Review

Food acquisition, food ingestion and food digestion by protists

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Food acquisition, food ingestion and food digestion are crucial for the survival of all living creatures, including, of course, unicellular organisms. The following article will concentrate on these three topics with respect to free-living protists.

I. Food acquisition (Fig. 1)

In general, two different types of food acquisition can be discerned in free-living protists: filter-feeding and predation (Fig. 1) (Hausmann and Hülsmann, 1996).

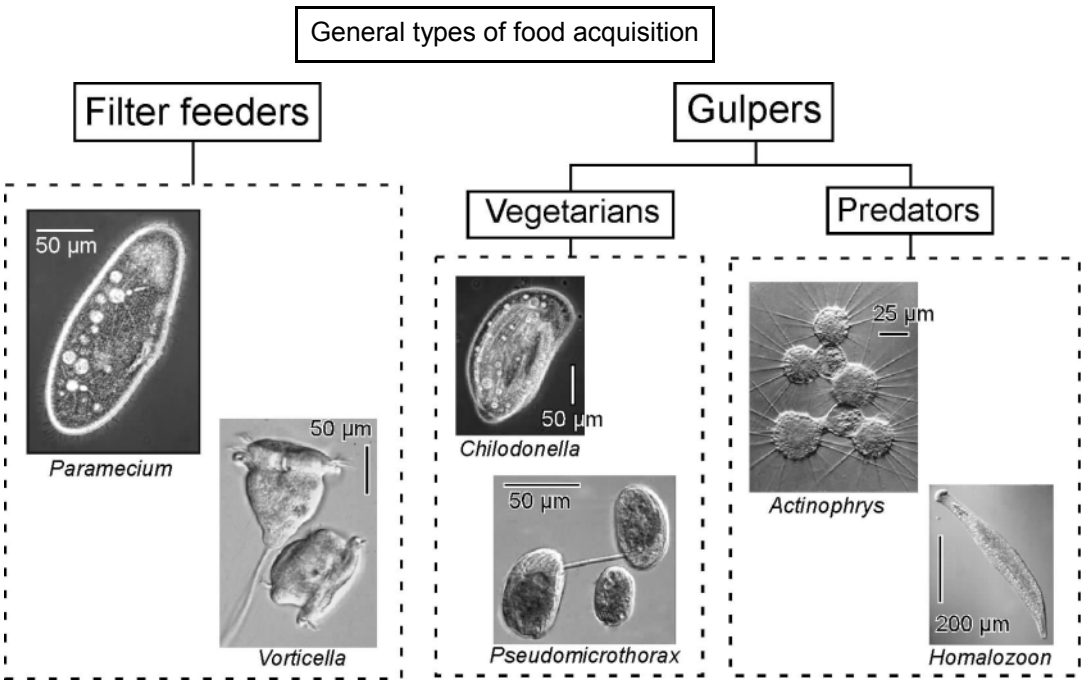

Fig. 1. Synopsis of general types of food acquisition in protists.

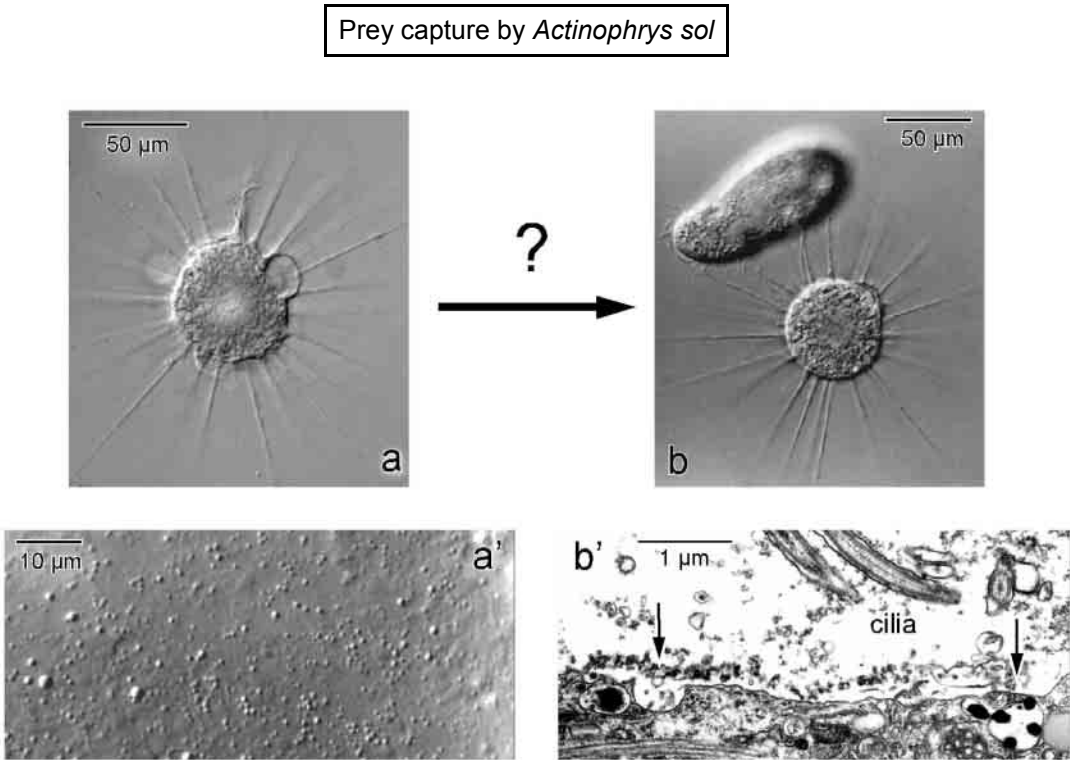

Fig. 2. Prey capture in the heliozoon *A. sol* (b) involves a high number of extrusomes (a') which are seen in the electron microscope in the process of transformation and discharge (b', arrows).

Filter-feeding is food acquisition in which suspended particles are concentrated through the action of flagella or cilia in the areas of the cell at which endocytosis can take place. The flagella / cilia direct a current of water to the cell and - especially in many ciliates - towards a filter device which concentrates food particles before their enclosure within a food vacuole. Well-known examples of filter feeders are ciliates such as paramecia (Ishida et al., 2001) as well as numerous flagellates (Boenigk and Arndt, 2000; Boenigk et al., 2001).

Predatorial feeding, in contrast, is characteristic for those protists which obtain their food through the action of special cell organelles, generally called extrusomes. These are cell elements with intravacuolar and dischargeable contents, derived from typical dictyosomes (Hausmann, 1978). In the context of this article the function of extrusomes in heliozoans and of toxicysts in ciliates will be discussed.

Extrusomes of *Actinophrys sol* (Fig. 2)

The capture of prey by the heliozoon *Actinophrys sol* follows the random contact between the axopods of the passively floating heliozoon and the actively swimming ciliate prey. The ciliate adheres to the predator despite the active beating of its cilia and numerous avoidance responses (Fig. 2 b). In the region of contact, individual cilia may be seen to adhere to axopodia or to each other (Patterson and Hausmann, 1981). A very similar situation has been reported for another heliozoon, *Echinosphaerium nucleofilum* (Suzaki et al., 1980).

It can be clearly seen using an electron micro-

Predatory *Homalozoon vermiculare*

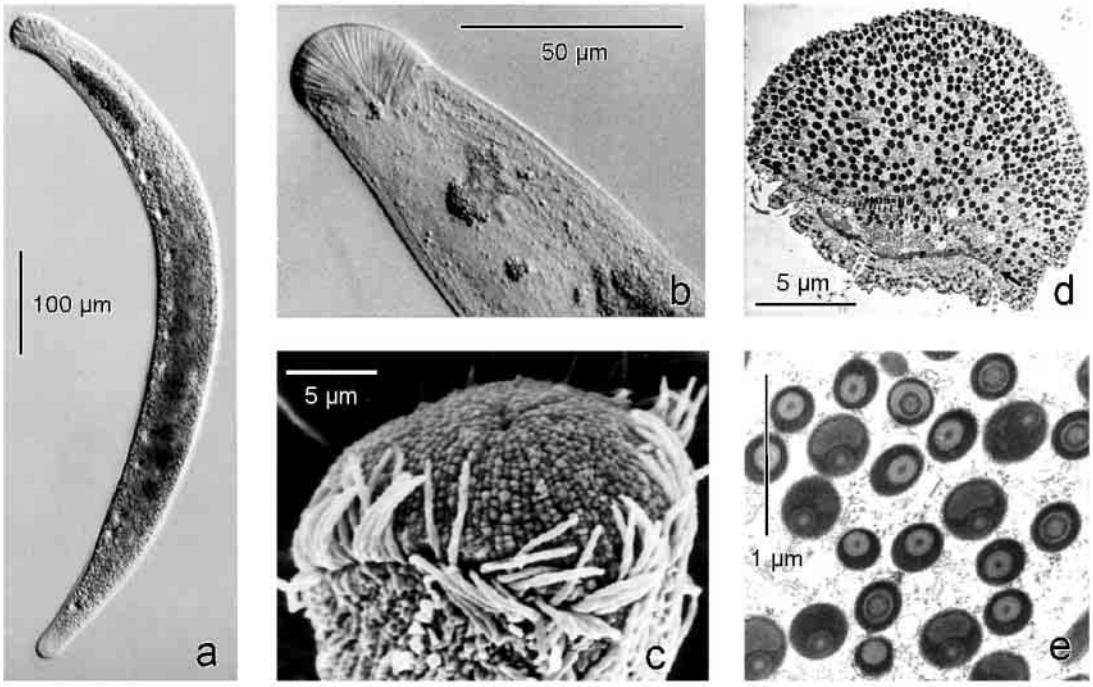

Fig. 3. The oral bulge of the ciliate *H. vermiculare* is filled with hundreds of toxicysts which appear in light micrographs as rood like structures (a + b) and in ultra-thin cross sections as organelles with a circular to oval profile (d) which are filled with more-or-less concentric structures (e).

scope that direct contact between the prey and predator is minimal, if it occurs at all. However, much extracellular material is typically found between the two cells (Fig. 2 b'). Obviously this material connects the two cells. At least a part of it originates from the discharged content of the numerous extrusomes of *A. sol* which can be recognised easily by light microscopy (Fig. 2 a'). The functional role of this discharged material has been investigated in great detail up to the molecular level by Sakaguchi et al. (2001) (see also article by M. Sakaguchi in this issue, pp. 35-39). In addition, large amounts of small pieces of membrane, arised from the plasmalemma of the predator, were also present. The membranes exist as fine threadlike pseudopodia extending from the surface of the predator (Hausmann and Patterson, 1982). Again, the extrusomes seem to play an essential role also in this process. It has not yet been clarified whether or not these morphologically more-or-less identical organelles represent different types of extrusomes with different functions.

In *Rhaphidiophrys contractilis*, a centrohelid heliozoon, so-called kinetocysts are implicated in the process of prey adhesion. The posterior part of the discharged kinetocyst is always attached to the plasma membrane of the heliozoon, while the anterior end is directed towards the prey, permitting close contact with the food organism (Sakaguchi et al., 2002) (see also article by M. Sakaguchi in this issue, pp. 35-39).

Toxicysts of *Homalozoon vermiculare* (Figs. 3-7)

Homalozoon vermiculare is a predatory ciliate

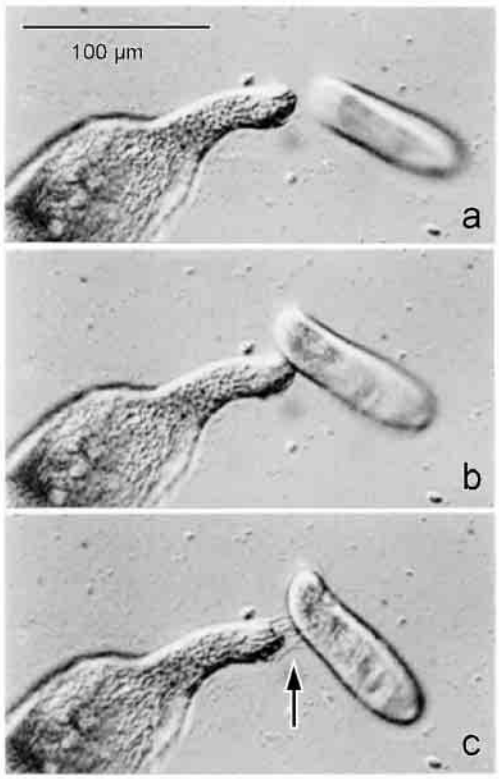

Fig. 4. The first step of food capture by *H. vermiculare* is mechanical contact with the prey ciliate *Colpidium* (a + b). Within milliseconds extruded toxicysts are detectable (c, arrow).

which feeds on other ciliates, e.g. on *Paramecium* or *Colpidium* (Fig. 4). Its oral bulge is filled with several hundred toxicysts (Fig. 3) (Kuhlmann et al., 1980). The resting toxicysts are detectable under a light microscope as rod-like elements (Fig. 3 a + b); in cross section they appear in electron micrographs as round to spherical organelles (Fig. 3 d + e) (Kuhlmann and Hausmann, 1980).

Toxicysts are used to immobilize and to kill the normally fast-swimming prey (Hausmann, 1978). The explosive extrusion of toxicysts occurs due to mechanical contact with the food organism and lasts only milliseconds (Fig. 4). In all likelihood, chemical recognition of the prey is also involved in this event. The tubules are forced into the prey organisms like hypodermic needles.

Resting toxicysts are morphologically complex organelles which contain fully developed structures inside a capsule which is located inside an extrusomal vesicle. Tubes lying within the capsule are filled with an ordered material which is probably a toxin. Furthermore, an amorphous substance is detectable in the vicinity of the capsule wall (Fig. 5 a - c, Fig. 6, resting stage).

The first step in the expulsion process of toxicysts is the fusion of the toxicyst membrane with the plasma membrane (Fig. 5 d), after which a tubule is discharged via evagination (Fig. 6, discharging stage). Alternatively, for certain ciliates a telescopic discharge of a tube is reported (Fig. 7). During or near the end of toxicyst expulsion a toxic material is secreted by the tubule (Fig. 7, toxifying). Only wild speculations exist about the force driving toxicyst ejection. In this context, however, the apparent similarities in structure, effect, and mode of function between the toxicysts of protozoa and the nematocysts of cnidarians should be mentioned, although the structures differ significantly in size.

Using darkfield microscopy, Krüger, the nestor of extrusomal research, postulated already in 1936 that three different categories of toxicysts exist: capsule, tubule and tube. He based his scheme on the ratio of the length of the different parts of the ejected extrusomes (compare Fig. 7). Any deviation from these ratios, if they exist, have not been reported so far.

There are still important questions which need to be answered for a better understanding of toxicyst function:

- What triggers the ejection of toxicysts?
- What is the origin of the forces needed for the expulsion of toxicysts?
- What is the nature of the extruded toxic agent?
- How are toxicysts synthesized in the cytoplasm and rearranged in the oral bulge?

Ultrastructural appearance of toxicysts

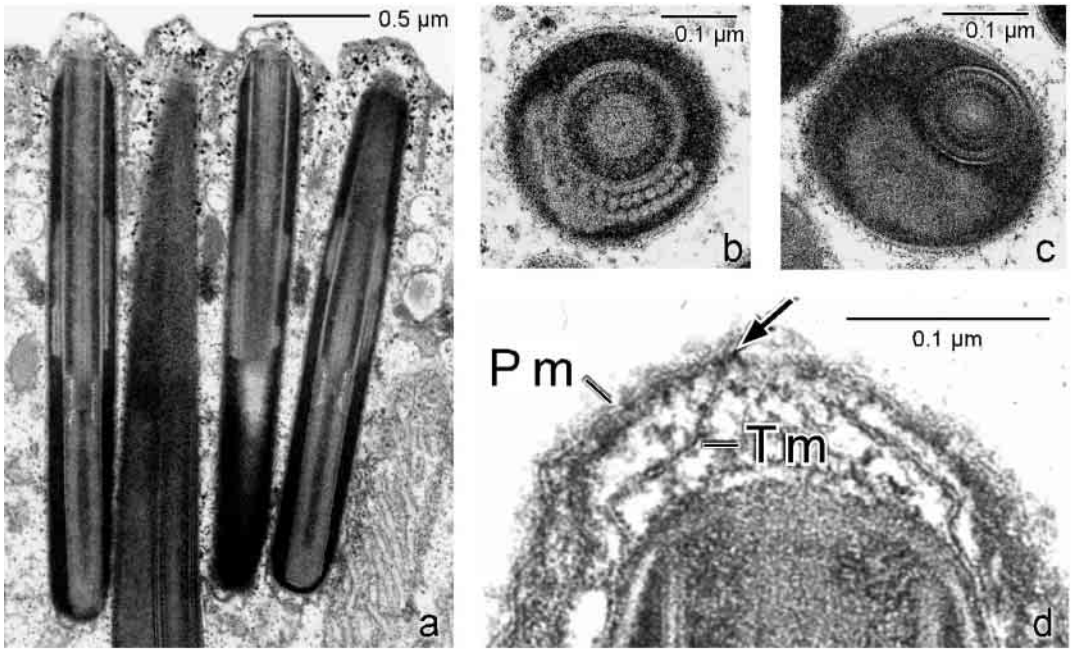

Fig. 5. Resting toxicysts at higher magnifications (a - c) revealing complex internal structures a part of which are inverted tubules. The first step of exocytotical toxicyst expulsion (d) is the fusion of the toxicyst membrane (Tm) with the plasma membrane (Pm).

II. Food ingestion (Fig. 8)

Food ingestion, the internalization of external material into a cell, is ruled by certain necessities. One of these is the fact that under normal, natural conditions, everything which is engulfed by a cell needs to be enclosed by a membrane, e.g. in the case of protistan phagocytosis by a food vacuolar membrane. The origin of this membrane is of great interest. From the light microscopical point of view, in some cases it seems to be trivial, in others merely mystical. For instance, *Amoeba proteus* easily engulfs prey organisms by an invagination of its plasma membrane. However, what occurs in *Actinophrys sol*, whose prey is enclosed in a giant food vacuole, the origin of which cannot be the plasma membrane at all, 10-15 minutes after the first contact (Fig. 8 a + b) (Linnenbach et al., 1983)?

Electron microscopical investigations of *A. sol* revealed that during the development of the food vacuole, extrusomes expand and fuse with each other and eventually with the plasma membrane (Fig. 8 c + d). Their investing membrane is thereby made available as food vacuole membrane (Hausmann and Patterson, 1982).

At first glance, food ingestion by ciliophora seems to be rather diverse. But on closer inspection, it obeys rules which seem to be generally valid for all phagocytosing ciliates (Radek and Hausmann, 1996): Food vacuoles are formed at the cytostome, i.e., the region in the oral apparatus bounded only by the plasma membrane, which is lacking alveoli and infraciliature. The membrane for the growing food vacuole is not derived from the plasma membrane but is delivered by pre-formed cytoplasmic

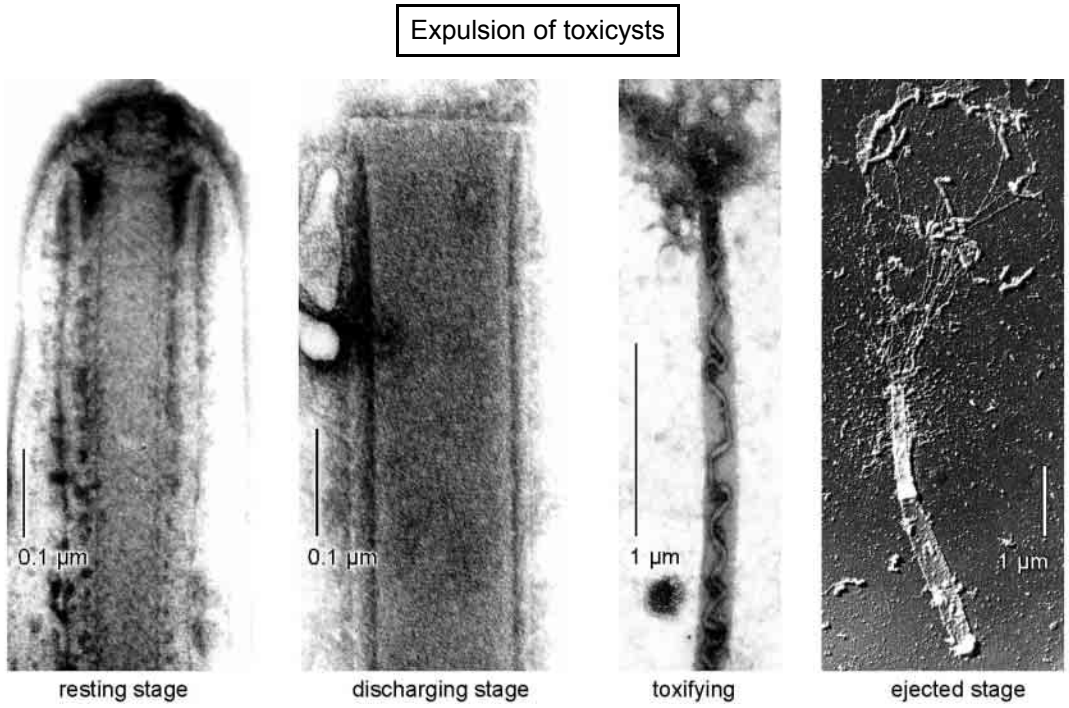

Fig. 6. Sequence of the expulsion of a toxicyst illustrated by selected stages of extrusion.

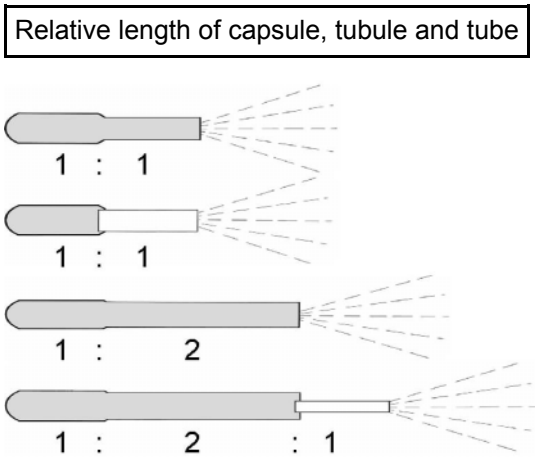

Fig. 7. Currently known length ratios of components of extruded toxicysts.

vesicles which fuse with the plasma membrane in the cytostomal region. *De novo* synthesis would be far too slow to make the large amounts of required membrane available within a relatively short period of time, i.e., in few seconds or minutes. The vesicles are transported towards the cytostome along ribbons of microtubules. In all likelihood, cytoplasmic dynein, a minus-end-directed microtubule-based motor, is responsible for the movement. However, in most cases due to the small size of these vesicles, their transport cannot be analyzed using light microscopy. Electron microscopical analyses of these events were needed.

In *Paramecium* these vesicles are very flat and without visible contents. In other ciliates such as *Climacostomum*, *Eufolliculina*, and *Euplotes*, the vesicles contain an electron-opaque material (at least in some cases digestive enzymes) which is

Food ingestion by *Actinophrys sol*

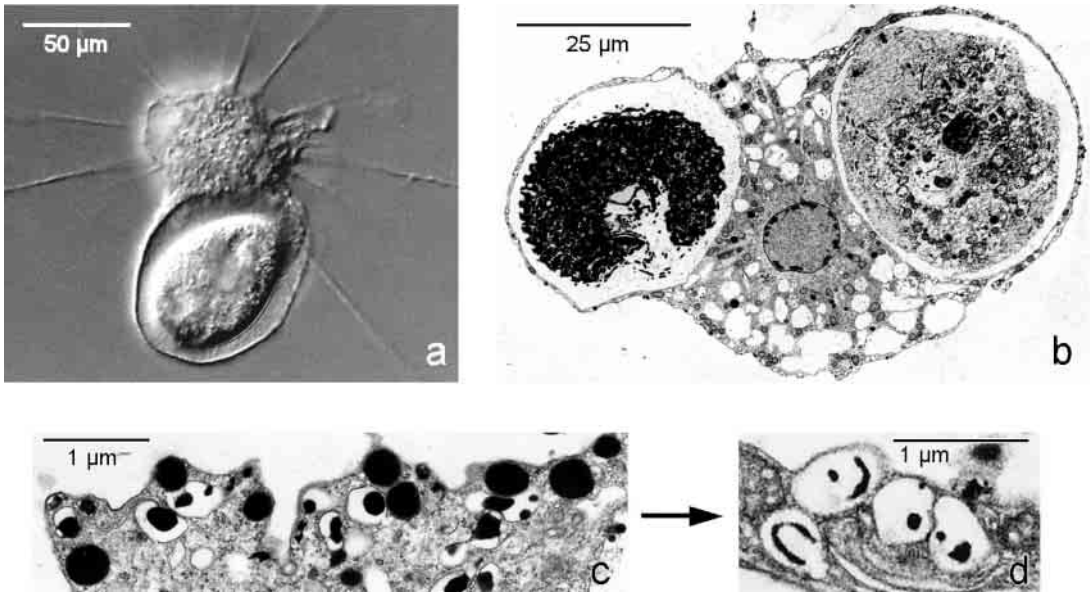

Fig. 8. *Actinophrys sol* with completed food vacuoles surrounding the prey (a + b). During food ingestion, compact resting extrusomes (c) transform to expanded stages (d) which fuse readily with each other and the plasma membrane, thus supplying sufficient membrane material for the nascent food vacuole.

released when the vesicles fuse with the cytostomal membrane.

The gulper *Pseudomicrothorax dubius* (Figs. 9-10)

A rather impressive example of ciliate phagocytosis is the food uptake by the gulper *Pseudomicrothorax* dubius. This ciliate is highly specialized to feed on cyanobacterial filaments (Fig. 9).

A complex cytopharyngeal basket of rods as well as sheets of microtubules and associated structures was developed to move algal filaments into a growing food vacuole at very high speeds (Fig. 10) (Hausmann and Peck, 1978). During food uptake, the membrane of the food vacuole increases rapidly at rates of up to 270 μm^2 per second. Vacuole growth results - again - from the fusion of membrane-bound, spherical vesicles (1 µm in diameter) which are similar to the flat vesicles seen in other ciliates. During phagocytosis, a fast streaming of these relatively large vesicles can be observed using light microscopy in the cytoplasm surrounding the basket (Fig. 11). The vesicles enter the lumen of the basket at its anterior end, in a zone where the wall of the basket is perforated (Hausmann and Peck, 1979).

III. Food digestion (Fig. 11)

The process of food digestion has been studied in great detail in ciliates like *Tetrahymena* (Nilsson, 1979) and particularly in *Paramecium* (Allen and Fok, 1993, 2000; Fok and Allen, 1993). Their general digestion processes correspond to those known for multicellular organisms.

However, at least for the ciliate *Pseudomicrothorax dubius* there exists a two-step digestive scenario. The second step seems to be comparable with

Food ingestion by the ciliate *P. dubius*

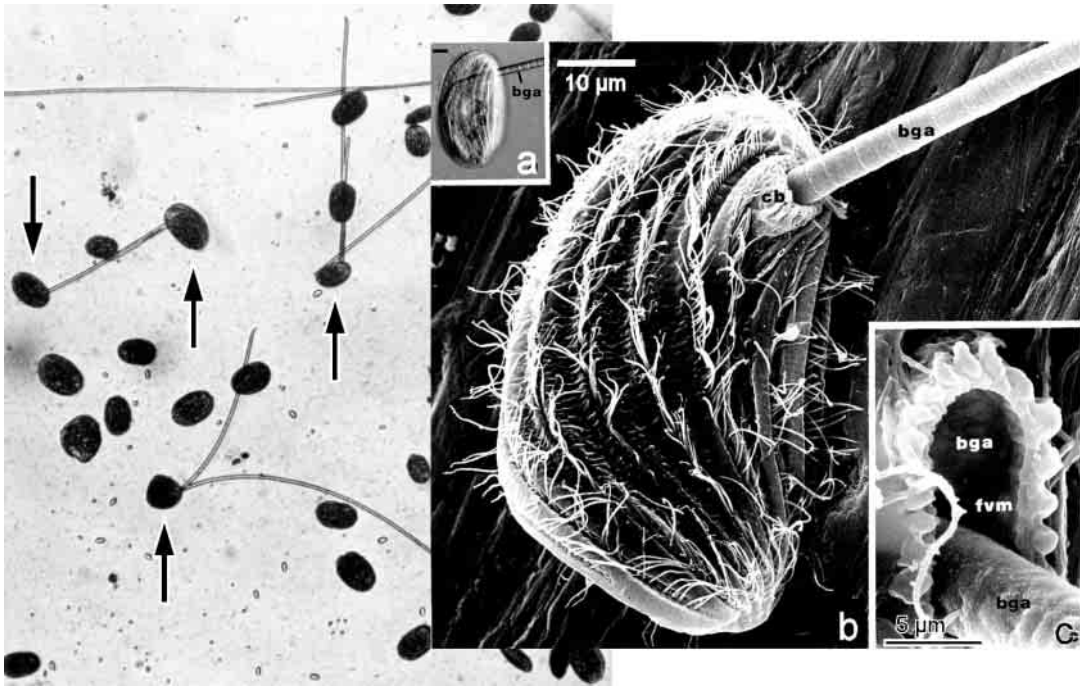

Fig. 9. Starved *P. dubius* cells start food uptake immediately after supply with cyanobacteria (left micrograph, arrows). The filaments (bga) are engulfed rapidly by a specially constructed ingestion apparatus, the cytopharyngeal basket (cb, right micrographs) (fvm – food vacuole membrane).

the well-known processes of unicellular and multicellular organisms, but the first step is very different. Due to the fusion of the aforementioned vesicles with the growing food vacuole membrane (Fig. 11 b), lysosomal hydrolases (AcPase) are liberated into the food vacuole. They are detectable in the cyanobacterial cytoplasm within less than one second after AcPase's entry into the food vacuole, and within a few seconds, the destruction of the wall of the cyanobacterial filament can be observed.

The benefits to *P. dubius* of rapid destruction of the cell walls of *Oscillatoria* are:

- softening of the rigid algal filament and therefore faster food uptake.
- fast local extracellular digestion of the cyanobacterial cell wall and subsequent folding of algal filaments before uptake and consequently ingestion of twice as much food.
- possibility of chemically "biting off" the algal filament when the filament is not driven into the cell but arrested in the cytopharyngeal basket; here the cell wall is destroyed due to the activity of the digestive enzymes and the remnant algal filament is pulled out of the oral apparatus.

Pseudomicrothorax dubius ingests a large volume of filamentous blue-green alga within a short time span (about one minute). Immediately after this rapid ingestion, the food is enclosed in a single, extremely large food vacuole, which fills up the ciliate almost entirely. During the following hour this giant food vacuole vesiculates. Finally, numerous small vacuoles 1-2 µm in diameter are formed.

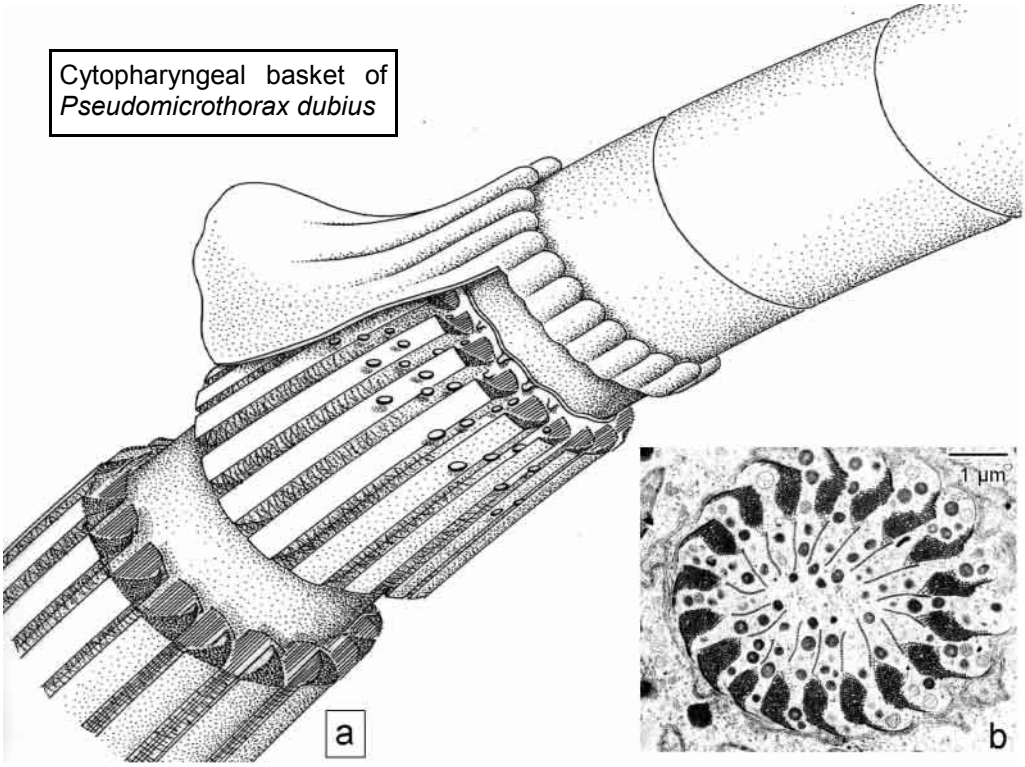

Fig. 10. The 3D-reconstruction of the cytopharyngeal basket of *P. dubius* (a) is based on numerous ultra-thin sections (b).

Simultaneously the content of the vacuoles is noticeably condensed. Only at this time does the digestion of the food start, as is indicated by numerous, highly active dictyosomes which now surround the periphery of the food vacuoles (Hausmann, 1980).

Conclusions

In the natural environment the nutrition of free-living protists mainly depends on the uptake of solid food. A variety of devices for food-trapping such as extrusomes has been developed in response to the kind of food preferred. For example, small particles such as bacteria are filtered out by the use of flagella and cilia which create water currents and at the same time, especially in ciliates, act as sieves for retaining food particles. Complex cytostomal apparatuses, sometimes in combination with a special food-finding behaviour, have been evolved for the ingestion of large food organisms. As a general rule, the food vacuole grows by fusion of small vesicles, partly extrusomes, with the cytostomal area.

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Rapid destruction of cyanobacterial cell walls by *Pseudomicrothorax dubius*

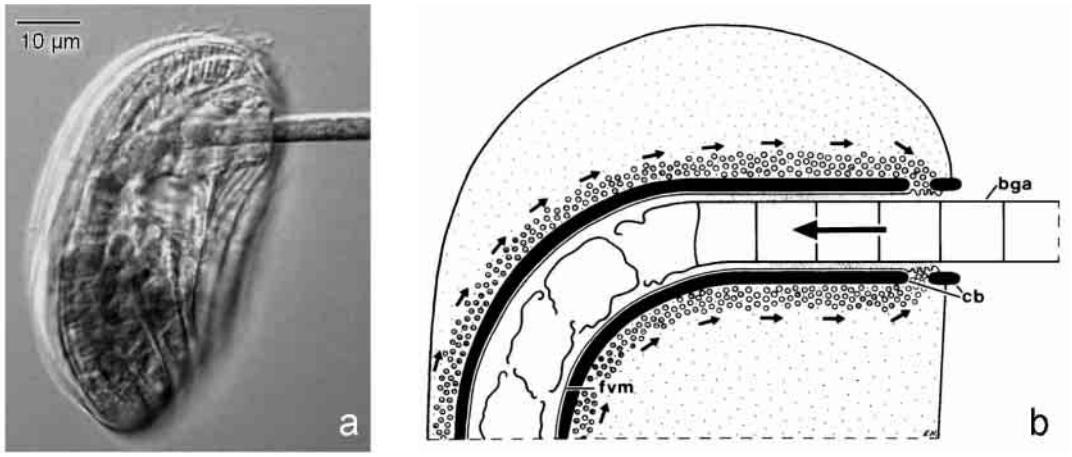

Fig. 11. Feeding *P. dubius* (a) and schematic representation of this process (b). The small arrows indicate the streaming of vesicles opposite to the direction of the ingestion of the blue-green alga (bga, big arrow). The vesicles enter the lumen of the cytopharyngeal basket (cb), fuse with the nascent food vacuole and supply the material for the increase of the membrane of this vacuole (fvm) and simultaneously secrete digestive enzymes into the food vacuole for the rapid destruction of the cyanobacterial cell walls.

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