Fig. 9). A few flattened vesicles, around 0.2 μm long, may also be seen next to this ball. Only short fragments of microtubules are present at this stage near the opaque beads at the cytoproct lips.

Discussion

The cytoproct of *Tetrahymena* can be distinguished from other pellicular features. It is a zone limited by a single membrane near the posterior end of the cell, bordered by an electron-opaque flange that seems to weld the margin of the encircling alveolar membranes to the plasma membrane. It also has associated microtubules which seem to originate in this electron-opaque material and pass individually, not as ribbons, into the endoplasm, where they frequently lie next to the cytoplasmic surface of food vacuoles. The contractile vacuole pore has been confused with the cytoproct in the past (plate VI in Nilsson, 1976), but these 2 closely spaced surface features are most readily distinguished by the helically arranged microtubules, which encompass the contractile vacuole pore but not the cytoproct, and by the ribbons of microtubules that radiate from the contractile vacuole pore (Hausmann & Allen, 1977), but not from the cytoproct. In addition, the closed cytoproct is only slightly indented into the cytoplasm and is much longer than wide, while each contractile vacuole pore, of which there are at least 2 per cell, has an indentation of about 0.5–1 μm and is always circular.

Food vacuole egestion and the subsequent closing of the cytoproct occur rapidly. Blum & Greenside (1976) have recently studied the egestion events in *Tetrahymena* with cinematography and report that the food vacuole may lie next to the cytoproct before egestion for at least 7 s. The actual defecation takes 0.04–0.08 s following a period of 2.3 s when the food vacuole noticeably pushes against the pellicle at the cytoproct. Our study extends these observations by showing that within 10–15 s after its opening the cytoproct is again closed and all the membrane of the egested food vacuole has been recycled back into the cell via endocytosis.

At the ultrastructural level the cycle of events occurring in *Tetrahymena pyriformis* corresponds closely to the events we described in *Paramecium caudatum* (Allen & Wolf, 1974). Microtubules extending into the endoplasm from the electron-opaque margins of the cytoproct may trap and guide the vacuoles to the cytoproct, as we postulated in the case of *Paramecium*. However, no bundles of microtubules from the nearby basal bodies were observed to supplement those from the cytoproct lips, as
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they do in *Paramecium*. The electron-opaque material at the cytoproct edges apparently serves as a microtubule-organizer region; similar electron-opaque material is known to have this function in other cell types (Tilney & Goddard, 1970). There is some suggestion that these microtubules may be linked to food vacuole membranes by bridges. Such bridges, if they are real, may be involved in the production of shearing forces that guide the vacuoles to the cytoproct and ultimately bring about membrane contact and fusion, as we proposed for *Paramecium* (Allen & Wolf, 1974).

Endocytosis of the old food vacuole membrane may begin even before egestion and appears to require a network of microfilaments. Such microfilaments are always present at the sites of channeling of the food vacuole membrane, as they are in *Paramecium* (Allen & Wolf, 1974) and as they are during pinocytosis at the plasma membrane of amoeba (Marshall & Nachmias, 1965; Stockem, 1977). Thus long finger-like channels with small diameters extend from the food vacuole membrane before the food vacuole contacts the cytoproct limiting membrane. Such protrusions could facilitate the crucial event of membrane fusion between the food vacuole membrane and plasma membrane as discussed by Poste & Allison (1973). However, the flat membranes can be seen to lie within 10 nm of each other for some time before fusion occurs. The actual fusion event has not been recorded in this system but presumably follows the same steps as those observed in a number of other cell systems (see Poste & Nicolson, 1978), all of which are still incompletely understood at the biochemical level.

Following membrane fusion, the opening presumably widens automatically either to the limits set by the size of the cytoproct or by the size of the food vacuole, whichever is smaller. Egestion seems to be a passive event resulting ultimately from the formation of an opening and the subsequent elimination of the space occupied by the egested contents as the food vacuole membrane is re-engulfed. Osmotic pressure within the cytoplasm may also play a less-essential part in defecation, such as causing the rounding of the cytoproct opening and the flattening of the vacuole. Currents developed by the cilia may also speed up the elimination process in living cells.

The biochemical nature of the network of microfilaments scattered around the food vacuoles has not been investigated. However, similar 5–7-nm-diameter microfilaments found in amoeba and many non-muscle cells of higher organisms are, for the most part, actin in nature (Pollard & Weihing, 1974; Korn, 1978). That *Tetrahymena pyriformis* does have actin has been demonstrated by immunofluorescent antibody techniques (unpublished observations of R. D. Allen and E. Lazarides). Some types of endocytosis seem to depend on actin-like microfilaments to form the channels and to constrict these channels so that their membranes will fuse, thereby releasing the endosomes (Allison & Davies, 1974; Silverstein, Steinman & Cohn, 1977). Thus we feel the microfilaments are likely to be involved in the active process of endocytosis and need not be implicated in a food vacuole-encompassing, active, contractile event that would lead to a rapid expulsion of the undigested vacuole contents. In fact, light-microscope observations of defecation do not reveal a forcible ejection of vacuole contents but a slower elimination of food vacuole contents requiring about 0.1 s (Blum & Greenside, 1976).
The beginning of endocytosis of the food vacuole membrane is not dependent on egestion since it begins before egestion has started. There must be some as yet unknown signal, which causes the polymerization of microfilaments specifically around food vacuoles as they arrive in the vicinity of the cytoproct. Interestingly, not all membranes near the cytoproct receive this complement of polymerized microfilaments. There is, for example, an absence of such microfilaments around the contractile vacuole. Since the membranes of these 2 types of vacuoles have different thicknesses which must reflect biochemical differences (for review and discussion see Allen, 1978), it may be that the thinner type of membrane, e.g. the contractile vacuole membrane, lacks the proteins to which microfilaments can attach. The polymerization of membrane-associated actin-like microfilaments does seem to be limited to thick membranes such as plasma membranes and phagosomes (see Korn, 1978).

Re-engulfment of the membrane necessarily leads to cytoproct closing, as it must in *Paramecium* also. However, a cytoproct ridge such as that found in *Paramecium* is not found in *Tetrahymena*. The fibrous mats lining the internal aspect of the alveoli within the ridge in *Paramecium* are not found in *Tetrahymena*; their absence here further implicates the role of these mats in the formation and maintenance of the ridge in *Paramecium*.

In *Tetrahymena* the fate of the re-engulfed membrane is not clear. In *Paramecium* the endosomes may be transformed into the diskoidal vesicles that move to, and accumulate at, the cytopharynx where they form a pool of membrane waiting to be incorporated into nascent food vacuoles (Allen, 1974). flattened vesicles of a more irregular shape than those in *Paramecium* are also found near the cytopharynx of *Tetrahymena* (Nilsson, 1976), but in fewer numbers. The origin of these vesicles is not known. Some flattened vesicles can be found at the periphery of the ball, composed of tubules and microfilaments, that remains under the recently closed cytoproct but there are a larger number of small spherical vesicles scattered near the food vacuole membrane remnants. Thus both flattened and spherical vesicles may be produced from the membrane remnants and the flattened vesicles may ultimately arrive at the cytopharynx. The fate of these spherical vesicles, which resemble transition vesicles originating from Golgi in other cell types, or micropinocytic vesicles, has not been determined.

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REFERENCES


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