The Journal of Experimental Biology 205, 2089–2097 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3971

In the polymorphic ciliate *Tetrahymena vorax*, the non-selective phagocytosis seen in microstomes changes to a highly selective process in macrostomes

Heidi K. Grønlien*, Trond Berg and Arne M. Løvlie
Department of Biology, University of Oslo, PO Box 1051 Blindern, N-0316 Oslo, Norway
*e-mail: h.k.gronlien@bio.uio.no

Accepted 22 April 2002

Summary

Ciliates use phagocytosis to acquire edible particles. The polymorphic ciliate *Tetrahymena vorax* appears in two forms ('microstomes' and 'macrostomes'). Transformation of microstomes into macrostomes takes place in the presence of the ciliate *Tetrahymena thermophila* and enables the macrostome to phagocytose the latter species. The non-specific, constitutive phagocytosis in microstomes thereby changes into a specific inducible process in macrostomes. The purpose of this study was to determine whether the phagocytotic process in macrostomes is specifically aimed at catching *T. thermophila*. The two forms of phagocytosis represent an interesting model system for studying the mechanism whereby phagosomes are formed. The macrostomal form capture deciliated and ciliated *Tetrahymena thermophila*, latex beads with diameters of 20.3 and 30.0 μm and small microstomal cells. However, the macrostomes select *T. thermophila* as a prey when they have the opportunity to choose between deciliated *T. thermophila* and latex beads and between *T. thermophila* and microstomes. The non-selective formation of phagosomes seen in microstomes changes to a highly selective process during the transformation to macrostomes. Unlike microstomes, macrostomes do not form a closed vacuole after capturing a latex bead, indicating that mechanical stimulation by the prey does not in itself trigger phagocytosis in the macrostomal form of *T. vorax*. Although macrostomes captured *T. thermophila* in preference to microstomes, phagocytosis of microstomes started immediately following capture, indicating that the substance/molecule that triggers the formation of the phagosome is not specific for *T. thermophila* cells.

After capturing a *T. thermophila* cell, the macrostomal cell, which normally swims in a forward direction, reverses direction and swims backwards for a short time before starting to rotate. Macrostomal cells did not change their swimming pattern after capturing a latex bead. We believe, therefore, that backward swimming is more likely to be related to signals resulting from phagocytosis than from mechanical stimulation of the pouch.

Cytochalasin B (10 μg ml^{-1}) inhibits phagocytosis in both microstomes and macrostomes, indicating that actin filaments play an active role in phagocytosis in both cell types. The antitubulin drug nocodazole (0.3–30 μmol l^{-1}) inhibits the formation of more than one phagosome in the macrostome, indicating that membrane transport to the oral apparatus in macrostomes is guided by microtubules. Nocodazole has no effect on the process of phagocytosis in microstomes.

Key words: *Tetrahymena vorax*, polymorph, microstome, macrostome, phagocytosis, phagosome, latex bead, *Tetrahymena thermophila*, cytochalasin B, nocodazole, microtubule, actin filament.

Introduction

Phagocytosis is a special form of endocytosis by which eukaryotic cells internalize particles (Silverstein et al., 1989). In mammals, cells specialized for phagocytosis are found mainly in the immune system (Brown, 1995). Protozoa such as ciliates use phagocytosis as a major pathway for acquiring nutrients such as bacteria and other food particles (Nilsson, 1979). In *Tetrahymena* spp., the oral apparatus, from which the phagosome grows, defines the anterior part of the cell. As the nascent phagosome grows, the most posterior part of the oral apparatus becomes filled with food particles that are swept in by beating of the oral ciliary membranelles (Nilsson, 1972). Stimulated by an as yet unknown trigger, the nascent phagosome becomes detached from the oral apparatus after the limiting membrane has been sealed off (Nilsson, 1976). In *Paramecium* spp., movement of the nascent phagosome is guided along postoral microtubular bundles, and this movement appears to be responsible for pulling the nascent phagosome away from the oral apparatus (Schroeder et al., 1990). The actual pinching off that results in the formation of a phagosome is dependent, at least in part, on the presence of actin and myosin (Cohen et al., 1984a,b).

The polymorphic ciliate *Tetrahymena vorax* appears in two forms, termed microstomes and macrostomes (Williams, 1961). When a microstome transforms into a macrostome, its

small oral apparatus is resorbed and replaced with a larger one of different shape termed a pouch (Buhse, 1966b; Smith, 1982a,b). Transformation occurs in the presence of another ciliate, *Tetrahymena thermophila*, and permits *T. vorax* to live as a carnivore preying on *T. thermophila* (Buhse, 1966a). The signal from *T. thermophila* that triggers this differentiation in *T. vorax* is a complex of iron and nucleic acid catabolites termed stomatin (Smith-Somerville et al., 2000).

Little is known about the process of phagocytosis in macrostomes. The transformation of microstomes results in cells that are able to capture, phagocytose and digest *T. thermophila*. Here, we have investigated whether the phagocytotic process in macrostomes is non-selective or whether it is specifically aimed at catching *T. thermophila*. Phagocytosis by microstomes seems to be non-selective. Food particles are swept into the oral apparatus by beating of the oral ciliary membranelles. The process may be compared with so-called macropinocytosis in macrophages, whereby relatively large volumes of medium are internalized in large endosomes (Swanson, 1989). If macrostomes select a certain ciliate for feeding, the capture of these cells may be initiated by binding of the prey to specific binding sites, similar to the interaction between receptor and prey that occurs before phagocytosis by macrophages and neutrophils. To obtain information about the selectivity of phagocytosis in macrostomes, we compared phagocytotic uptake of *T. thermophila* cells, microstomes and latex beads by macrostomal cells. To further characterize phagocytosis by macrostomes, we studied the effects of drugs that affect the cytoskeleton (microtubules, microfilament) in these cells. In parallel control experiments, we assessed the effects of the same drugs on phagocytosis by microstomes.

Materials and methods

Latex beads of uniform size (diameter varied by less 4 %) were obtained from Dynal Particles, Lillestrøm, Norway. They were washed three times in sterilized, deionized water. We tested four different sizes of bead with diameters of 3.0, 20.3, 30.0 and 41.3 μm. The concentrations of latex beads and of *T. thermophila* were determined microscopically using a Sedgwick–Raffer chamber. The experimental saline solution was prepared following the method of Dryl (1959). Cytochalasin B and nocodazole were obtained from Sigma (USA) and were dissolved in dimethyl sulphoxide (DMSO) to obtain stock solutions of 10 and 33 mmol l^{-1}, respectively.

Animals

Tetrahymena vorax strain V2 and *Tetrahymena thermophila* were kept at room temperature (21–23 °C) in standard growth medium (Plesner et al., 1964). The experiments were performed on cells in the mid-logarithmic phase of exponential growth (Grelland, 1988). Cell densities were approximately 5×10^5 cells ml^{-1} for *T. vorax* and 8×10^5 cells ml^{-1} for *T. thermophila*. The size of the elongated microstomal cell was approximately 60 μm×20 μm. The size of the more spherical *T. thermophila* was approximately 35 μm×25 μm. The size of the *T. vorax* cells was approximately the same as that of interphase *T. thermophila* cells immediately after division.

Transformation of microstomes into macrostomes

Transformation of microstomes into macrostomes is initiated by a transforming substance called stomatin that is released into the medium by *T. thermophila* (Smith-Somerville et al., 2000; Buhse, 1967). To obtain a stomatin-containing solution, 100 ml of the *T. thermophila* culture was centrifuged to pellet the cells, and the cells were subsequently suspended in 50 ml of deionized water and incubated for 3 h at room temperature. The suspension was centrifuged to remove the cells, and the supernatant was filtered through a 0.45 μm pore diameter filter. The stomatin-containing solution was added to the *T. vorax* culture in a ratio of 1:10 (v/v). The first macrostomes appeared after approximately 5 h (at 23 °C), and macrostomes continued to be formed for the next 5–7 h. The first division of a macrostome into microstome daughters occurred after 15–17 h. In these experiments, we used macrostomal cells that had been in the stomatin solution for 8 h; approximately 20 % of the cells had been transformed to macrostomes at this point. The size of the macrostomal cell was approximately 120 μm×80 μm.

Prey selection and formation of phagosomes in macrostomes

To study the process of prey selection and the formation of phagosomes in macrostomes, the cells were incubated with latex beads or with ciliated or deciliated *T. thermophila* cells. *T. thermophila* was mechanically deciliated using the procedure described by Hawkins (1975). To determine the prey concentration that gave optimal phagocytotic uptake, dose/response curves for both latex beads and deciliated *T. thermophila* were obtained (data not shown). The optimal concentrations were 10^6 beads ml^{-1} and 10^5 cells ml^{-1}. To compare phagocytosis of latex beads and deciliated *T. thermophila* by macrostomes, the cells were exposed to the same concentrations (10^6 prey ml^{-1}) of the two types of prey. The macrostomes were exposed to latex beads and deciliated *T. thermophila* cells for 30 min with manual agitation every 10 min to keep the prey in suspension. The deciliated cells were immobile during the experiments. This is in accordance with results reported by Rannestad (1974), who found that deciliated *T. thermophila* stay immobile for 40 min after deciliation. At the end of the incubation period, the cells were quickly but gently mixed with formaldehyde to a final concentration 0.4 % (the formaldehyde was methanol-free; Code F017/3 from TAAB Laboratories, England, UK). In one set of experiments, repeated three times, the numbers of latex beads in the pouch and/or *T. thermophila* in the food vacuoles in 100 macrostomes were counted using light microscopy (magnification 20×).

In the experiments in which phagosome formation in individual macrostomes was studied, macrostomal cells were selected with a manually operated micropipette and placed in separate drops under liquid paraffin in Petri dishes. A single macrostomal cell was placed in the droplet together with either

five small microstomal cells (approximately 35 μm×20 μm) or a mixture of 25 small microstomes and five *T. thermophila* cells. All prey were manually collected using a suction pipette. The *T. thermophila* cells were marked with Texas-Red-labelled ovalbumin, and the cells were observed with both a fluorescence and a light microscope. The Texas-Red-labelled *T. thermophila* culture was washed three times before the cells were added to the droplets. The behaviour of the animals was recorded using a video camera recording at 25 frames s^{-1}.

Drug treatments

To determine the effects of cytochalasin B and nocodazole on phagocytosis in microstomes and macrostomes, cells were first preincubated with the drugs for 8 h and 2 h, respectively. Microstomes were exposed to carmine particles (50 μg ml^{-1}) for 6 min, and three-quarters of the culture was quickly but gently fixed with formaldehyde (final concentration 0.4 %). In one set of experiments, repeated three times, labelled food vacuoles were counted in 100 microstomes. The remaining 25 % of the cells were washed five times in Dryl solution and allowed to stand for 30 min before being fixed in formaldehyde (0.4 %). Macrostomes were allowed to feed on *T. thermophila* cells (10^5 cells ml^{-1}) for 30 min before they were gently fixed in formaldehyde (final concentration 0.4 %). In one set of experiments, repeated three times, food vacuoles were counted in 100 macrostomes.

Results

Macrostomes select T. thermophila *in preference to latex beads*

Macrostomes were incubated with latex beads with diameters of 3.0, 20.3, 30.0 and 41.3 μm. The macrostomes did not capture latex beads with diameters of 3.0 and 41.3 μm, but approximately 30 % of the cells captured latex beads with diameters of 20.3 and 30.0 μm (Fig. 1). The maximal number of latex beads in the pouch during a 30 min exposure to beads with a diameter of 30 μm was six (data not shown). These experiments were performed in growth medium because the transformation back to microstomes started when the macrostomal cells were exposed to Dryl solution. Microstomes cultured in a Dryl solution only captured beads with a diameter of 3.0 μm (data not shown).

We next compared the capture by macrostomes of latex beads with the capture of *T. thermophila*. Latex beads with a diameter of 30 μm were chosen since this size is nearest that of *T. thermophila*. We used deciliated *T. thermophila* since swimming *T. thermophila* cells may conceivably be easier to capture than immobilised cells. The rate of phagocytosis, given as the number of digestive vacuoles formed in 30 min, is more than twice as high for ciliated (2.6±0.1; mean ± S.E.M., *N*=4) as for deciliated (0.93±0.3; *N*=6) prey.

Deciliated *T. thermophila* or latex beads (30.0 μm indiameter) were added to *T. vorax* cultures 8 h after exposure to stomatin-containing solution. Prey concentrations were 10^6 ml^{-1}. The control results presented in Fig. 2 show that the

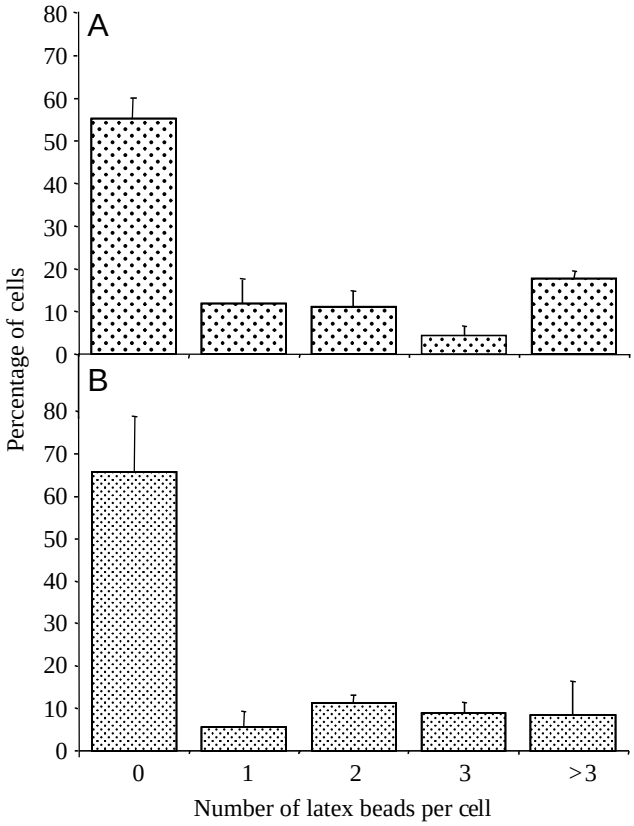

Fig. 1. The distribution of latex beads in the pouch of macrostomes. Macrostomes were allowed to prey on latex beads with a diameter of (A) 20.3 μm and (B) 30.0 μm. The macrostomes did not differentiate between these two sizes. The macrostomes did not capture latex beads with a diameter of 3.0 and 41.3 μm. In all experiments, the concentration of beads was 10^6 ml^{-1}. The experiment was repeated three times, and 50 cells are counted in each experiment. Values are expressed as the mean + S.E.M.

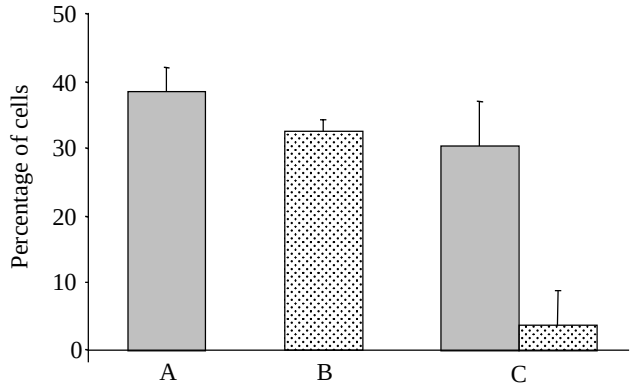

Fig. 2. The percentage of macrostome cells that captured either deciliated *Tetrahymena thermophila* (grey columns) or latex beads with a diameter of 30 μm (stippled columns) in a medium containing (A) only deciliated *T. thermophila*, (B) only latex beads and (C) both deciliated *T. thermophila* and latex beads. The macrostomes consistently selected the cells rather than the beads. In all experiments, the concentration of the prey was 10^6 ml^{-1}. The experiment was repeated three times, and 100 cells were counted in each experiment. Values are expressed as the mean + S.E.M.

percentage of macrostomes capturing deciliated *T. thermophila* when exposed for 30 min to cells only was 37.7±0.9 % (mean ± S.E.M.) (Fig. 2) and that the percentage of macrostomes capturing latex beads when exposed for 30 min to beads only was 30.7±3.5 % (Fig. 2). If the medium contained both deciliated cells and latex beads (final concentration 10^6 prey ml^{-1}), the macrostomes consistently selected cells rather than beads (Fig. 2). The percentage of macrostomes capturing deciliated *T. thermophila* in this medium during the 30 min exposure was 32.7±6.1 %, and the percentage capturing latex beads was 4.0±5.1 %.

In an immature macrostome, the prey attaches to the mouth for 2–5 s, but the macrostomal cell is unable to engulf it since the mouth is not yet fully developed (data not shown). We did not observe this abortive interaction between macrostomes and prey when immature macrostomes were exposed to latex beads.

To determine whether the macrostome recognizes a signal that is specific for *T. thermophila*, a macrostomal cell was placed in a droplet of medium (under liquid paraffin) together with either a mixture of small microstomes and *T. thermophila* or only small microstomes. If the droplet contained only microstomes, the macrostomal cell did phagocytose microstomal cells. In another set of experiments, six droplets contained a mixture of 25 microstomes and five *T. thermophila* each, in addition to the macrostome. In this experiment, the effect of the size difference between a normal microstome and *T. thermophila* cells was reduced by manually collecting microstomal cells of a size close to that of a *T. thermophila* cell (approximately 35 µm×20 µm) using a suction pipette. In all six droplets, the macrostome selected exclusively *T. thermophila*.

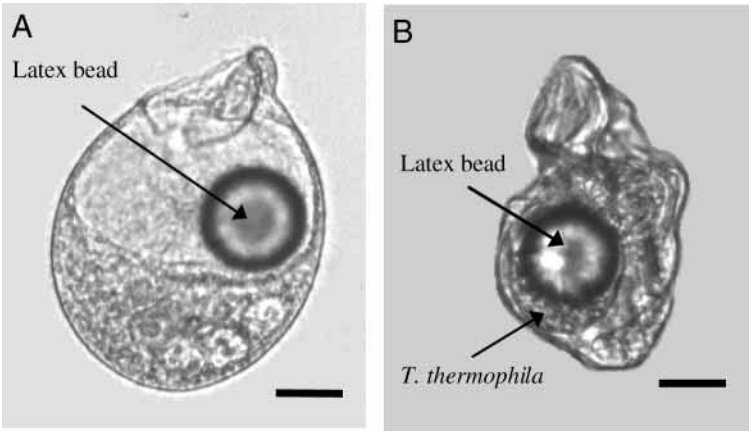

Fig. 3. (A) A macrostomal *Tetrahymena vorax* that has captured a latex bead with a diameter of 30 µm. The picture was taken 30 min after capture of the bead; there was no sign of phagocytosis of the latex bead. Scale bar, 20 µm. (B) A macrostomal cell *Tetrahymena vorax* that has phagocytosed a latex bead together with a *T. thermophila* cell. The picture was taken 10 min after adding *T. thermophila* cells and latex beads to the medium. Scale bar, 20 µm.

The macrostome does not phagocytose latex beads

We do not know the mechanisms whereby phagocytosis is initiated in ciliates (Allen and Fok, 2000). In the presence of only latex beads in the medium, macrostomes captured but did not phagocytose the beads (Fig. 3A). When both latex beads and *T. thermophila* were present in the medium, a bead was occasionally phagocytosed together with the cell (Fig. 3B). This was, however, a rare event. At least 300 macrostomal cells were observed during these experiments, and concurrent uptake of beads and cells was observed only four times. Since the latex beads were of the same size as *T. thermophila*, the results indicate that the signal for phagocytosis in macrostomes is not merely mechanical but probably due to signal molecules associated with or released from *T. thermophila*.

Behavioural response to phagocytosis in macrostomes

When *T. thermophila* has been captured in the pouch, the macrostomal cell continues to swim in the same direction before starting a short backward swim that is followed by cell rotation (Fig. 4). The time from capture to the reverse reaction was found to be 1.7±0.6 s (*N*=8). The total duration of the chain of events depicted was 28.3±5.2 s (means ± S.E.M., *N*=8). The macrostomal cells did not change their swimming pattern after capturing a latex bead. At the same time as the macrostome swims backwards in response to phagosome formation, the anterior part of the cell contracts. We believe, therefore, that backward swimming is related to the process of phagocytosis.

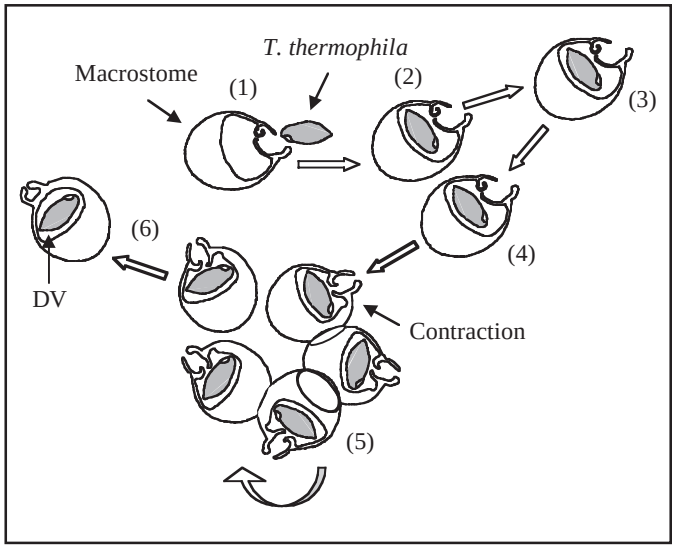

Fig. 4. Diagram of the behavioural response of a macrostomal cell of *Tetrahymena vorax* after capturing a *T. thermophila* cell. The macrostome selects a *T. thermophila* (1) and captures it (2). The macrostome continues to swim in the same direction for 1.7±0.6 s (mean ± S.E.M., *N*=8) (3) before briefly swimming backwards and the anterior part starts to contract (4); this is followed by cell rotation (5). The total duration of the chain of events depicted (1–5) was 28.3±5.2 s (mean ± S.E.M., *N*=8). The cell then swims forward again, the prey is in a digestive vacuole (DV) (6). It normally takes 10–13 min before phagocytosis restarts.

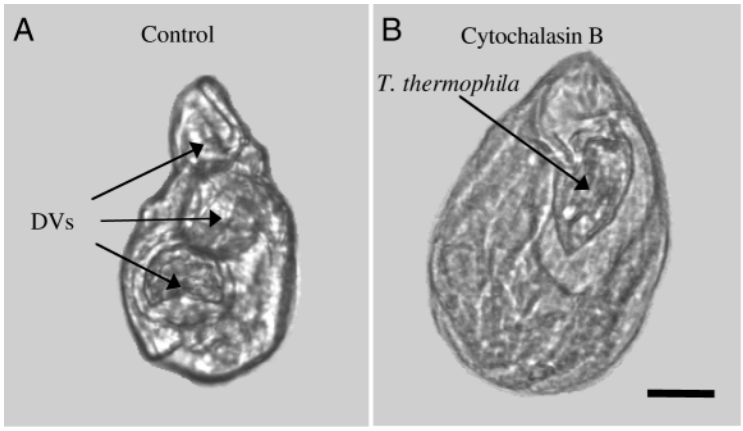

Fig. 5. The effect of cytochalasin B (10 μg ml^{-1}) on phagocytosis in a macrostomal cell of *Tetrahymena vorax*. Both pictures are taken 30 min after the addition of *T. thermophila* to the medium. In the control cell (A), phagocytosis of three *T. thermophila* cells has occurred. In the cytochalasin-B-treated cell (B), the *T. thermophila* has been captured but no phagocytosis has taken place. DVs, digestive vacuoles. Scale bar, 20 μm.

Table 1. *The rate of phagocytosis by macrostomes and microstomes treated with cytochalasin B*

	Control cells	Cytochalasin-B-stimulated cells
Rate of phagocytosis by macrostomes (number of DVs per 30 min)	2.4±0.2 (3)	0±0 (3)
Rate of phagocytosis by microstomes (number of DVs per 6 min)	3.9±0.1 (3)	0.2±0.2 (3)

The rate of phagocytosis was measured as the number of digestive vacuoles (DVs) formed.

In all experiments, the concentration of *Tetrahymena thermophila* was 10^5 cells ml^{-1}.

In each experiment, 100 cells were counted.

Data are expressed as the mean ± S.E.M. (*N*).

Cytochalasin B concentration was 10 μg ml^{-1}.

Effects of cytoskeletal inhibitors on phagocytosis in macrostomes and microstomes

To further characterize phagocytosis in macrostomes, we studied the effects of cytochalasin B, an inhibitor of microfilament polymerization, and nocodazole, an inhibitor of microtubules, on phagocytosis by macrostomes. The drugs were dissolved in DMSO, and the final DMSO concentration in the cell suspensions was far below the critical concentration (2.5 % v/v) for suppressing phagocytosis in *Tetrahymena pyriformis* (Nilsson, 1974). The effects of these drugs on phagocytosis in microstomes were studied in parallel experiments.

Cytochalasin B, which is a potent inhibitor of actin polymerization (Wessels et al., 1971), has been shown to inhibit the separation of the nascent phagosome from the oral apparatus in ciliates (Nilsson et al., 1973; Allen and Fok, 1985; Fok et al., 1985; Leakey et al., 1994). Actin polymerization may be required just before the nascent phagosome starts to move along the microtubular bundle (Cohen et al., 1984a,b; Allen and Fok, 2000). To test the effects of cytochalasin B on phagocytosis in macrostomes, the drug (final concentration 10 μg ml^{-1}) was added to the culture together with stomatin-containing solution 8 h before phagocytosis was initiated. The long preincubation period was chosen because it has been shown that, to achieve a maximal effect, the cells need to be exposed to the drug for at least 8 h (Leakey et al., 1994). As a control, we tested the effect of the drug on the microstomes in the same culture. Cytochalasin B was found to inhibit phagosome formation in both macrostomes and microstomes (Table 1). In cytochalasin-B-treated macrostomal cells, a *T. thermophila* cell was often captured but the prey continued to swim in the pouch (Fig. 5) it was also frequently able to escape. These results indicate that the formation of the phagosome requires actin assembly in the macrostomal form of *T. vorax*.

In *Paramecium* spp., phagosomes arise in the lower part of the oral apparatus and grow as discoidal vesicles fuse with the membrane of the oral apparatus (Allen, 1974). The discoidal vesicles are transported to the oral apparatus along microtubular ribbons (Schroeder et al., 1990) anchored to the membrane of the oral apparatus (Allen, 1974). In *Tetrahymena* spp., these ribbons are less well developed; they are shorter and the number of microtubules per ribbon is smaller than in *Paramecium* (R. D. Allen, personal communication).

To determine whether phagocytosis in the two forms of *T. vorax* is dependent on microtubules, we used nocodazole, which has been shown to depolymerize microtubules (Adelman et al., 1968). As depicted in Fig. 6, phagocytosis in microstomal cells was not significantly (*t*-test, $P>0.05$) inhibited by nocodazole (final concentration 5 μmol l^{-1}). However, the movement of the phagosomes was prevented by the drug, and the phagosomes stayed near the oral apparatus. To count the number of phagosomes, the cells were washed five times in Dryl solution after the 6 min incubation period with carmine particles and allowed to stand for 30 min before being fixed in formaldehyde (0.4 %). In macrostomes, the concentrations of nocodazole used were 0.3, 3.0 and 30.0 μmol l^{-1}. The nocodazole-treated macrostomes rarely formed more than one phagosome (Fig. 7), possibly as a result of the inhibition of membrane transport (in the form of discoidal vesicles transported along microtubules) to the pouch. Membrane transport in microstomal cells did not seem to be affected by nocodazole since the drug did not inhibit phagocytosis in these cells.

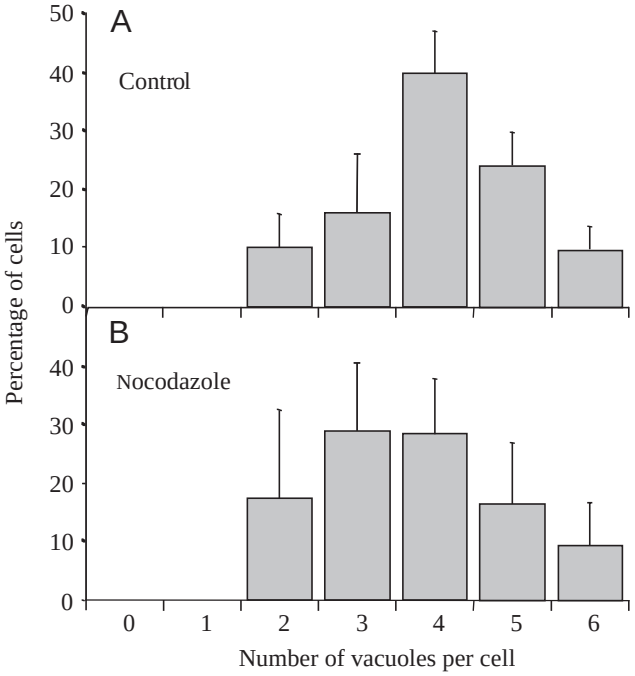

Fig. 6. The distribution of digestive vacuoles (A) in untreated microstomes (control) and (B) in cells treated with 5 μmol l^{-1} nocodazole. No significant differences were observed between the control and treated cells. In all experiments, the concentration of *T. thermophila* was 10^5 cells ml^{-1}. The experiment was repeated three times, and 100 microstomal cells were counted in each experiment. Values are expressed as the mean + S.E.M.

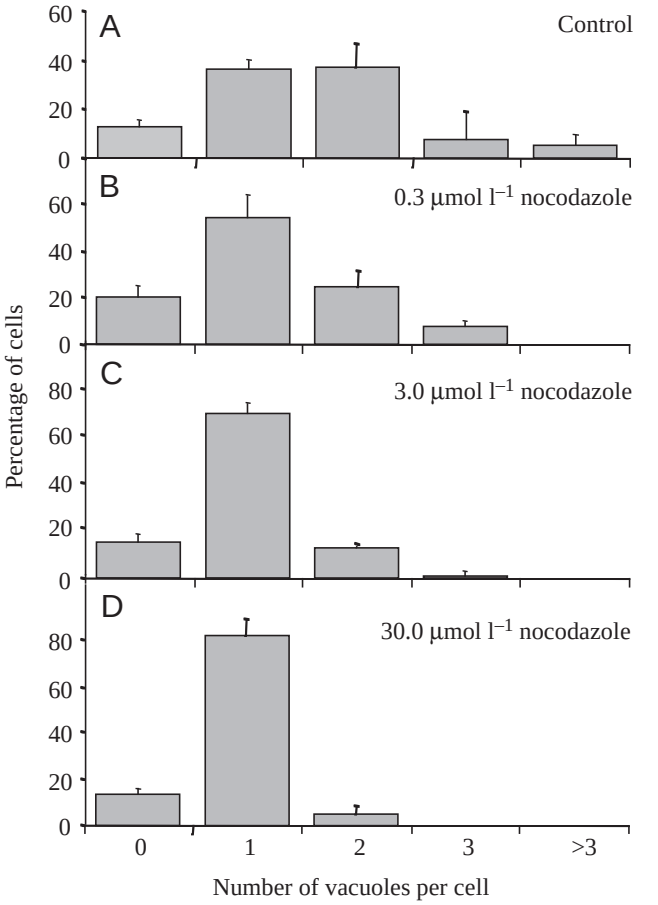

Fig. 7. The distribution of digestive vacuoles in macrostomes in untreated microstomes (A) (control) and in cells treated with 0.3 μmol l^{-1} (B), 3.0 μmol l^{-1} (C) and 30.0 μmol l^{-1} (D) nocodazole. Nocodazole largely prevented macrostomes from forming more than one digestive vacuole. In all experiments, the concentration of *T. thermophila* was 10^5 cells ml^{-1}. The experiment was repeated three times, and 100 microstomal cells were counted in each experiment. Values are expressed as the mean + S.E.M.

Discussion

Prey capture by macrostomes and microstomes

The data presented here indicate that the macrostome form of the polymorphic ciliate *T. vorax* selects *T. thermophila* as a prey when it has the opportunity to chose between *T. thermophila* and latex beads and between *T. thermophila* and microstomes. It has been suggested that some ciliates may select edible particles from mixtures of edible and inedible particles. *T. pyriformis*, for instance, engulfs bacteria in preference to colloidal gold, but gold is taken in when no bacteria are present (Elliott and Clemmons, 1966). In a droplet containing small microstomes and *T. thermophila* cells, the macrostomal cells chose *T. thermophila* even if the ratio was 5:1 in favour of microstomes. These data show that the selection of *T. thermophila* cannot be due only to recognition of digestible substances and suggest that *T. thermophila* exposes or releases a substance that is easily recognized by the macrostome. It remains to be determined whether this putative substance acts as a chemotactic molecule or whether the macrostome selects *T. thermophila* only after random contact.

The percentage of macrostomes in the process of capturing prey during 30 min of incubation with either deciliated *T. thermophila* or latex beads was approximately 30 % (Fig. 2), which may seem low. It should be noted, however, that all cells with a large pouch were counted. Thus, both immature and dividing macrostomes, which do not phagocytose, were counted. In immature macrostomal cells, which are incapable of engulfing prey since the pouch is not completely developed, the *T. thermophila* remained bound for 2–5 s to the anterior surface of the cell. There were no signs of any interaction between the anterior part of an immature macrostome cell and a latex bead, supporting the idea that macrostomes recognize *T. thermophila* selectively.

Phagocytosis in macrostomes is not triggered by mechanical stimulation

The mechanism whereby phagosomes are pinched off from the oral apparatus in ciliates is unknown. It has been suggested that the formation of food vacuoles is induced by the mechanical action of captured particles (Allen and Fok, 2000). *Tetrahymena pyriformis* incubated with polystyrene latex particles and natural food particles (bacteria) accepted the polystyrene latex particles as readily as the bacteria, and acid

phosphatase appeared normally in the vacuoles (Muller et al., 1965). Microstomal cells cultured in Dryl formed phagosomes when they were exposed to latex beads with a diameter of 3.0 μm (data not shown). Phagocytosis of particles in microstomes therefore seems to be a rather non-selective process. Food particles are swept into the oral apparatus by the beating of the oral ciliary membranelles, and mechanical stimulation by the prey may subsequently trigger phagocytosis.

The non-selective phagocytosis seen in microstomes changes to a highly selective process during the transformation to macrostomes. In macrostomal cells, as in microstomal cells, latex beads were captured in the oral apparatus but whereas the oral apparatus containing beads separates to form a closed vacuole in microstomes, this step does not take place in macrostomes (Fig. 3A), indicating that mechanical stimulation by the prey does not in itself trigger phagocytosis in the macrostomal form of *T. vorax*. Although macrostomes captured *T. thermophila* in preference to microstomes, phagocytosis of microstomes started immediately following capture, indicating that the substance/molecule that triggers the formation of the phagosome is not specific for *T. thermophila* cells. However, *T. thermophila* seem to contain 'signal' molecules with higher affinity or in greater amounts than those in the microstomes. The macrostomes may conceivably contain receptors in their pouch that need to be activated by signal molecules associated with or released from the prey in order to stimulate phagocytosis. The actual phagocytosis of the prey may be initiated by binding to receptor sites similar to those that mediate phagocytosis in macrophages and neutrophils (Aderem and Underhill, 1999). Receptor-mediated phagocytosis in macrophages is a well-documented process initiated by signals that originate from the receptors involved in binding the particle (Aderem and Underhill, 1999). Two-dimensional electrophoresis of the macrostome oral apparatus of *T. vorax* has revealed approximately 55 polypeptides in addition to the set of approximately 145 polypeptides also found in the microstome oral apparatus (Gulliksen et al., 1984). Some of these additional peptides in macrostomes may correspond to receptor proteins and signal proteins.

The closing of the food vacuole is dependent on actin filaments

Little is known about the molecular mechanisms that lead to the closing of a phagosome in ciliates. In 'higher' Eukaryota, receptors (e.g. Fc receptors) mediating phagocytosis are expressed on macrophages and neutrophils. Receptor clustering occurs upon particle binding, and this generates a phagocytotic signal that leads to the activation of kinases (including scr and syk and phosphatidylinositol 3-kinase) (Ninomiya et al., 1994). Monomeric GTPases, in cooperation with phosphatidylinositol 3-kinase, can modulate the assembly of the submembranous actin filament system, leading to particle internalization (Araki et al., 1996). Cytochalasin B, a potent inhibitor of actin polymerization, inhibits the detachment of the nascent phagosome from the oral apparatus in both microstomes and macrostomes. This is in accordance with earlier observations of cytochalasin-B-treated *Paramecium* spp. and *T. thermophila* (Nilsson et al., 1973; Allen and Fok, 1985; Fok et al., 1985). It has been suggested that actin polymerization may be required just before the commencement of the movement of the nascent phagosome along the postoral microtubular bundle (Schroeder et al., 1990). Antibodies against heavy meromyosin (which decorates actin) label the periphery of phagosomes during and shortly after phagosome movement (Cohen et al., 1984a,b), indicating that the pinching off of the nascent phagosome is dependent on actin microfilaments.

Membrane transport to the pouch in macrostomes occurs along microtubules

Microtubules are an important constituent of the architecture of the oral apparatus in *Tetrahymena* spp. The microtubules form a deep fibre bundle that extends far into the cytoplasm, and the food vacuoles are guided along these tubules away from the oral apparatus (Nilsson and Williams, 1966). New membrane is incorporated into the membrane of the oral apparatus during phagocytosis. Small vesicles are present in the oral region of *Tetrahymena* spp. (Nilsson and Williams, 1966; Nilsson, 1976), and their membranes resemble those of the food vacuoles (Nilsson, 1979). In *Paramecium* spp., the membrane of the nascent phagosome is provided by discoidal vesicles that fuse with the membrane of the oral apparatus (Allen, 1974). The discoidal vesicles are transported to the oral apparatus along microtubular ribbons (Schroeder et al., 1990) anchored to the membrane of the oral apparatus (Allen, 1974). It has been suggested that vesicles retrieved from food vacuoles during processing as well as after fusion with the cytoproct may be reused for vacuole formation (Allen and Fok, 1980). Nocodazole, an inhibitor of microtubule formation, did not affect phagocytosis in microstomes in our studies (Fig. 6). It is possible, however, that nocodazole does not depolymerize microtubules in *Tetrahymena* spp. The microtubular ribbons in the oral region of *Paramecium* spp. are unaffected by nocodazole (R. D. Allen, personal communication). The absence of effect could also be due to a large membrane pool already docked to the oral apparatus.

Surprisingly, nocodazole greatly inhibited the formation of more than one phagosome in macrostomes (Fig. 7); this could be due to inhibition of membrane transport along microtubules to the pouch. However, the need for membrane during phagocytosis to form the pouch may require the retrieval and recycling of membrane from previously formed phagosomes before the cell can capture another prey.

Backward swimming after capture of a T. thermophila *cell is probably related to the process of phagocytosis*

When a *T. thermophila* has been engulfed in the pouch, the macrostomal cell, which normally swims in a forward direction, reverses direction and swims backwards for a short time before starting to rotate (Fig. 4). This backward swimming could be due to the avoidance reaction normally

seen after mechanical stimulation of the anterior part of the cell. This behaviour has been studied in detail in *Paramecium* spp. (Naitoh and Eckert, 1969). Touching the front end of the animal opens stretch-sensitive Ca^{2+} channels and generates a depolarizing receptor potential that leads to a reversal of the ciliary beat and backward swimming. *T. vorax*, in both its microstome and macrostome forms, display a similar avoidance reaction to mechanical obstacles (H. K. Grønlien, unpublished data). If the observed backward swimming were related to the stimulation of mechanosensitive channels in the pouch by the prey, we might expect backward swimming to occur after capture of a latex bead. However, since the macrostomal cells did not change their swimming pattern after capturing a latex bead, we believe that backward swimming is more likely to be related to signals resulting from phagocytosis. At the same time as the macrostome swims backward in response to phagosome formation, the anterior part of the cell contracts.

Backward swimming may be the result of an increased internal Ca^{2+} concentration, which will cause reversal of the cilia (Naitoh and Eckert, 1969). It has been shown that Ca^{2+} plays a role in the initiation of pouch closure to form a closed vacuole in the macrostomal form of *T. vorax* (Sherman et al., 1982). This observation supports the idea that influx of Ca^{2+} to the cytosol is one step in the signal-transduction process during phagocytosis in macrostomes. Contractile proteins have also been reported to be associated with the oral region of *Pseudomicrothorax dupius* (Hauser et al., 1980). In *Tetrahymena* spp., three different calmodulin proteins have been found (Takemasa et al., 1989, 1990), and the cytosol of *Tetrahymena* spp. contains Ca^{2+}-activated ATPases (Chua et al., 1977). The increased cytosolic Ca^{2+} concentration may be a result of influx of Ca^{2+} from the environment and/or from intracellular organelles. Abundant alveoli, the main storage organelles of Ca^{2+} (Stelly et al., 1991), are associated with the oral apparatus in *Tetrahymena pyriformis* (Nilsson and Williams, 1966). Ca^{2+} chelators (EDTA and EGTA) are known to cause collapse and resorption of the pouch in the macrostomal form of *T. vorax* (Sherman et al., 1982).

The authors wish to thank The Research Council of Norway for financial support. We thank Dr Richard Allen for helpful comments and suggestions.

References

Adelman M. R., Borisy, G. G., Shelanski, M. L., Weisenberg, R. C. and Taylor, E. W. (1968). Cytoplasmic filaments and tubules. *Fedn. Proc.* **27**, 1186–1193.

Aderem, A. and Underhill, D. M. (1999). Mechanisms of phagocytosis in macrophages. *Annu. Rev. Immunol.* **17**, 593–623.

Allen, R. D. (1974). Food vacuole membrane growth with microtubule-associated membrane transport in *Paramecium*. *J. Cell Biol.* **63**, 904–922.

Allen, R. D. and Fok, A. K. (1980). Membrane recycling and endocytosis in *Paramecium* confirmed by horseradish peroxidase pulse–chase studies. *J. Cell Sci.* **45**, 131–145.

Allen, R. D. and Fok, A. K. (1985). Modulation of the digestive lysosomal system in *Paramecium caudatum*. III. Morphological effects of cytochalasin B. *Eur. J. Cell Biol*. **37**, 35–43.

Allen, R. D. and Fok, A. K. (2000). Membrane trafficking and processing in *Paramecium*. *Int. Rev. Cytol.* **198**, 277–318.

Araki, N., Johnson, M. T. and Swanson, J. A. (1996). A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages. *J. Cell Biol.* **135**, 1249–1260.

Brown, E. J. (1995). Phagocytosis. *BioEssays* **17**, 109–117.

Buhse, H. E., Jr (1966a). An analysis of macrostome production in *Tetrahymena vorax*, strain V2-type. *J. Protozool*. **13**, 429–435.

Buhse, H. E., Jr (1966b). Oral morphogenesis during transformation from microstome to macrostome and macrostome to microstome in *Tetrahymena vorax*, strain V2 type S. *Trans. Am. Microsc. Soc*. **85**, 305–313.

Buhse, H. E., Jr (1967). Microstome–macrostome transformation in *Tetrahymena vorax*, strain V2 type S induced by a transforming principle, stomatin. *J. Protozool*. **14**, 608–613.

Chua, B., Elson, C. and Shrago, E. (1977). Purification and properities of a cytosol Ca^{2+}-activated ATPase from *Tetrahymena pyriformis*. *J. Biol. Chem.* **252**, 7548–7554.

Cohen, J., Garreau de Loubresse, N. and Beisson, J. (1984a). Actin mobilization during phagocytosis in *Paramecium*. *J. Submicrosc. Cytol*. **16**, 103–104.

Cohen, J., Garreau de Loubresse, N. and Beisson, J. (1984b). Actin microfilaments in *Paramecium*: Localization and role in intracellular movements. *Cell Motil*. **4**, 443–468.

Dryl, S. (1959). Antigenic transformation in *Paramecium aurelia* after homologous antiserum treatment during autogami and conjugation. *J. Protozool. (Suppl.)* **6**, 25.

Elliott, A. M. and Clemmons, G. L. (1966). An ultrastructural study of ingestion and digestion in *Tetrahymena pyriformis*. *J. Protozool.* **13**, 311–323.

Fok, A. K., Leung, S. S., Chun, D. P. and Allen, R. D. (1985). Modulation of the digestive lysosomal system in *Paramecium caudatum*. II. Physiological effects of cytochalasin B, colchicine and trifluoperazine. *Eur. J. Cell Biol.* **37**, 27–34.

Grelland, E. M. (1988). Different growth phenotypes of *Tetrahymena-vorax* V-2 microstomes. I. Changed control of cell-cycle passage. *Eur. J. Protistol*. **23**, 309–316.

Gulliksen, O. M., Løvlie, A. M. and Kvamme, L. (1984). Different polypeptides in two homologous cellular structures: The microstomal and the macrostomal oral apparatus of *Tetrahymena vorax*. *Dev. Biol.* **103**, 511–516.

Hauser, M., Hausmann, K. and Jockusch, M. (1980). Demonstration of tubulin, actin and α-actinin by immunofluorescence in the microtubule–microfilament complex of the cytopharyngeal basket of the ciliate *Pseudomicrothorax dupius*. *Exp. Cell Res.* **125**, 265–274.

Hawkins, S. E. (1975). The regeneration of cilia in *Tetrahymena*. In *Laboratory Manual of Cell Biology* (ed. D. O. Hall and S. E. Hawkins), pp. 124–126. London: The English Universities Press.

Leakey, R. J. G., Wills, S. A. and Murray, A. W. A. (1994). Can cytochalasin B be used as an inhibitor of feeding in grazing experiments on ciliates? *Eur. J. Protistol*. **30**, 309–315.

Muller, M., Rohlich, P. and Toro, I. (1965). *Tetrahymena pyriformis* mechanical trigger the phagocytosis. *J. Protozool*. **12**, 27–34.

Naitoh, Y. and Eckert, R. (1969). Ionic mechanisms controlling behavioural responses in *Paramecium* to mechanical stimulation. *Science* **164**, 963–965.

Nilsson, J. R. (1972). Further studies on vacuole formation in *Tetrahymena pyriformis* GL. *C.R. Trav. Lab. Carlsberg* **39**, 83–110.

Nilsson, J. R. (1974). Effects of DMSO on vacuole formation, contractile vacuole function and nuclear division in *Tetrahymena pyriformis* GL. *J. Cell Sci.* **16**, 39–47.

Nilsson, J. R. (1976). Physiological and structural studies on *Tetrahymena pyriformis* GL. *C.R. Trav. Lab. Carlsberg* **40**, 215–355.

Nilsson, J. R. (1979). Phagotrophy in *Tetrahymena*. In *Biochemistry and Physiology of Protozoa* (ed. M. Levandowsky and S. H. Hunter), pp. 339–379. New York: Academic Press.

Nilsson, J. R., Ricketts, T. R. and Zeuthen, E. (1973). Effects of cytochalasin B on cell division and vacuole formation in *Tetrahymena pyriformis* GL. *Exp. Cell Res.* **79**, 456–459.

Nilsson, J. R. and Williams, N. E. (1966). An electron microscope study of the oral apparatus of *Tetrahymena pyriformis*. *C.R. Trav. Lab. Carlsberg* **35**, 119–141.

Ninomiya, N., Hazeki, K., Fukui, Y., Seya, T., Okada, T., Hazeki, O. and Ui, M. (1994). Involvement of phosphatidylinositol 3-kinase in FC-gamma receptor signaling. *J. Biol. Chem.* **269**, 22732–22737.

Plesner, P., Rasmussen, L. and Zeuthen, E. (1964). Techniques used in the study of synchronous *Tetrahymena*. In *Synchrony in Cell Division and Growth* (ed. E. Zeuthen), pp. 544–563. New York: Interscience.

Rannestad, J. (1974). The regeneration of cilia in partially deciliated *Tetrahymena*. *J. Cell Biol.* **63**, 1009–1017.

Schroeder, C. C., Fok, A. K. and Allen, R. D. (1990). Vesicle transport along the microtubular ribbons and isolation of cytoplasmic dynein from *Paramecium*. *J. Cell Biol.* **111**, 2553–2562.

Sherman, G. B., Buhse, H. E., Jr and Smith, H. E. (1982). Physiological studies on the cytopharyngeal pouch, a prey receptacle in the carnivorous macrostomal form of *Tetrahymena vorax*. *J. Protozool*. **29**, 360–365.

Silverstein, S. C., Greenberg, S., Di Virgilo, F. and Steinberg, T. H. (1989). In *Fundamental Immunology* (ed. W. E. Paul), pp. 703–719. New York: Raven Press.

Smith, H. E. (1982a). Oral apparatus structure in the microstomal form of *Tetrahymena vorax*. *Trans. Am. Microsc. Soc.* **101**, 36–58.

Smith, H. E. (1982b). Oral apparatus structure in the carnivorous macrostomal form of *Tetrahymena vorax*. *J. Protozool.* **29**, 616–627.

Smith-Somerville, H. E., Hardman, J. K., Timkovich, R., Ray, W. J., Rose, K. E., Ryals, P. E., Gibbons, S. H. and Buhse, H. E., Jr (2000). A complex of iron and nucleic acid catabolites is a signal that trigger differentiation in a freshwater protozoan. *Proc. Natl. Acad. Sci. USA* **97**, 7325–7330.

Stelly, N., Mauger, J. P., Claret, M. and Adoutte, A. (1991). Cortical alveoli of *Paramecium* – A vast submembranous calcium storage compartment. *J. Cell Biol*. **113**, 103–112.

Swanson, J. A. (1989). Phorbol esters stimulate macropinocytosis and solute flow through macrophages. *J. Cell Sci*. **94**, 135–142.

Takamasa, T., Ohnishi, K., Kobayashi, T., Takagi, T., Konishi, K. and Watanabe, Y. (1989). Cloning and seqencing of the gene for *Tetrahymena* calcium-binding 25-kDa protein (TCBP-25). *J. Biol. Chem*. **264**, 19293–19301.

Takamasa, T., Takagi, T., Kobayashi, T., Konishi, K. and Watanabe, Y. (1990). The 3rd calmodulin family protein in *Tetrahymena* – Cloning of the cDNA for *Tetrahymena* calcium-binding protein of 23 kDa (TCBP-23). *J. Biol. Chem*. **265**, 2514–2517.

Wessells, N. K., Spooner, B. S., Ash, J. F., Bradley, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. T. and Yamaa, K. (1971). Microfilaments in cellular and developmental processes. *Science* **171**, 135–143.

Williams, N. E. (1961). Polymorphism in *Tetrahymena vorax*. *J. Protozool*. **8**, 403–410.