The Journal of Experimental Biology 214, 2258-2266

doi:10.1242/jeb.055897

RESEARCH ARTICLE

Microstome–macrostome transformation in the polymorphic ciliate *Tetrahymena vorax* leads to mechanosensitivity associated with prey-capture behaviour

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Accepted 25 March 2011

SUMMARY

Ciliates feed by phagocytosis. Some ciliate species, such as *Tetrahymena vorax*, are polymorphic, a strategy that provides more flexible food utilization. Cells of the microstomal morph of *T. vorax* feed on bacteria, organic particles and organic solutes in a non-selective manner, whereas macrostome cells are predators that consume specific prey ciliates. In the present study, we investigated a possible correlation between phagocytosis and mechanosensitivity in macrostome *T. vorax*. Microstome cells seem to be insensitive to mechanical stimulation whereas macrostome cells depolarise in response to mechanical stimulation of the anterior part of the cell. The amplitude of the receptor potential induced by either a prey ciliate or a 5 μm push by a glass needle was sufficient to elicit a regenerative Ca^{2+} spike. The difference in mechanosensitivity of the two forms correlates with the swimming behaviour when hitting an obstacle; microstome cells swim alongside the obstacle whereas macrostome cells swim backwards, turn and resume forward swimming. Macrostome cells prevented from backward swimming and the subsequent turn failed to capture prey cells in their pouch. Macrostome cells consumed heterospecific prey ciliates preferentially over conspecific microstome cells. This selectivity is not due to electrical membrane responses elicited by physical contact. Both microstome and macrostome cells accumulated in an area containing putative substances released from heterospecific prey ciliates, but the substances did not elicit any electrophysiological membrane responses. We conclude that the mechanosensitivity of macrostome cells is associated with the prey-capture behaviour, whereas the selective phagocytosis is probably due to chemo-attraction to heterospecific prey ciliates.

Key words: *Tetrahymena vorax*, polymorphism, microstome, macrostome, mechanical stimulation, mechanosensitivity, phagocytosis, electrophysiology, chemosensitivity, chemo-accumulation, ciliate, *Tetrahymena thermophila*.

INTRODUCTION

Unicellular organisms such as ciliates employ a variety of swimming strategies in response to the numerous challenges in their aqueous environment (Jennings, 1906). One of these challenges is competition for limited food resources. Several ciliate species have developed polymorphism, which provides more flexibility regarding food utilization (Kidder et al., 1940; Corliss, 1953; Corliss, 1957; Williams, 1961; Williams et al., 1984; Washburn et al., 1988; Kopp and Tollrian, 2003). The ciliate *Tetrahymena vorax* is polymorphic and exists as two alternative mobile morphs – microstomal and macrostomal – in addition to a cyst form (Williams, 1961). Recently, a third distinct morph, tailed microstomal, was described in *T. vorax* populations kept in media containing bacteria (Banerji and Morin, 2009). This morph is absent in axenic solutions (Kidder et al., 1940). Cells from each of the three mobile morphs can transform into any of the others (Banerji and Morin, 2009). Microstome cells feed on bacteria, organic particles and dissolved organic compounds, whereas macrostome cells are predators that consume other ciliates.

When a microstome cell transforms into a macrostome cell, its small oral apparatus is resorbed and replaced with a larger and differently shaped structure termed a pouch (Buhse, 1966; Smith, 1982a; Smith, 1982b). The cue that triggers the transformation in *T. vorax* is released by potential prey ciliates, such as *Tetrahymena thermophila* and *Colpidium striatum*. The cue is a mixture termed stomatin, which contains numerous components including Fe^{2+} and nucleic acid catabolites (Smith-Somerville et al., 2000; Arauz et al., 2009). Receptors for inducing the transformation have not yet been indentified, but a rapid increase in cytosolic Ca^{2+} concentration is an important step in the transmembrane signalling cascade (Ryals et al., 1999; Ryals et al., 2008).

Microstome cells consume bacteria and organic particles in a rather non-selective manner (Kidder et al., 1940; Grønlien et al., 2002), in which food particles are swept into the oral apparatus by beating of oral ciliary membranelles (Smith, 1982b). Involvement of ligand-receptor interactions in the subsequent unselective phagocytosis is unknown, and it has been postulated that mechanical stimulation by the particles triggers the phagocytosis in filter feeders such as microstome *T. vorax* and *Paramecium* spp. (Allen and Fok, 2000; Grønlien et al., 2002). In contrast to microstome cells, phagocytosis in macrostome *T. vorax* cells is highly selective, i.e. other ciliate species that are competitors for bacteria and organic particles are preferentially selected compared to conspecific cells (Grønlien et al., 2002; Banerji and Morin, 2009).

Ruptured bodies of prey ciliates failed to induce phagocytosis in macrostome *T. vorax* cells (Kidder et al., 1940), indicating that mechanical stimulation by the prey does play a role in phagosome formation. However, such mechanical stimulation alone is not

sufficient to trigger phagosome formation in macrostome cells (Grønlien et al., 2002).

Several species of ciliates display mechanoreceptor potentials. In *Paramecium* spp. (Naitoh and Eckert, 1969; Eckert et al., 1972), *T. thermophila* (Takahashi et al., 1980), *Stentor coeruleus* (Wood, 1982) and *Stylonychia* spp. (dePeyer and Machemer, 1978), mechanical stimulation of the anterior part of the cell opens mechanosensitive Ca^{2+} channels in the somatic membrane and generates a depolarising receptor potential. In *Paramecium*, the somatic depolarisation opens voltage-dependent Ca^{2+} channels in the cilia, leading to inward Ca^{2+} current and regenerative Ca^{2+} responses (Eckert et al., 1972; Ogura and Takahashi, 1976; Machemer and Ogura, 1979). An increase in the intraciliary $[Ca^{2+}]$ to more than 10^{-6} mol l^{-1} induces a reversal of the ciliary beat and backward swimming (Naitoh and Kaneko, 1972; Naitoh et al., 1972; Naitoh and Kaneko, 1973; Iwadate and Nakaoka, 2008). As intraciliary Ca^{2+} is sequestered and a normal cytosolic Ca^{2+} level is restored (reviewed in Plattner and Klauke, 2001; Plattner et al., 2006; Sehring et al., 2009), the cell will resume forward swimming in a different direction. In this manner, obstacles that the cells bump into are avoided (Naitoh, 1974).

Membrane responses to mechanical stimulation have not been previously explored in macrostome *T. vorax* cells. In the present study, we investigated mechanoreceptor potentials in microstome and macrostome cells of *T. vorax*. Cells of these morphs display strikingly different responses to mechanical stimulation, and we suggest a possible correlation between mechanosensitivity and phagocytosis in macrostome *T. vorax* cells.

MATERIALS AND METHODS

Cells

We studied behavioural and electrophysiological responses in polymorphous *Tetrahymena vorax* Kidder 1941, strain V_2S. For induction of the transformation between the microstome and the macrostome form, and as prey species for the macrostome form, we used *Tetrahymena thermophila*, Nanney and McCoy 1952 (Buhse, 1967; Smith-Somerville et al., 2000). Both strains were obtained from the American Type Culture Collection. The two species were kept in separate cultures at 19°C in standard axenic culture medium (Plesner et al., 1964), which is essentially a balanced salt solution with nutrients in the form of 2 g l^{-1} yeast extract and 20 g l^{-1} proteose peptone (protein hydrolysate from meat) (Sigma-Aldrich, St Louis, MO, USA). The experiments were performed on cells in the mid-logarithmic phase of exponential growth. The cell density was then approximately 5×10^5 cells ml^{-1} for a *T. vorax* culture and 8×10^5 cells ml^{-1} for a *T. thermophila* culture.

In a *T. vorax* strain V_2S culture grown in axenic standard conditions, the microstomal morph predominates and only approximately 10% of the cells are macrostomal. As noted, *T. vorax* cells do not transform into tailed microstome cells in axenic solutions (Kidder et al., 1940). In the present study, infected cultures with tailed microstome cells were discarded. The sizes of the microstome and macrostome cells were approximately 60×20 and 100×70 μm, respectively. The size of the more spherical *T. thermophila* cells was approximately 35×25 μm.

Transformation of microstome cells into macrostome cells

Transformation of microstome *T. vorax* cells into macrostome cells was induced by adding a sample of *T. vorax* culture to a *T. thermophila* culture at a ratio of 1:100 (v/v). The first noticeable increase in the number of macrostome cells then appeared after approximately 5 h (at 23°C), and macrostome cells continued to be formed for the next 72 h. In the experiments, we used *T. vorax* cells that had been in the mixed culture for approximately 72 h. At this time, approximately 100% of the *T. vorax* cells were macrostomal and the culture was devoid of *T. thermophila* cells. The first division of a macrostome cell into microstome daughter cells occurred after approximately 90 h.

Experimental solutions

The registration solution contained (final concentrations in mmol l^{-1}): 1 NaCl, 1 KCl, 1 $CaCl_2$, 1 Tris/HCl (pH 7.2). The test solutions were filtrates from *T. thermophila* and microstome *T. vorax* cultures in which the ordinary growth medium was replaced with registration solution. To obtain the test solutions, 10 ml of the cell cultures were gently centrifuged at approximately 10 ***g*** for 1 min to form a loose pellet. The cells were then suspended in 10 ml registration solution and washed twice by repeating the same procedure. Finally, the cells were incubated in 5 ml registration solution for 3 h at room temperature. The suspension was then centrifuged at approximately 1000 ***g*** for 15 min to pellet the cells, and the supernatant was filtrated through a 0.45 μm pore diameter filter. The filtrate from the *T. thermophila* culture was confirmed to induce transformation of microstome to macrostome *T. vorax* cells before experimental application.

Prior to the experiments, cells in standard culture medium (2 ml) were gently centrifuged at approximately 10 ***g*** for 30 s to form a loose pellet. The cells were then suspended in the registration solution and washed twice by repeating the same procedure. The cells were finally suspended in 2 ml of the registration solution. Prior to the recordings, the cells were kept in the registration solution for 1 h. The experiments were performed at room temperature (21–23°C).

Electrophysiological recordings

Current clamp recordings were performed at room temperature on the stage of an inverted microscope. The microelectrode was positioned with a hydraulic micromanipulator (MO-103, Narishige Scientific Instrument Lab, Tokyo, Japan). During the recordings, the microstome and macrostome cells were kept stationary by suction micropipette with tip diameters of 15 and 30 μm, respectively. The pipette was positioned with a motorized micromanipulator (MS 314, Märzhäuser Wetzlar GmbH and Co., Wetzlar, Germany) and connected to ambient, subatmospheric or superatmospheric pressure *via* a solenoid valve (Jonsson and Sand, 1987). A selected cell was sucked onto the tip of the pipette by activating the solenoid valve using a manual trigger. The subatmospheric catching pressure was between –1.0 and –1.5 kPa, whereas the holding pressure during the recordings was reduced to –0.5 kPa. The pipette was preferentially placed midway along the longitudinal axis of the cell in order to minimize mechanical excitation. The coating of the cells tended to clog the pipette when a trapped cell was released. Between each catch, the pipette was therefore cleaned by a short flush of registration solution through the tip by connecting the pipette to a superatmospheric pressure of approximately 5 kPa.

The electrical membrane properties were studied using conventional microelectrodes and standard equipment for current clamp recordings. The electrodes were filled with 4 mol l^{-1} potassium acetate adjusted to pH 7.2 with acetic acid, and the electrode resistance was 30–60 MΩ. Electrode penetration of the cell was facilitated by passing a short pulse of high-frequency oscillating current through the electrode. The analogue signals from the current clamp amplifier were fed into an A/D converter (Digidata 1320A, Axon Instruments,

Union City, CA, USA) connected to a PC. The PClamp (Axon Instruments, Union City, CA, USA) software package was used for data collection and storage, to generate command signals for current injection, and for data processing and analysis.

Stimulation

Mechanoreceptor potentials were recorded from immobilized cells that were mechanically stimulated by a glass needle with a polished, blunt tip with a diameter of 10 µm. The tip of the glass needle was positioned less than 2 µm from the cell membrane on the anterior part, posterior part and along the lateral part of the cell (Fig. 1). The glass needle was operated by a motorized micromanipulator (Merthaüser MS 314), and the stimulus was a rapid advancement of the needle in a single step. The step amplitude was approximately 7 µm and the glass needle was withdrawn after approximately 1 s.

Electrophysiological responses during physical contact between a macrostome cell and a possible prey organism were recorded when the prey cell (*T. thermophila* cell, microstome cell and macrostome cell) randomly touched the anterior part of the immobilized macrostome cell.

Electrophysiological responses to filtrates from *T. thermophila* and *T. vorax* cultures were recorded by ejecting the test solutions onto an immobilized cell through a glass micropipette with tip diameter of approximately 10 µm. The tip of the pipette was positioned approximately 75 µm from the cell using a micromanipulator. The cell was flushed with test solution by engaging a solenoid valve, which connected the pipette to a superatmospheric pressure of 1–2 kPa. Control experiments were performed by using this procedure with pure registration solution in the pipette.

Behavioural recordings

Possible chemo-accumulation responses to the test solutions were assessed by recording the time-dependent accumulation of cells close to a point source of test solution, as previously described (Grønlien et al., 2010). A total of 60 washed cells, either macrostome cells or microstome cells, were placed in a circular cell chamber with a volume of 230 µl and a surface area of 95 mm^2. The chamber contained 200 µl registration solution and was topped with paraffin oil to prevent evaporation of water. The experiments were performed on the stage of an inverted microscope fitted with a video camera connected to a video recorder. The behaviour of the cells was recorded at 25 frames s^{-1}.

A glass micropipette with a tip diameter of approximately 25 µm was filled with the test solution, and the tip of the pipette was positioned centrally in the chamber, approximately 50 µm from the bottom. The level of solution within the pipette was kept 5–10 mm above the level that would result from capillary suction, thus ensuring a constant leakage of solution out of the pipette. In the area surrounding the pipette tip, the concentration of the attractant was considerably less than in the test solution, and declined with distance to the pipette. During a period of 15 min, approximately 8 µl of test solution leaked into the cell chamber. In order to quantify the chemo-accumulating potency of the test solution, the number of cells within a radius of 0.95 mm from the tip of the leakage pipette, i.e. approximately 3% of the surface area of the cell chamber, was counted every 10 s during the 14th minute after placing the pipette in the cell chamber. As a relative measure of chemo-accumulation, the mean number of counted cells during this 1 min period was expressed as the percentage of the total number of cells in the chamber. Leakage of pure registration solution was used as a control. For each test solution, three independent experiments were conducted.

In the present study, we measured changes in the swimming pattern, i.e. swimming speed and frequency of turns, by manual tracking of cells displayed on a screen at a magnification of 126×. A change in direction of more than 90 deg was defined as a turn. A directional change of a multiple of this angle was still categorized as one turn if the turn was smooth and uninterrupted. The swimming paths between turns were relatively straight, and the speed between turns was approximately constant. Thus, the cells resumed full speed immediately after turning, with periods of acceleration too short to be resolved by our video system. The duration of tracks between turns was at least 0.5 s and the horizontal position of a cell was determined with an accuracy of approximately 6 µm, giving a resolution of the measurements of mean swimming speed better than 12 µm s^{-1}.

In order to determine the sustained swimming behaviour of chemo-accumulated cells, recordings from the 14th minute after the start of the experiments were replayed frame by frame. Individual cells were tracked for a period of 1–5 s, during which the frequency of turns and the swimming speed between turns were determined. If a cell collided with an obstacle or another cell, only data prior to the collision were included. For each test solution, the experiment was conducted three times, and 10 of the aggregated cells were tracked in each experiment. The cells to be tracked were chosen from a still picture prior to tracking, attempting to achieve an even spatial distribution of the selected cells. In the control recordings of dispersed cells prior to exposure to the attractant, all the cells in the observation area were tracked.

Statistics

The significance of the results was tested using a Student's *t*-test ($P<0.05$). Values are presented as means ± s.d.

RESULTS

Microstome–macrostome transformation induces mechanosensitivity in *T. vorax*

Fig. 1Ai–iii presents representative recordings of the membrane potential in microstome *T. vorax* cells stimulated by touching the cell with a glass needle at various positions along the cell. Surprisingly, microstome *T. vorax* cells did not display any electrophysiological responses to mechanical stimulation of any part of the cell. All six microstome cells included in this series of experiments exhibited a similar lack of responses.

Fig. 1Bi shows a typical recoding of the membrane potential of a macrostome cell during similar mechanical stimulation of the anterior part of the cell. In contrast to the microstome cells, the macrostome cell displayed a pronounced depolarisation in response to a touch by the glass needle. As in other ciliates, moderate depolarisations may elicit regenerative Ca^{2+} spikes in *T. vorax* cells (Connolly and Kerkut, 1981; Jansen and Sand, 1995; Bruskeland and Sand, 1999; Grønlien et al., 2001). Therefore, it is likely that the observed depolarisation is caused by an initial receptor potential that elicits a regenerative Ca^{2+} spike. These depolarizing events are merged and were not separated in the present experiments. In ciliates, voltage-gated Ca^{2+} channels are generally localized in the ciliary membrane, and this is also the case in *T. vorax* (Grønlien et al., 2001). A Ca^{2+} spike induces ciliary reversal and a brief period of backward swimming (Naitoh and Kaneko, 1972; Naitoh and Kaneko, 1973; Ogura and Takahashi, 1976). For a cell trapped on the suction pipette in the present experiments, this ciliary reversal was evident from a twisting movement of the cell. All the six macrostome cells included in this series of experiments were depolarised by mechanical stimulation of the anterior part of the cell. Measurements of the amplitude of the receptor potentials would have required blockage of the Ca^{2+} spikes.

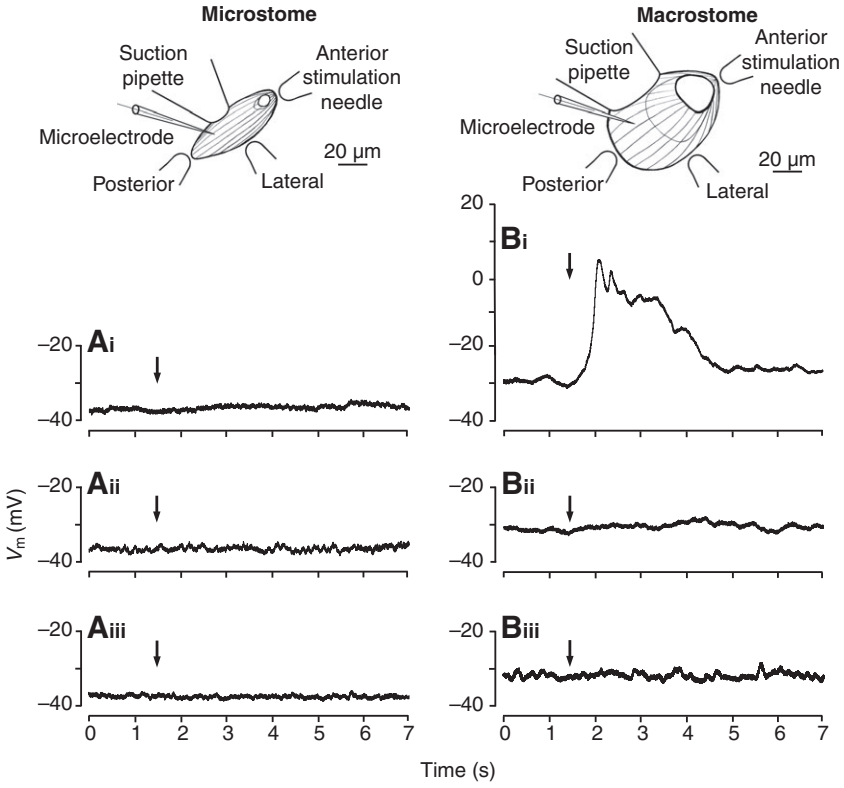

Fig. 1. Membrane potential (V_m) recordings from mechanically stimulated microstome and macrostome *Tetrahymena vorax* cells. Upper panels display the various positions of the glass needle used to stimulate the cells. The needle was initially advanced to barely touch the cell at the indicated locations. The cell was stimulated by a further 7 μm rapid advancement of the needle, followed by withdrawal of the needle after 1 s. A suction micropipette kept the cells stationary during the recordings.
(A) Recordings from microstome cells stimulated at the anterior (Ai), posterior (Aii) and lateral (Aiii) part of the cell.
(B) Recordings from macrostome cells stimulated at the anterior (Bi), posterior (Bii) and lateral (Biii) part of the cell.
Arrows indicate the start of the mechanical stimulation. Only mechanical stimulation of the anterior membrane of the macrostome cell evoked a depolarising receptor potential, which in turn triggered a regenerative Ca^{2+} spike.

However, these experiments were of a qualitative nature, and the relationship between stimulus strength and amplitude of the receptor potential was not determined. The maximum amplitude of the post-stimulus depolarisation of the tested cells, caused by both the receptor current and the subsequent current through voltage-dependent Ca^{2+} channels, was 23.0±6.8 mV (N=6). In no cases were electrophysiological responses observed during stimulation of the posterior part of the macrostome cells or along their lateral parts (Fig. 1Bii,iii).

These results demonstrate that, during transformation from the microstome to the macrostome morph, the cell acquires mechanosensitivity in its anterior part.

The difference in mechanosensitivity of the two morphs is reflected in the swimming behaviour

In free-swimming *T. vorax* cells, the difference in mechanosensitivity of the two forms is asserted in the swimming behaviour. When a microstome cell bumps into an obstacle, the cell gradually turns in a new direction and swims continuously forward alongside the obstacle (Fig. 2A). The swimming patterns of 20 microstome cells bumping into obstacles were analyzed, and all cells displayed a similar response. In contrast, a macrostome cell hitting an obstacle immediately responds by briefly swimming backwards. It then turns and resumes forward swimming in a different direction (Fig. 2B). The swimming patterns of 20 macrostome cells hitting an obstacle were analyzed, and all cells displayed a similar response.

The observed behaviour in macrostome cells is strikingly similar to the avoidance response previously described in *Paramecium* cells colliding with objects (Jennings, 1906). In *Paramecium*, this behaviour is linked to the depolarising receptor potentials and the associated Ca^{2+} spikes generated by mechanical stimulation of the anterior part of the cell (Naitoh and Eckert, 1969). Therefore, it is reasonable to suggest that the different behavioural responses in microstome and macrostome *T. vorax* cells colliding with obstacles are due to the induced mechanosensitivity in the macrostome cells.

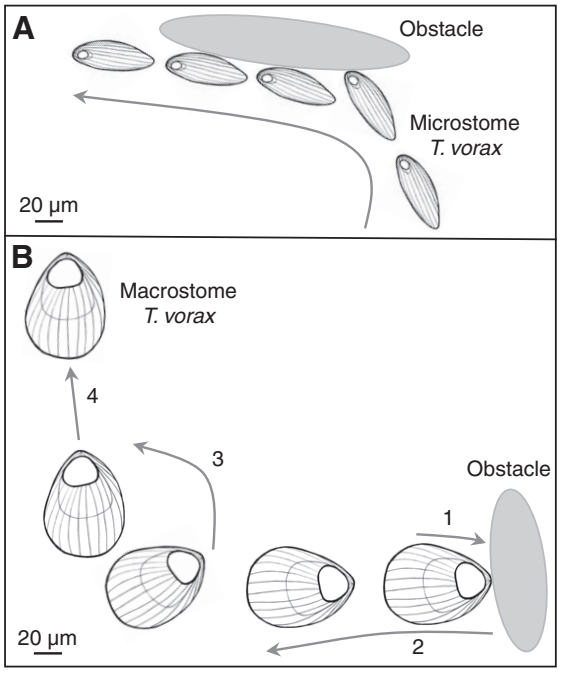

Fig. 2. Diagram of behavioural responses of a microstome (A) and a macrostome (B) *Tetrahymena vorax* cell colliding with an obstacle. The arrows show the swimming paths, and the numbers in B indicate the sequence of the swimming pattern.

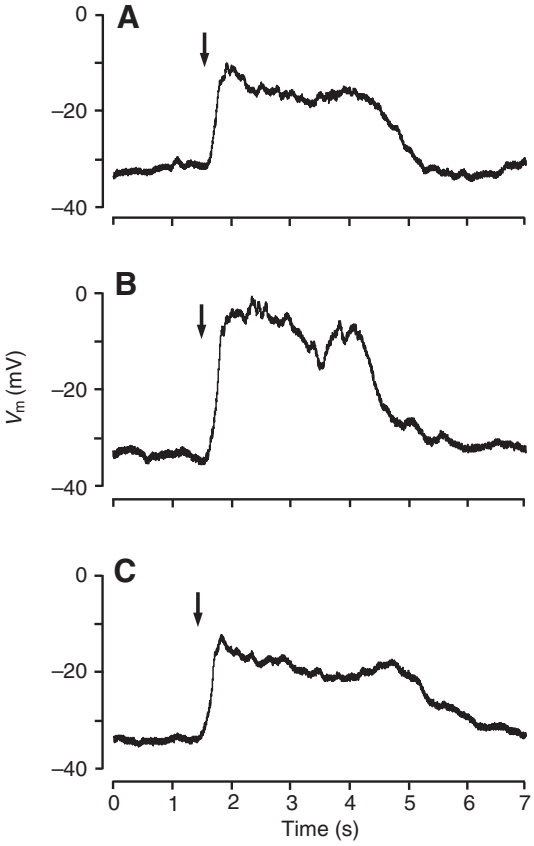

Fig. 3. Membrane responses of a macrostome *Tetrahymena vorax* cell to other cells contacting its anterior membrane. Contacts were made by (A) a *T. thermophila* cell, (B) a microstome *T. vorax* cell and (C) a macrostome *T. vorax* cell. The arrows indicate the time of initial physical contact, and the traces show the membrane potential. A suction micropipette kept the cell stationary during the recording. Note that the different cells contacting the anterior membrane evoked similar depolarisations, which reflect receptor potentials and associated regenerative Ca^{2+} spikes.

Possible relationship between mechanosensitivity and phagocytosis in macrostome cells

A previous study has shown that microstome and macrostome *T. vorax* cells use different mechanisms for capture and internalization of food, i.e. the non-specific, constitutive phagocytosis in microstome cells changes into a specific, inducible process in macrostome cells (Grønlien et al., 2002). A macrostome cell is able to internalize both *T. thermophila* cells and microstome *T. vorax* cells, but *T. thermophila* cells are preferentially selected in a mixture of both types of prey (Grønlien et al., 2002).

To test whether the food selectivity in macrostome *T. vorax* cells is due to membrane responses during physical contact between the macrostome cell and the prey, we exposed macrostome cells to *T. thermophila* cells, microstome cells and macrostome cells while the examined macrostome cell was kept stationary by a suction micropipette and penetrated by a recording electrode. Random touches by *T. thermophila*, microstome and macrostome cells to the anterior part of the tested macrostome cell were observed, and the membrane responses were monitored. None of the five macrostome cells that were kept stationary on a pipette in this series of experiments captured a prey cell in its pouch during the recordings.

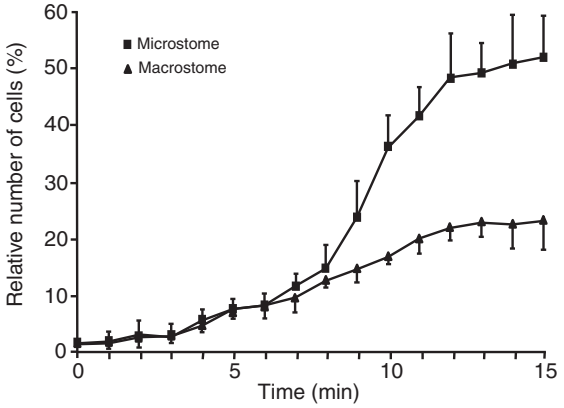

Fig. 4. Aggregation of microstome and macrostome *Tetrahymena vorax* cells close to a point source of *T. thermophila* filtrate as a function of time. The ordinate shows the relative number of cells within the observation area (3% of the chamber surface area) surrounding the tip of a leakage pipette containing a filtrate from a *T. thermophila* culture. The experiment was conducted three times and the values are presented for the microstomal morph as means + s.d. and for the macrostomal morph as means – s.d.

Fig. 3 shows representative recordings from one of the macrostome cells during physical contact between different prey cells and the anterior part of the cell. Such contact always induced a depolarisation. The mean value of the depolarisation evoked by contact with a *T. thermophila* cell was 22.4±3.6 mV (N=5), whereas the corresponding values for contact with a microstome and a macrostome *T. vorax* cell were 23.9±3.1 mV (N=5) and 24.4±3.2 mV (N=5), respectively. As previously noted, these depolarisations were probably composed of receptor potentials and associated regenerative Ca^{2+} spikes (Fig. 3). As with stimulation by a needle, the contacts induced a twisting movement of the cells trapped on the suction micropipette, presumably due to reversal of the ciliary beat caused by Ca^{2+} influx into the cilia (Ogura and Takahashi, 1976). Physical contacts at sites other than the anterior part did not induce membrane responses in the tested macrostome cells. In a parallel series of experiments, five microstome *T. vorax* cells were examined. None of these displayed any detectable electrophysiological responses upon physical contact with other cells.

These results indicate that macrostome *T. vorax* cells probably do not select *T. thermophila* cells as prey on the basis of electrophysiological responses upon physical contact. However, in all the examined macrostome cells, we observed a twisting movement of the cell when prey organisms made contact with the anterior part of the cell. These observations are in accordance with a previous study of the importance of initial backward swimming in macrostome *T. vorax* cells in order to phagocytose a prey (Grønlien et al., 2002).

The selective phagocytosis in macrostome cells is probably due to chemical cues

In order to elucidate whether the specificity of phagocytosis observed in macrostome *T. vorax* cells could be due to chemical selection prior to capture, we compared chemo-accumulating responses to filtrates from a *T. thermophila* culture and a microstome *T. vorax* culture, both in the early logarithmic growth phase.

Fig. 4 shows the time-dependent aggregation of macrostome cells close to the tip of a leakage pipette containing filtrate from a *T. thermophila* culture. The number of cells in the observation area

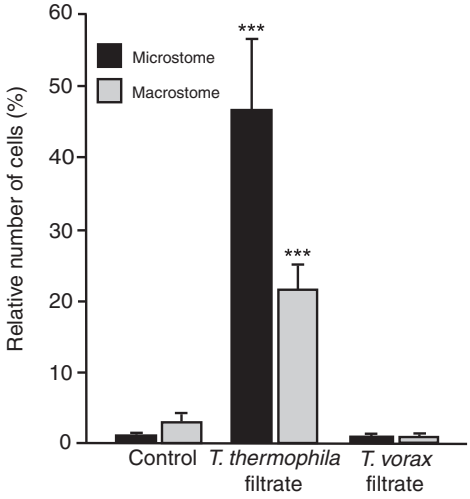

Fig. 5. Chemo-accumulating potencies of test solutions on microstome and macrostome *Tetrahymena vorax* cells. The ordinate shows the relative number of cells within the observation area (3% of the chamber surface area) during the 14th minute of exposure to a test solution leaking from a centrally positioned micropipette. The test solutions were filtrates from a *T. thermophila* culture and a *T. vorax* culture. Control values were obtained in tests with pure registration solution in the pipette (control). The experiment was conducted three times for each morph and the values are presented as means + s.d. ***, highly significant difference (P<0.0001) compared with control values.

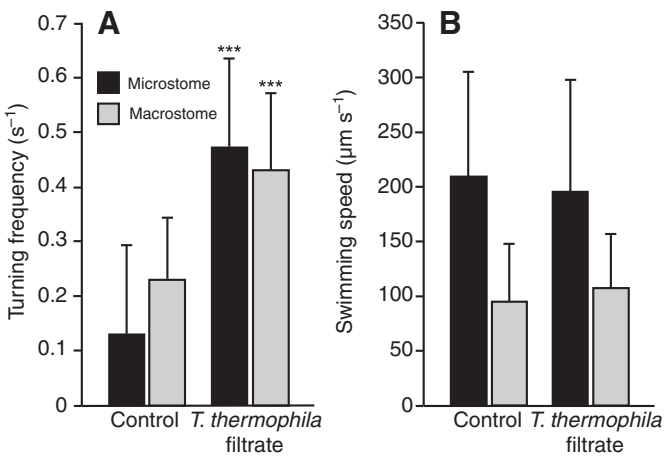

Fig. 6. Swimming behaviour of chemo-accumulated microstome and macrostome *T. vorax* cells. Turning frequency (A) and swimming speed between turns (B) were recorded in cells aggregated close to the tip of a leakage pipette containing a filtrate from a *T. thermophila* culture during the 14th minute of exposure. Control values were obtained in tests with pure registration solution in the pipette. The chamber contained 60 cells in the chemo-accumulating experiments. The experiment was performed three times for each morph and the values are presented as means + s.d. ***, highly significant difference (P<0.0001) compared with control values.

increased toward a relatively stable level during the 15 min observation period. In three parallel tests of *T. thermophila* filtrate, the mean number of macrostome cells present within the observation area (3% of the chamber surface area) during the 14th minute of the test period was 21.5±3.3% (N=3) of the total number of cells in the chamber (Fig. 5). The corresponding number in experiments where macrostome cells were stimulated by microstome *T. vorax* filtrate was 0.6±0.3% (N=3). In control experiments using leakage pipettes containing only registration solution, 2.9±1.0% (N=3) of the total number of cells in the chamber was within the observation area (Fig. 5). This result demonstrates that macrostome cells are attracted to chemicals released from or associated with *T. thermophila* cells, whereas microstome *T. vorax* filtrate had no chemo-accumulating effect on macrostome cells.

To demonstrate the swimming behaviour of macrostome cells accumulated in areas where *T. thermophila* cells are present, we studied the frequency of turns and the swimming speed between successive turns in macrostome cells stimulated by *T. thermophila* filtrate. Because the degree of chemo-accumulation was estimated from recordings during the 14th minute after start of exposure to the filtrate, the measurement of chemo-accumulating responses was restricted to the same time period. However, as no accumulation of cells was observed when testing the response to *T. vorax* filtrate or in the control experiments, a longer observation period was required to obtain data from a sufficient number of cells in these cases. Fig. 6 presents the results of the analysis of turning frequency and swimming speed for macrostome cells stimulated by either registration solution (control) or a filtrate from a *T. thermophila* culture. The turning frequency of the chemo-accumulated cells was two times higher than that of the control cells, whereas no difference was observed in swimming speed. We have previously shown that increased cell density *per se* increases the turning frequency of microstome *T. vorax* (Grønlien et al., 2010). However, this effect is probably too modest to fully account for the elevated turning frequency of macrostome cells in the present experiments. Thus, we suggest that chemo-attractants in a *T. thermophila* filtrate increase the turning frequency without affecting the swimming speed in accumulated macrostome *T. vorax* cells. Chemo-accumulation associated with increased turning frequency, but unchanged swimming speed, has also been reported in the microstome morph of *T. vorax* (Grønlien et al., 2010) and in *T. thermophila* (Lampert et al., 2011).

If a macrostome cell encounters a region where *T. thermophila* cells are present, the turning frequency increases and the macrostome cell will tend to stay in this region. This positive chemo-accumulating response to *T. thermophila* cells increases the probability that a macrostome cell will select and internalize *T. thermophila* cells.

Microstome *T. vorax* cells display chemo-accumulation to *T. thermophila* filtrate

Microstome *T. vorax* cells do not engulf *T. thermophila* cells. However, they transform to macrostome cells in the presence of *T. thermophila* cells. Therefore, it was interesting to elucidate whether the chemo-attraction to *T. thermophila* cells seen in macrostome cells is a feature in microstome cells. Therefore, we performed similar tests as those described in the previous section also for microstome *T. vorax* cells, and Fig. 4 also shows the time-dependent aggregation of microstome cells close to the tip of a leakage pipette containing filtrate from a *T. thermophila* culture. The number of cells in the observation area increased to a relatively stable level during the 15 min exposure period. In the three parallel tests of *T. thermophila* filtrate, the mean number of microstome cells present within the observation area (3% of the chamber surface area) during the 14th minute of the test period was 46.6±10.0% (N=3) of the total number of cells in the chamber (Fig. 5). The control value,

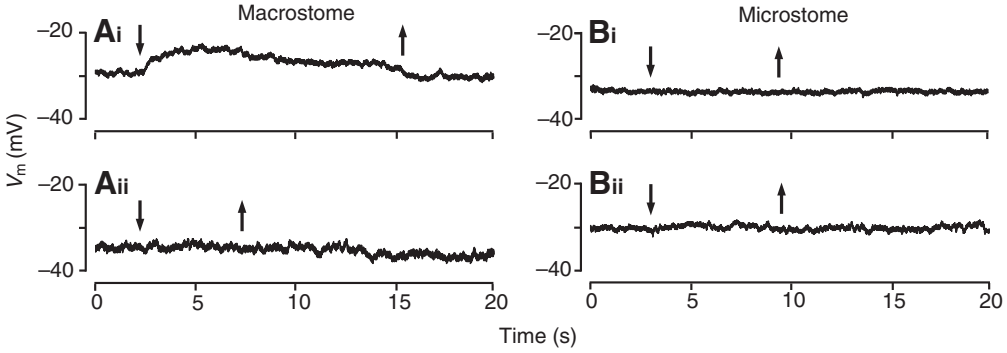

Fig. 7. Lack of membrane response of *Tetrahymena vorax* cells stimulated by *T. thermophila* filtrate. (Ai) Control recording from a macrostome cell stimulated by ejection of registration solution at the anterior part of the cell. Note the depolarising response, which may be due to mechanostimulation caused by the ejection. (Aii) Lack of membrane response to ejection of *T. thermophila* filtrate at the lateral part of the macrostome cell. Diffusion and convection movements of compounds in the filtrate would subsequently cause chemo-stimulation of the anterior part of the cell. In microstome cells, the membrane potential was unchanged during both ejection of registration solution at the anterior part of the cell (Bi) and ejection of *T. thermophila* filtrate at the lateral part of the cell (Bii). Downward arrows indicate the start of ejection of test solution onto the recorded cell, and upward arrows indicate termination of the ejection. The traces show the membrane potential. A suction micropipette kept the cells stationary during the recording.

using leakage pipettes with registration solution, was 0.7±0.2% (N=3). Thus, microstome *T. vorax* cells displayed even stronger attraction to *T. thermophila* filtrate than did the macrostome cells. However, a filtrate from a microstome *T. vorax* culture had no chemo-accumulating effect on microstome or macrostome cells (Fig. 5). As was also the case for macrostome *T. vorax* cells, microstome cells that were accumulated in a region with *T. thermophila* filtrate displayed increased turning frequency and unchanged swimming speed (Fig. 6).

Measured by our chemo-accumulation assay, the maximal chemo-accumulation to *T. thermophila* filtrate was approximately twice as high in microstome cells as in macrostome cells. There were also some differences in swimming behaviour in the two morphs. In normal registration solution, the microstome cells had a lower turning frequency (0.13±0.16 s^{-1}, N=30) and a higher swimming speed (215±95 μm s^{-1}, N=30) than macrostome cells (0.23±0.11 s^{-1} and 95±55 μm s^{-1}, N=30; Fig. 6).

These results demonstrate that chemo-attraction to *T. thermophila* cells is a feature of both morphs of *T. vorax*, and indicate that macrostome cells take advantage of this feature by seeking out and selecting *T. thermophila* cells in preference to cannibalistic capture of microstome *T. vorax* cells.

Chemo-attractants in *T. thermophila* filtrate do not alter the membrane potential of *T. vorax* cells

Compounds released from *T. thermophila* cells are attractants to both microstome and macrostome *T. vorax* cells. In microstome *T. vorax* cells, even a strong attractant like L-cysteine does not alter the membrane potential (Grønlien et al., 2010). However, chemo-attractants hyperpolarise *Paramecium* cells (Van Houten, 1979). Because macrostome *T. vorax* cells resemble *Paramecium* in swimming behaviour, we investigated whether compounds released from *T. thermophila* cells affect the membrane potential of macrostome cells. This was studied by pressure ejection of *T. thermophila* filtrate onto current-clamped cells kept stationary by a suction micropipette. Fig. 7A shows representative recordings from this series of experiments. Control ejections of registration solution aimed at the anterior membrane elicited modest depolarisation (Fig. 7Ai), which might be a mechanoreceptor potential induced by the fluid flow during ejection. Possible chemoreceptor potentials evoked by ejection of *T. thermophila* filtrate onto the anterior membrane might thus be camouflaged by mechanoreceptor potentials. Ejection of registration solution onto the lateral parts of the cell caused no change in membrane potential. Therefore, ejection of *T. thermophila* filtrate was restricted to the lateral parts of the cell, and stimulation of the anterior part was then based on diffusion and convection movements of the attractants. The membrane potential did not change in response to such ejection of *T. thermophila* filtrate in any of the five tested macrostome *T. vorax* cells (Fig. 7Aii). Similar tests were also performed on five microstome *T. vorax* cells, and none of these displayed any detectable membrane response to ejection of *T. thermophila* filtrate (Fig. 7Bii).

These results indicate that the chemo-attractants in the *T. thermophila* filtrate do not evoke changes in the membrane potential of either macrostome or microstome *T. vorax* cells, which suggests that the membrane potential does not regulate chemo-accumulation in *T. vorax*.

DISCUSSION

Mechanosensitivity in *T. vorax* cells

The present data show that microstome and macrostome *T. vorax* cells employ different swimming strategies in response to challenges in their aqueous environment. For example, when microstome cells encounter an obstacle in their swimming path, they continue their forward swimming and thus tend to swim alongside the obstacle (Fig. 2A). In contrast to microstome cells, macrostome cells colliding with an obstacle immediately respond by brief backward swimming, a pronounced turn and resumed forward swimming in a different direction (Fig. 2B). This swimming behaviour is strikingly similar to the avoidance reaction seen in *Paramecium* (Jennings, 1906).

The differences in swimming behaviour between microstome and macrostome cells are reflected in the electrophysiological membrane responses to mechanical stimulation (Fig. 1). In microstome *T. vorax* cells, there were no detectable changes in membrane potential during mechanical stimulation of any part of the cell (Fig. 1A). In contrast, macrostome *T. vorax* cells displayed a pronounced depolarisation in response to mechanical stimulation of the anterior membrane (Fig. 1Bi). The depolarising receptor potential elicited a regenerative Ca^{2+} spike (Fig. 1Bi), which in macrostome cells most likely induced ciliary reversal visualized as a twist of the cell on the pipette. In several species of ciliates, ciliary reversal is induced by Ca^{2+} influx through voltage-sensitive Ca^{2+} channels in the cilia (Naitoh and Kaneko, 1972; Naitoh et al., 1972; Onimaru et al., 1980; Wood,

1982; Grønlien et al., 2001). In *Paramecium*, the mechanoreceptors are located in the soma membrane, and maximal depolarising receptor potential is elicited by stimulation of the anterior end of the cell (Ogura and Machemer, 1980). When the posterior end is mechanically stimulated, *Paramecium* cells display hyperpolarising receptor potentials (Naitoh and Eckert, 1969). However, in macrostome *T. vorax* cells, the mechanosensitivity is restricted to the anterior membrane, as no electrophysiological responses were observed during stimulation by other parts of the cell (Fig. 1Bii,iii).

Previously, both depolarising and hyperpolarising receptor potentials in response to mechanical stimulation of microstome *T. vorax* cells have been reported (Connolly and Kerkut, 1981). However, these membrane responses were not consistent and no strict regional separation between hyperpolarising and depolarising responses were observed, in contrast to the situation in *Paramecium* (Naitoh and Eckert, 1969; Onimaru et al., 1980). In our studies on the electrophysiological properties of microstome *T. vorax* cells (Jansen and Sand, 1995; Bruskeland and Sand, 1999; Grønlien et al., 2001), we found that the membrane resistance of microstome *T. vorax* was approximately 350 MΩ, whereas Connolly and Kerkut reported values of approximately 44 MΩ (Connolly and Kerkut, 1981). This very low value indicates that the cells were rather damaged by the electrode penetrations. Thus, a possible interpretation of their inconsistent 'receptor potentials' might be that push on the penetrated cell affected the poor recording conditions, i.e. the degree of shunting by the damaged membrane around the electrode. When studying the swimming behaviour of intact microstome *T. vorax* cells, Connolly and Kerkut (Connolly and Kerkut, 1981) reported lack of avoidance responses to mechanical contact, in contrast to *Paramecium*, which is in agreement with our observations (Fig. 2A).

Despite the lack of mechanoreceptor potentials in microstome *T. vorax* cells, we cannot exclude that the swimming behaviour upon bumping into an obstacle is a consequence of mechanically induced changes in ciliary motility. However, the cell also tended to follow the outline of the obstacle simply by continued forward swimming. Deformation of the cytoskeleton beneath the cell membrane may trigger intracellular signalling (Asparuhova et al., 2009), but little is known about the signal transduction related to mechanosensitivity in ciliates. However, in the ciliate *Stentor coeruleus*, G-proteins modulate the sensitivity to mechanical stimuli (Marino and Wood, 1993; Marino et al., 2001), and the actin cytoskeleton is involved in mechanical stimulus transduction (Wood and Kilderry, 1997). It is possible that mechanically induced intracellular signalling in microstome cells may occur without changing the membrane potential, but this topic needs to be explored in future studies.

Phagocytosis in *T. vorax* cells

In order to accomplish phagocytosis, the macrostome *T. vorax* cells need to capture a prey cell in the anterior pouch, and the phagocytosis is always accompanied by a brief backward swimming followed by rotation (Grønlien et al., 2002). In microstome *T. vorax* cells, food particles are swept into the oral apparatus by beating of oral ciliary membranelles (Smith, 1982b), and no particular change in swimming behaviour during phagocytosis is observed (Grønlien et al., 2002). In contrast, the beating of the membranelles is probably insufficient for a macrostome cell to capture the prey cell. In this context, it is noteworthy that macrostome cells restrained from swimming by a suction micropipette were unable to capture a prey cell in the pouch, even when it was in physical contact with the prey cell. The prey cells evoked depolarising receptor potentials and subsequent Ca^{2+} spikes in the macrostome cell upon physical contact with the anterior end of the cell (Fig. 3). The stimulation induced a twisting movement of the macrostome cell attached to the pipette, presumably due to ciliary reversal induced by the Ca^{2+} influx linked to the Ca^{2+} spikes. However, these observations are not definite proof of a causal link between phagocytosis of macrostome *T. vorax* cells and backward swimming and rotation.

In macrostome *T. vorax*, Ca^{2+} also plays a role in the formation of a closed vacuole by initiating the pouch closure (Sherman et al., 1982). However, Ca^{2+} influx through voltage-sensitive channels in the cilia as a consequence of mechanical stimulation is not an exclusive trigger signal for phagosome formation, as captured latex beads are not internalized in macrostome *T. vorax* cells (Grønlien et al., 2002). Ligand-receptor-mediated phagocytosis is also insufficient to explain the internalization of prey cells in macrostome cells, because dead or ruptured prey cells fail to form a phagosome (Kidder et al., 1940; Grønlien et al., 2002). Therefore, we conclude that in the carnivorous macrostome *T. vorax*, the induced mechanosensitivity may facilitate the initial capturing of the prey cell, whereas the internalization of the prey is probably triggered by a combination of mechanical and chemical stimulation by the prey captured in the pouch.

Carnivorous macrostome *T. vorax* cells preferentially select *T. thermophila* cells over other prey cells (Grønlien et al., 2002). This selection is probably not based on electrophysiological responses upon physical contact between prey cell and macrostome cell, as the depolarising receptor potentials induced by different prey cells were apparently identical (Fig. 3). However, the macrostome cells did accumulate in an area where a *T. thermophila* filtrate was present, but were not attracted to a filtrate from a microstome *T. vorax* culture (Fig. 5). Therefore, macrostome cells recognize specific substances released from *T. thermophila* cells. This kairomone could be the complex mixture, i.e. stomatin that induces transformation in *T. vorax* (Williams, 1961; Buhse, 1966; Smith-Somerville et al., 2000; Arauz et al., 2009). Chemo-attraction to potential prey cells seems to be the main mechanism for food selection also in the carnivorous protozoa *Didinum* and *Coleps* (Seravin and Orlovskaja, 1977).

Macrostome *T. vorax* cells stayed accumulated close to a point source of a filtrate from *T. thermophila* by increasing their turning frequency without changing their swimming speed (Fig. 6). This observation is in accordance with a previous report on the microstomal morph of *T. vorax* (Grønlien et al., 2010). In *Paramecium*, the chemo-attractive behaviour is governed by changes in membrane potential (Van Houten, 1979). However, in both microstome *T. vorax* cells (Grønlien et al., 2010) and in *T. thermophila* cells (Lampert et al., 2011), there is no unambiguous correlation between chemo-accumulating responses and membrane potential, which is in agreement with the present observation that neither macrostome nor microstome *T. vorax* cells displayed electrophysiological responses to stimulation by the *T. thermophila* filtrate (Fig. 7).

Because macrostome *T. vorax* cells are carnivores preying on *T. thermophila* cells, the observed chemo-attraction to *T. thermophila* cells increases the probability of a macrostome cell finding adequate food. However, microstome *T. vorax* cells were also attracted to a filtrate from a *T. thermophila* cell culture (Figs 4, 5). This attraction would presumably also increase the probability of cannibalistic capture of microstome *T. vorax* cells, as macrostome cells do phagocytose microstome cells (Grønlien et al., 2002). However, Banerji and Morin (Banerji and Morin, 2009) have observed synchronous transformation of microstome cells into either macrostome cells or tailed microstome cells. Transformation to the latter morph may constitute a defence against potential cannibalism

by macrostome cells by reducing the susceptibility to consumption (Banerji and Morin, 2009). Transformation to tailed microstome cells requires a growth medium containing bacteria (Kidder et al., 1940), whereas we used an axenic growth medium. In nature, free-living ciliates are of course surrounded by bacteria. The simultaneous transformation into either the macrostome or the tailed microstome morph may be an evolutionary adaptation to reduce intraspecific competition and take full advantage of the available resources. Similar strategies also occur in polymorph multicellular organisms (Fisher, 1958; Ehlinger, 1990; Dudgeon and Buss, 1996).

Conclusions

Our data show that, during transformation from the microstome to the macrostome morph of *T. vorax*, the cell acquires mechanosensitivity in its anterior part. As a consequence of the induced mechanosensitivity, macrostome and microstome cells display different behaviour when colliding with obstacles. The mechanoreceptor potentials and the associated Ca^{2+} spikes also seem to be linked to the prey-capturing behaviour of macrostome cells. The selective phagocytosis observed in macrostome *T. vorax* cells is at least partially due to chemo-attraction to putative substances released from *T. thermophila* cells prior to capture.

ACKNOWLEDGEMENTS

We thank the two anonymous reviewers for their suggestions for improvements of the manuscript. This work was supported by The Research Council of Norway (RCN) grant 15927.

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