

## Ultrastructural Events in the Predator-induced Defence Response of *Colpidium kleini* (Ciliophora: Hymenostomatia)

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**Summary.** The hymenostome ciliate *Colpidium kleini* undergoes a morphological defence response when in the presence of the predatory ciliate *Lembadion bullinum*, with cells of *C. kleini* becoming shorter and wider and thus more difficult for *L. bullinum* to ingest. The ultrastructural events associated with this morphological transformation were investigated by transmission electron microscopy. A number of differences between defended and undefended *C. kleini* cells were observed with defended cells showing: an increased localization of mitochondria in the peripheral regions of the cell cortex; a mobilization of mucocysts; a change in the appearance of the plasmalemma. Possible explanations for these ultrastructural events are discussed.

**Key words:** ciliate, *Lembadion bullinum*, morphological transformation, mucocyst, TEM.

### INTRODUCTION

Predator-induced defence responses in ciliates have been the subject of several studies since the phenomenon was first reported by Kuhlmann and Heckmann (1985). While most of these studies have dealt primarily with morphological responses, others have focused on changes in life history, behaviour, trophic strategy and growth kinetics (for reviews see Wicklow 1997, Kuhlmann *et al.* 1999). Predator-induced responses have only been reported in three classes of ciliates:

Hypotrichia, Stichotrichia and Hymenostomia. A recent investigation failed to extend the range of ciliate groups with a capacity for predator-induced change (Fyda *et al.* 2005).

Only two genera of hymenostomes are known to exhibit predator-induced defence responses: *Lambornella* and *Colpidium*. In the case of *Lambornella* the response takes the form of a trophic shift from free-living trophonts to parasitic theronts (Washburn *et al.* 1988). By contrast, cells of *Colpidium kleini* and *C. colpoda* increase in volume and become more spherical in shape in the presence of the predatory peniculine ciliate *Lembadion bullinum* (Fyda 1998).

Ultrastructural investigations have rarely been carried out on defended ciliate morphotypes so the changes

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that occur at the subcellular level are poorly understood. Notable exceptions are the studies by Jerka-Dziadosz *et al.* (1987) and by Wicklow (1997) on the hypotrich *Euplotes octocarintaus* and the stichotrich *Onychodromus quadricornutus* respectively. Hitherto there have been no ultrastructural studies of defended morphotypes among the hymenostomes. In this paper we present preliminary observations on ultrastructural differences between defended and undefended cells of *Colpidium kleini*.

## MATERIALS AND METHODS

Stock cultures of *Colpidium kleini* and *Lembadion bullinum* from the Department of Hydrobiology, Jagiellonian University, Kraków were used as the primary source of experimental organisms. Clonal cultures of each were established for use in the present study. All *C. kleini* cells examined during the present study, both defended and undefended, were derived from the same clone. Defended cells of *C. kleini* were obtained according to Fyda (1998). Predator and prey were combined in the same Petri dish and incubated for 48 h. Undefended *C. kleini* were obtained by incubating in the absence of *Lembadion*. Cells were harvested by micropipette and prepared for examination by transmission electron microscopy (TEM) according to the method of Kennaway and Lewis (2004). All cells were treated identically, the only variable being whether or not they had been exposed to the predator *Lembadion*. Ultrathin sections of at least three defended and three undefended cells of *C. kleini* were examined using a Hitachi H7100 TEM.

## RESULTS AND DISCUSSION

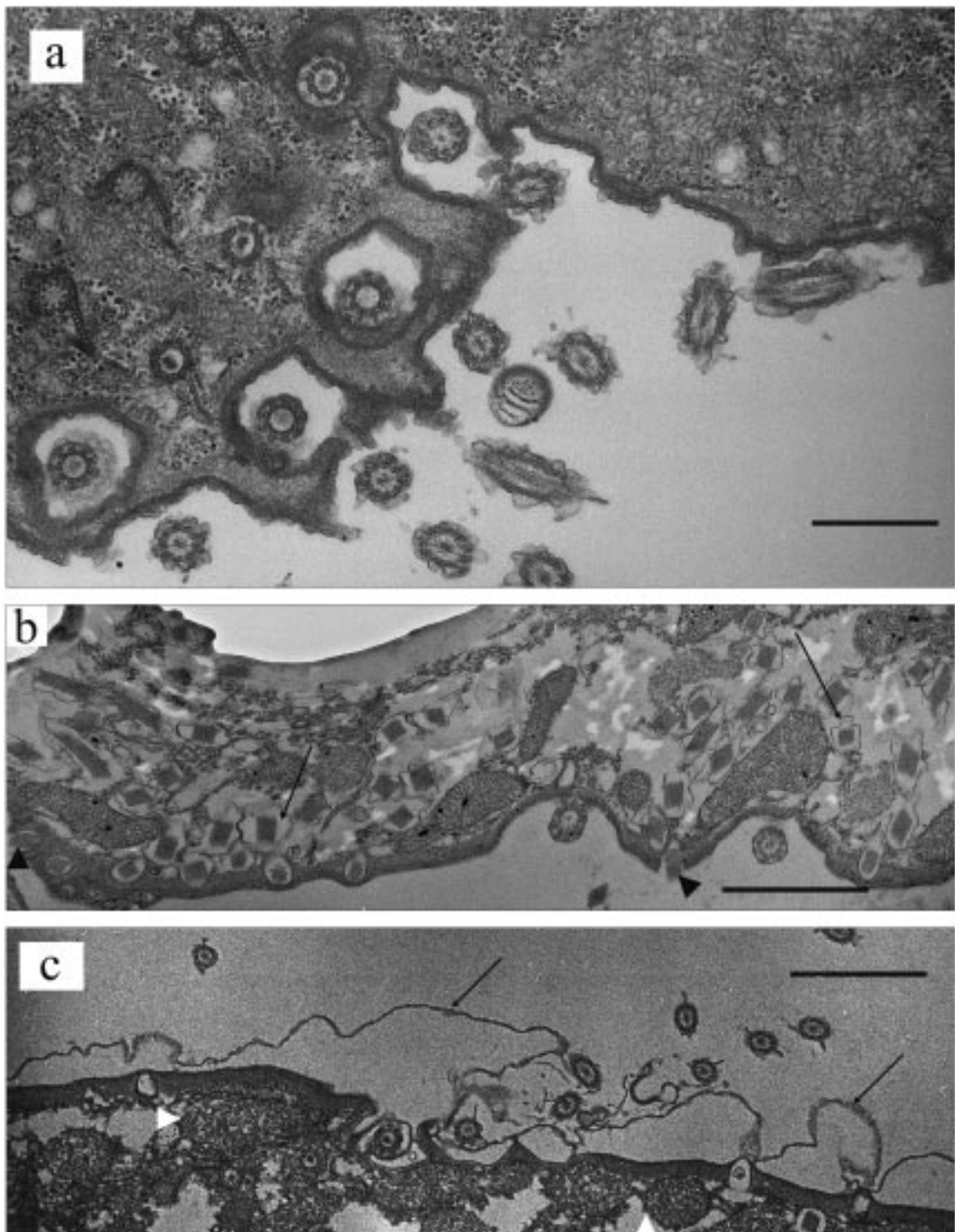
The ultrastructure of the cortical regions of typical defended and undefended *Colpidium kleini* cells are shown in Figures 1a-c. From these it can be seen that a number of changes occur at the ultrastructural level during the defence response, specifically with respect to the distribution of mitochondria, the appearance of the plasmalemma (plasma membrane) and the number and activity of the mucocysts. Although the three micrographs do not necessarily show the same region of the cell, examination of whole-cell sections reveal the same ultrastructural differences in the cortex of defended vs undefended cells throughout the organism.

In defended morphotypes the mitochondria appear to be localized in the cortical region (Fig. 1c). It has long been known that many mitochondria in ciliates localize underneath the pellicle (Fauré-Fremiet 1910, Horning 1927) and some are closely associated with the epiplasm (Aufderheide 1983). It has been postulated that the

cortical mitochondria are involved in active interchange processes in protozoa (Doyle 1935). The expansion of the cortex which gives rise to the cell enlargement during the defence response in *C. kleini* presumably entails increased activity in this region of the cell and might thus account for the cortical localization of the mitochondria.

The second ultrastructural feature that characterises transformed *Colpidium kleini* is the presence of large numbers of mucocysts in the cortical and sub-cortical regions of the cell. Mucocysts are a type of extrusome, the main function of which is to export material to the cell surface. They comprise a paracrystalline contents bounded by a membrane. Mucocysts are produced in the endoplasm and migrate to the cell periphery where they dock with the cell membrane. The mucocyst membrane then fuses with the cell membrane releasing its contents to the outside (Paulin 1996). Figure 1b shows examples of these mucocysts in the cortex of a partly defended *C. kleini* cell. Some appear to be fusing with the plasmalemma thus discharging their contents to the outside (Fig. 1b, arrowheads). Enhanced uptake of Alcian Blue in defended cells provides further support for the presence of higher numbers of mucocysts (data not shown). By contrast, mucocysts were not observed in the control (undefended) cell (Fig. 1a). A variety of functions have been suggested for mucocysts in different ciliates some of which, e.g. defence against predators, are still at the hypothesis level while others, e.g. secretion of material for cyst wall production, are supported by compelling evidence (for reviews, see Hausmann 1978, Rosati and Modeo 2003). It is noteworthy that increased rates of encystment have recently been reported in the hypotrich *Euplotes muscorum* when in the presence of certain predators (Fyda *et al.* 2005). This suggests that the mobilization of mucocysts is indeed involved during the predator-induced response in *E. muscorum*. For *C. kleini*, however, there is no evidence of predator-induced cyst formation so the fate of the contents of the mucocysts once it has been exported from the cell, and its role in the defence response, are unknown. One possibility is that once secreted, the mucocyst contents may coat the cell thereby blocking chemical receptor sites on the cell surface thus compromising the *Lembadion* prey recognition system.

A third feature that distinguishes defended cells of *Colpidium kleini* from undefended cells is the expansion of the plasmalemma. In undefended cells the plasmalemma lies in close proximity to the outer and inner alveolar membranes forming the typical three-mem-



**Figs 1a-c.** *Colpidium kleini*, transmission electron micrographs of cortical regions of defended and undefended cells. **a** - undefended cell. **b** - cell undergoing induced morphological change. Note the mucocysts (arrows) some of which appear to have fused with the cell membrane thus discharging their contents to the outside (arrowheads). **c** - defended cell. Note the expanded plasmalemma (arrows) and localization of mitochondria beneath the pellicle (arrowheads). Scale bars: 1 μm (a); 750 nm (b, c).

brane structure that bounds the ciliate pellicle (Fig. 1a). In transformed cells the plasma membrane is expanded to such an extent that large spaces are formed between it and the outer alveolar membrane (Fig. 1c).

In addition to their assumed protective function, the mucocysts may also play a role in facilitating the shape change that characterizes the defence response in *Colpidium kleini* which involves an increase in cell diameter and therefore an expansion of the cell surface area (Fyda 1998). One mechanism by which this may occur would be if, following fusion of the mucocyst membrane with the plasmalemma, the vacuolar membrane is retained at the cell surface rather than being immediately recycled within the cell. In this way, the mucocysts may provide at least part of the additional membranous material required for the cell expansion to occur. The same process may also allow for the expansion of the plasmalemma shown in Fig. 1c. In his review of protist extrusomes, Hausmann (1978) notes that the fate of the mucocyst membrane following discharge is unknown.

Previous ultrastructural studies of predator-induced defensive morphotypes in ciliates have been restricted to the lateral wings and the dorsal spines that develop in transformed cells of *Euplotes octocarinatus* and *Onychodromus quadricornutus* respectively (Jerka-Dziadosz *et al.* 1987, Wicklow 1997). Both these studies report the appearance of additional microtubules in the sub-pellicular region of the cell, the function of which is thought to be that of supporting the expanding cell cortex. In the case of *Euplotes octocarinatus* there is an increase in the number of microtubular triads on both the dorsal and ventral surfaces of the lateral wings and extra single microtubules between the dorsal triads (Jerka-Dziadosz *et al.* 1987), while in *Onychodromus quadricornutus* bundles of microtubules develop along the axis of the defensive spines (Wicklow 1997). Although no change in the number of cortical microtubules was observed in the present study, unpublished investigations of defended *C. colpoda* cells, i.e. using an antibody directed against acetylated tubulin (TELU 348), have shown that elongation of both the transverse and postciliary microtubules occurs (Iftode, pers. comm.).

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