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STRUCTURE, FLUORESCENT PROPERTIES AND PROPOSED FUNCTION IN PHOTOTAXIS OF THE STIGMA APPARATUS IN THE CILIATE *CHLAMYDODON MNEMOSYNE*

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Summary

***Chlamydodon mnemosyne*, a brackish-water ciliate which feeds on cyanobacteria, is capable of sensing the direction of light. Cells are negatively phototactic in the well-fed state and tend to swim towards the light source when mildly starved. Severely starved cells normally fail to show phototactic responses. An autofluorescent substance, which is present in all life cycle stages, occurs in, or immediately beneath, the plasma membrane of this ciliate. It is located in the anterior left side of a cell, in the same region where mildly starved cells accumulate small orange globules that form a structure known as the stigma. The diameter of the whole area where the autofluorescent substance is located appears to be smaller than the stigma; typically, it consists of two rows of blue-green fluorescence, each row subdivided into 5–10 squares. Since the blue-green autofluorescence is excited by both blue (450–490 nm) and near-ultraviolet (340–380 nm) light, it possibly originates from flavin- and/or pterin-like molecules. We suggest that the autofluorescent substance located in or beneath the plasma membrane of *Chlamydodon mnemosyne* acts as a photoreceptor pigment in phototaxis and that photo-orientation of this ciliate is triggered by a combined mechanism involving the photoreceptor and either the stigma or a number of light-absorbing food vacuoles as a shading device.**

Key words: phototaxis, stigma, photoreceptive apparatus, ultrastructure, autofluorescence, *Chlamydodon mnemosyne*.

Introduction

Phototaxis *sensu strictu*, which is the perception of the light vector followed by oriented locomotion with respect to the light direction (Diehn et al., 1977; Machemer and Teunis, 1996), has been demonstrated not only in a large number of flagellated algae (for reviews, see Nultsch and Häder, 1988; Kreimer, 1994), but also in several ciliates (for a review, see Kuhlmann, 1998a). The cyrtophorid ciliate *Chlamydodon mnemosyne*, which feeds on cyanobacteria, shows opposing phototactic behaviour patterns under different nutritional conditions (Kuhlmann and Hemmersbach-Krause, 1993a). Well-fed cells, which possess numerous bluish food vacuoles, show negative phototaxis, while positive phototaxis predominates among slightly starved individuals. Cells in the latter state are transparent apart from a stigma (also called the ‘eyespot’) at their anterior left side composed of several hundred orange globules. Since, in earlier experiments, severely starved cells that had reduced the size of their stigma showed only very weak phototactic responses, the stigma of *Chlamydodon mnemosyne* is assumed to be part of the photoreceptive apparatus of this ciliate. It is possible that the stigma has an important accessory role in signal generation and modulation for phototaxis (Kuhlmann, 1998a,b). However, apart from some suggestions of possible shading effects, the mechanism of the spatial discrimination of light is still unknown.

In comparison with flagellated algae, in which the structure of the different types of photoreceptive organelles has been studied in considerable depth at both the light and electron microscopical levels (for a review, see Kreimer, 1994), our knowledge of the types of photoreceptive apparatus found in ciliates is limited. *Chlamydodon mnemosyne*, as well as some other stigma-forming species (Kuhlmann and Hemmersbach-Krause, 1993b; Kuhlmann, 1998a), appear to be appropriate organisms to unravel the mechanisms of photoperception and the signal-transduction and processing chains, consisting of cascades of biophysical and/or biochemical processes, that couple the photoreceptive apparatus to the effector (the cilia).

In this paper, in addition to ultrastructural studies focused on the stigma of *Chlamydodon mnemosyne*, we report recent findings of a blue-light-induced autofluorescence in, or immediately beneath, a well-defined area of the plasma membrane of this ciliate. This area possibly represents the photoreceptor site. The morphology and function of specialized structures such as the photoreceptive apparatus

must be closely linked (Kreimer, 1994). Therefore, on the basis of several characteristics of the putative photoreceptive organelle of *Chlamydodon mnemosyne*, we discuss the question of whether the apparatus fulfils the common functional demands for detection of the light direction. On the basis of new results referring to orientational experiments with *Chlamydodon mnemosyne* cells, we present a model that seems to explain the various facets of phototactic orientation of this ciliate.

Materials and methods

Chlamydodon mnemosyne Ehrenberg was isolated from a brackish-water pond near Carolinensiel, located on the North Sea coast of Germany (Kuhlmann and Hemmersbach-Krause, 1993a). Cells were cultivated at 20 °C with indirect daylight illumination in a mixture of North Sea water and soil medium (Kuhlmann and Hemmersbach-Krause, 1993a) diluted 1:1 with synthetic medium ('SM': distilled water plus 1.5 mmol l^{-1} NaCl, 0.05 mmol l^{-1} KCl, 0.4 mmol l^{-1} $CaCl_2$, 0.05 mmol l^{-1} $MgCl_2$, 0.05 mmol l^{-1} $MgSO_4$, 2.0 mmol l^{-1} sodium phosphate buffer, pH 6.8). The cells were fed with *Anabaena cylindrica*, a filamentous cyanobacterium. Approximately 5 h after feeding, the remnants of the cyanobacterium were removed by filtration of the culture through eight layers of commercially available gauze (Fuhrmann Verbandstoffe GmbH, D-53819 Neunkirchen, Germany/Art.-no. 13002).

To examine the general structure of *Chlamydodon mnemosyne*, living cells in different nutritional states were pipetted into a depression slide and observed at low magnification (40–80×) with a Zeiss binocular microscope. For phase-contrast and fluorescence microscopic observations, cells were transferred to a microscope slide, covered with a coverslip and immobilised by removal of excess culture fluid with the help of filter paper. To determine the number of food vacuoles or stigma-forming globules in single cells, specimens of *C. mnemosyne* were squashed by finger pressure on the coverslip.

Photographs were taken using a fluorescence microscope with an integrated photosystem (Leitz DM RD, Leica, D-35578 Wetzlar, Germany). A 50 W mercury gas discharge lamp served as the light source for fluorescence excitation. For excitation with near-ultraviolet and blue light, filter cubes A and I3 (Leica) were used, respectively. Filter cube A consists of an excitation band-pass filter with short- and long-wave half-power points at 340 and 380 nm, a dichromatic mirror (reflection short-pass filter, 400 nm) and a suppression filter (long-pass filter, 425 nm). Filter cube I3 is composed of an excitation band-pass filter with short- and long-wave half-power points at 450 and 490 nm, a dichromatic mirror (reflection short-pass filter, 510 nm) and a suppression filter (long-pass filter, 515 nm).

For ultrastructural examination, 10^6 cells taken from a Fernbach flask were fixed with 2.5 % (v/v) glutaraldehyde in 100 mmol l^{-1} Pipes buffer at pH 7.2 for 30 min and postfixed in 1 % (v/v) OsO_4 for 30 min. After washing, the cells were dehydrated in an ascending series of ethanol concentrations. Material was embedded in Epon 812 and was cured for 48 h at 60 °C. Sections were cut with a diamond knife, stained with 4 % (v/v) uranyl acetate for 30 min and 0.2 % lead citrate for 2 min, and examined with a Siemens Elmiscope 101 at 80 kV.

Photobehaviour of the ciliates was analysed in uni- and bilateral white light of 10 klx, produced by one or two halogen slide projectors, respectively. Cells were placed in a horizontal open cuvette (76 mm×30 mm), which was filled to a depth of 2 mm with exhausted culture medium. The cuvette was placed on a Zeiss microscope stage equipped with a CCD video camera (see Kuhlmann, 1993). Angular degrees of orientation of individual cells were measured over a distance of 3–6 mm.

Results

General morphology of Chlamydodon mnemosyne

Earlier workers have provided some fundamental information concerning the morphology and morphogenesis of the cortical structures of different species of *Chlamydodon* sp. (Ehrenberg, 1838; MacDougall, 1928; Kahl, 1932; Kaneda, 1953, 1960a,b; Dragesco, 1966). Recently, C. F. Bardele (University of Tübingen, Germany) started transmission electron microscopical investigations of the same strain of *Chlamydodon* that we have used in this study (C. F. Bardele, unpublished results). However, our knowledge of the putative photoreceptive organelle, the stigma together with an adjacent part of the plasma membrane, is still very limited. It, therefore, appeared desirable to focus our morphological studies on the stigma and the stigma area of *Chlamydodon mnemosyne* and, in addition, we investigated the shape of the cells with respect to their nutritional condition.

The species used in the present study measures between approximately 55 and 110 μm in length, and between 40 and 70 μm in width, depending on the nutritional condition of the cells. As in all species of *Chlamydodon*, cells are dorso-ventrally flattened and are ciliated on the ventral side only. The ventral part of *Chlamydodon mnemosyne* is bounded by the so-called 'cross-striped band', which characterizes this ciliate (MacDougall, 1928; Kaneda, 1960a). A ventral and a side view of *C. mnemosyne*, showing the cross-striped band, the pharyngeal basket, the cilia and the position of the stigma, are presented in Fig. 1.

The shape of *C. mnemosyne* depends on its nutritional state: while the dorsal and ventral surfaces of well-fed cells are always convex, the surfaces of starved cells are flat or even slightly concave. In ventral view, the broadest part of a well-fed cell is its equator, whereas starved cells are broadest in their anterior half, because of the slightly prominent stigma area (Fig. 1). The shape of the cell could be of importance with regard to the inversion of phototaxis in *Chlamydodon mnemosyne* (see Discussion).

The putative photoreceptive organelle: structural aspects

The stigma of *C. mnemosyne* forms in individuals that have been starved for 24–48 h. Typically, the stigma-forming

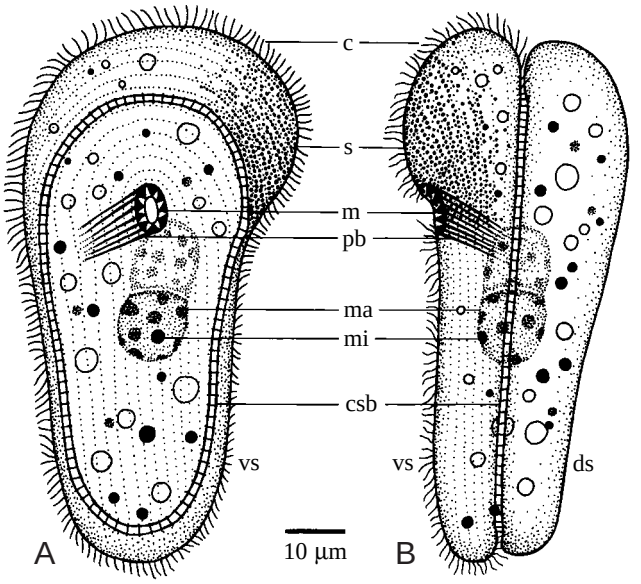

Fig. 1. *Chlamydodon mnemosyne*, slightly starved (anterior cell pole at the top of the figure). (A) Ventral view. (B) Side view. c, cilia; csb, cross-striped band; ds, dorsal side of the cell; m, mouth; ma, macronucleus; mi, micronucleus; pb, pharyngeal basket; s, stigma; vs, ventral side of the cell.

globules are regularly arranged beneath five or six rows of cilia. During prolonged starvation, the number of these globules decreases successively. Continuously starved individuals that have lost their orange globules appear almost transparent. Well-fed cells, in which the stigma is not apparent, are coloured blue-green owing to the presence of their food vacuoles, which contain cyanobacteria.

In the transmission electron microscope, the stigma is visible in longitudinal sections through the left side of a slightly starved cell (Fig. 2). From Fig. 2A,B, it is evident that the electron-dense stigma-forming globules accumulate close to the cell surface. In contrast to their appearance under the optical microscope, the globules visualized by electron microscopy are of irregular shape and no longer appear to be arranged in lines; their diameter is approximately 0.3±0.07 µm (mean ± S.D., N=100). As seen at higher magnifications, the globules are not surrounded by a membrane. The whole stigma area is covered by the pellicle and separated from the inner cytoplasm by a band of mitochondria.

Autofluorescence of the stigma-forming globules and the cell surface of Chlamydodon mnemosyne

Depending on the wavelength of the excitation light and the nutritional status of the cells, *Chlamydodon mnemosyne* cells exhibit different patterns of autofluorescence (Fig. 3). Two days after food uptake, the cells are slightly starved. When excited with blue light (450–490 nm), they show an intense green autofluorescence from approximately 200 globules (Fig. 3A). Most of these globules are oriented along five or six rows of cilia. Since the stigma is degraded during prolonged starvation, the cells do not usually possess any green-fluorescing globules when starved for a period of more than 7 days. At this stage, a greenish background fluorescence can be detected over the entire cytoplasm (Fig. 3C). When filamentous cyanobacteria are added to cultures of severely starved cells, *Chlamydodon mnemosyne* immediately starts phagocytosis. Within 10 min, numerous food vacuoles are formed. These show an intense red autofluorescence due to their content of chlorophyll and phycocyanin-producing cyanobacteria (Fig. 3E).

A different pattern of autofluorescence occurs upon excitation with ultraviolet light (340–380 nm). While light of this wavelength does not induce the fluorescence of stigma granules, another autofluorescent structure is detected within the stigma area of the cell (Fig. 3B). The area covered by the whole autofluorescent structure is always a little smaller than that of the stigma (compare Fig. 3A with Fig. 3B). It consists of two blue-green-fluorescing rows that appear to be associated with the area of the plasma membrane overlying the stigma.

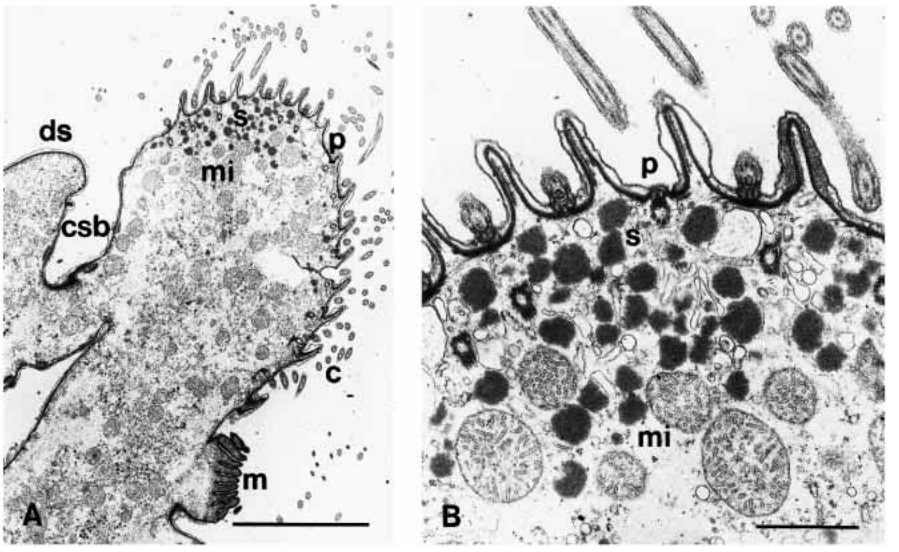

Fig. 2. Transmission electron micrographs of longitudinal sections through a slightly starved cell of *Chlamydodon mnemosyne*. (A) Anterior ventral half of the cell (overview). (B) Part of the stigma area at higher magnification. c, cilia; csb, cross-striped band; ds, dorsal side of the cell; m, mouth; mi, band of mitochondria; p, pellicle; s, stigma area. Scale bars, 5 µm in A, 1 µm in B.

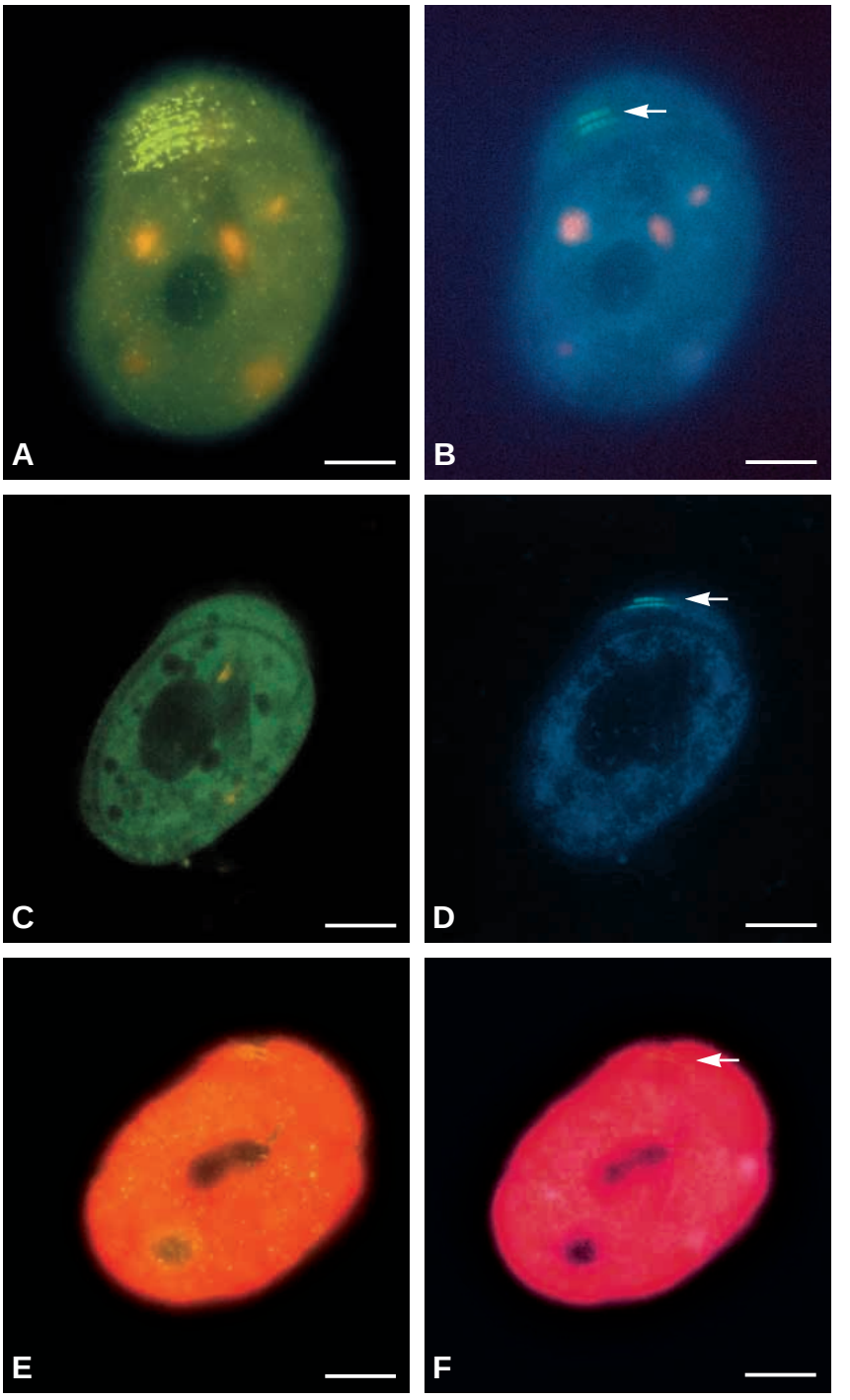

Fig. 3. Autofluorescence of *Chlamydodon mnemosyne* during different nutritional conditions. Slightly starved (A,B), severely starved (C,D) and recently fed (E,F) cells were excited first with near-ultraviolet light (B,D,F) and then with blue light (A,C,E). (A) Autofluorescence of granules forming the stigma becomes visible only in slightly starved cells under excitation with blue light. The stigma is assumed to play a role as a shading device for phototactic orientation. (B) A rapidly fading autofluorescent structure (arrow) is detected in ultraviolet-excited cells and is probably associated with the plasma membrane overlying the stigma. This structure presumably represents the photoreceptor of the organism. (C) Severely starved cells do not contain stigma granules because cells degrade their stigma upon prolonged starvation. (D) The rapidly fading autofluorescence (arrow) is still present in severely starved cells. (E) After food uptake, the red fluorescence of food vacuoles predominates. (F) Even with the strong background signal from food vacuoles, the autofluorescent structure (arrow) can still be detected in, or immediately beneath, the membrane of well-fed cells. Scale bars, 20 μm.

Each row is composed of 5–10 fluorescent squares. Irradiation of the cells with light at this wavelength causes the blue-green autofluorescence to fade rapidly so that it disappears within 10–30 s. Under our experimental conditions, no recovery of fluorescence was observed. When blue light was used for excitation instead of ultraviolet light, the same autofluorescent structure became visible and was similarily bleached (not shown). Thus, the absence of the fading autofluorescence in Fig. 3A is the result of previous irradiation of this cell with ultraviolet light.

In contrast to the temporary appearance of the stigma-forming globules, the rapidly fading autofluorescence occurred independently of the cellular feeding state. It was still detected in/below the plasma membrane of severely starved cells overlying the region where the stigma had formerly been present (Fig. 3D). The rapidly fading autofluorescence was also visible in recently fed cells, although the much stronger fluorescence of the cyanobacteria-containing food vacuoles made observations more difficult (Fig. 3F).

Orientation of Chlamydodon mnemosyne *in unilateral white light*

Initial investigations of the phototactic orientation of *C. mnemosyne* in unilateral light have been carried out by Kuhlmann and Hemmersbach-Krause (1993a; circular histograms of the orientation of well-fed and mildly starved cells are included in that paper). Here, we present new data on the orientation of cells under different nutritional conditions where varying numbers of either food vacuoles or orange globules had been formed. To simplify the evaluation of data, we did not determine the exact angular degrees of orientation of the swimming cells. Instead, we distinguished between (1) cells swimming either towards or away from the light source (i.e. into either the sector 0±45 ° or the sector 180±45 °), and (2) cells swimming more or less perpendicular to the light (into either the sector 90±45 ° or the sector 270±45 °). If the cells were positively or negatively phototactic, the number of cells in category 1 should be significantly larger than the number of cells in category 2. If, however, the cells oriented randomly, both populations should be of similar size. Usually, 100 cells of a predefined nutritional state were tested for orientation. In some experiments, single cells were tested repeatedly (20 times).

The orientational responses of *C. mnemosyne* during different nutritional states are summarized in Fig. 4A. While in well-fed cells phototactic orientation is evident, this capacity gradually decreases in mildly starved cells and almost disappears after a period of 8 days in severely starved cells.

A well-developed stigma is visible in the cells 2–3 days after feeding with cyanobacteria. The stigma becomes smaller and sometimes disappears completely in cells starved for more than 3 days. The relationship between phototactic orientation and the number of stigma-forming globules per cell is shown in Fig. 4B. While those cells having fewer than approximately 20 globules showed random (or almost random) orientation in unilateral white light, individuals with at least 100 globules preferred to swim towards or away from the light source in most of the 20 successive experiments.

Evidence that food vacuoles in addition to the stigma-forming globules play an important role in photo-orientation of *C. mnemosyne* is strengthened by the following experimental results. When severely starved cells, almost free of stigma-forming globules and unable to produce phototactic responses, were fed with cyanobacteria, they regained the capacity for phototaxis within only 5–20 min, coinciding with the time taken to form several bluish-coloured food vacuoles (Fig. 5A). Moreover, light-induced orientation was always better in ciliates that had ingested 50 or more cyanobacteria than in individuals with only a few cyanobacteria present in their food vacuoles (single *Anabaena cylindrica* cells, not trichomes or food vacuoles, were counted) (Fig. 5B).

Orientation of Chlamydodon mnemosyne *in bilateral white light*

In an additional series of experiments, two identical light sources were used instead of one. The light sources were placed opposite each other, at a greater distance from the experimental chamber compared with the previous experiments (so that the light intensity measured close to the experimental chamber was the same as under unilateral illumination).

The results of the experiments undertaken with two light sources are shown in Fig. 6. While starved cells, irrespective of the degree of starvation, were unable to develop phototactic responses, well-fed cells showed a preference to swim perpendicular to the directions of the lights. It was noted that the well-fed cells accumulated at those walls of the experimental chamber oriented parallel to the light beams.

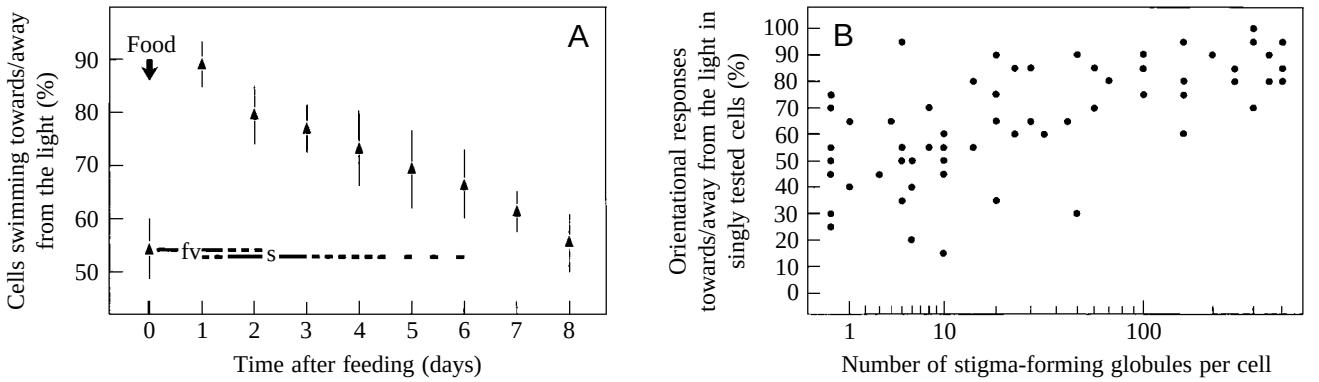

Fig. 4. Orientation of *Chlamydodon mnemosyne* in unilateral white light, followed over a period of 8 days after feeding. (A) On the first and second days after feeding, numerous bluish food vacuoles (fv) are present in each cell. A typical stigma (s), made up of small orange globules, is present on the second day; the stigma gradually becomes smaller during days 4–6 after feeding. After 7 or 8 days, the cells are almost transparent again; only in exceptional cases are a few stigma-forming globules still present. Percentages on the ordinate are derived from the numbers of cells that either swam towards or away from the light source (into either the sector 0±45 ° or the sector 180±45 °). Each data point is based on the orientation of 5×100 cells, tested in five successive experiments (error bars show S.D.). (B) Orientation of singly tested, starved cells (5–8 days after food uptake) with various numbers of stigma-forming globules. Each data point represents the percentage of orientational responses (movement into either the sector 0±45 ° or the sector 180±45 °) of an individual cell tested in 20 successive experiments. At the end of each series of experiments, the number of stigma-forming globules present in each cell was determined (error approximately ±10 %).

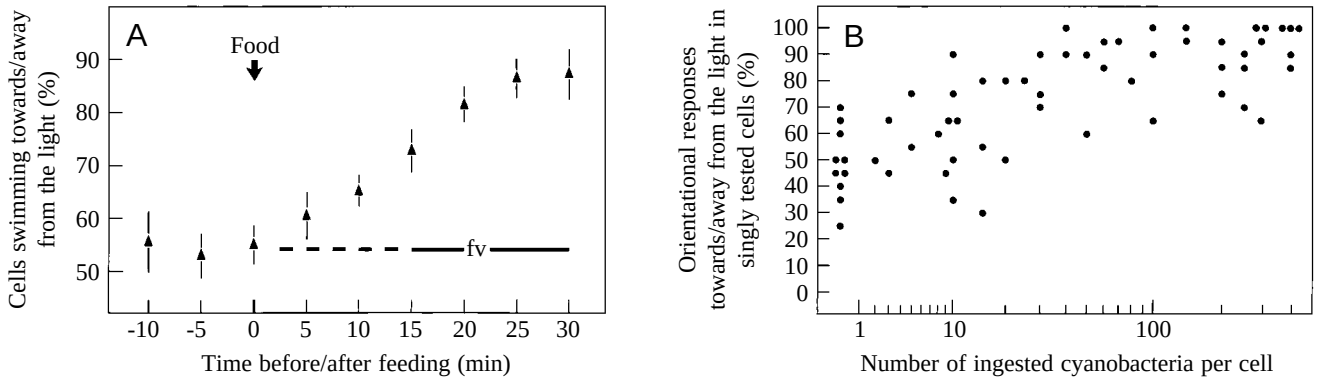

Fig. 5. Orientation of *Chlamydodon mnemosyne* in unilateral white light, followed for a period of 10 min before, and 30 min after, feeding with cyanobacteria. (A) Food vacuoles (fv) are formed by the almost transparent *Chlamydodon mnemosyne* cells within 2–3 min of adding a suspension of cyanobacteria to the culture. Fifteen minutes later, most of the ciliates have a deep blue colour due to the presence of numerous food vacuoles (for further explanation, see Fig. 4A). (B) Orientation of cells tested singly (isolated from a *Chlamydodon mnemosyne* culture 2–30 min after adding a suspension of cyanobacteria) with various numbers of food vacuoles (for further explanation, see Fig. 4B). At the end of each series of experiments, the number of cyanobacteria present in the food vacuoles of each cell was calculated (error approximately ±33 %).

Discussion

The photoreceptive apparatus of unicellular eukaryotes exhibits an enormous structural variation and probably results from numerous parallel lines of evolution. The ultrastructure and the functioning of these specialized structures are closely linked (Kreimer, 1994). Although different classes of phototactic protists possess systems with different ultrastructural designs, three characteristics are common to the protozoan photoreceptive apparatus. (1) It is usually a single structure. Only during sexual or asexual reproduction are two photoreceptive organelles sometimes present. (2) It is located in a defined position within the cell. Although the absolute position shows great variation, it is most commonly located in the anterior half of a cell, often roughly perpendicular to the axis of the swimming path of the cell. Sometimes there is a close association with microtubules or microtubular bands and roots. (3) It is a complex cell organelle, consisting of two or more functionally different parts. In the case of stigmata, which are common in green flagellates, carotenoid-rich lipid globules and the membrane-bound photoreceptor molecules can be distinguished. In most of the functional photoreceptive structures of photomotile flagellates, the photoreceptor is in front of the globules that form the stigma.

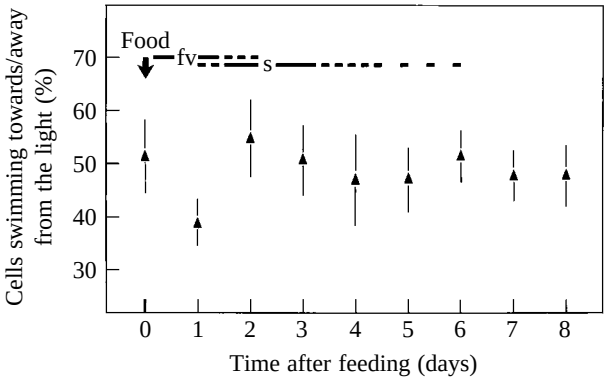

Fig. 6. Orientation of *Chlamydodon mnemosyne* in bilateral white light, followed over a period of 8 days after feeding. Two identical light sources were placed opposite one another (directions 0 ° and 180 °) (for further explanation, see Fig. 4A).

The stigma of *C. mnemosyne* is believed to be part of the photoreceptive apparatus of this ciliate. Doubtless it plays an important role in photo-orientation but, as pointed out above (see Introduction), the stigma is obviously not identical with the photoreceptor site. Our recent finding that there is an autofluorescent area in, or beneath, the plasma membrane adjacent to the stigma of *C. mnemosyne* strongly supports the hypothesis that this area is identical with the photoreceptor site and that the stigma acts as a signal-modulating device. Thus, the photoreceptive apparatus of *Chlamydodon mnemosyne* fulfils the common functional demands for a direction-sensitive photoreceptor, since the whole apparatus (1) is a singular structure, (2) is found in a defined position within the cell, and (3) consists of different subunits.

Since near-ultraviolet (340–380 nm) and blue (450–490 nm) light induce the blue-green, rapidly fading autofluorescence, the fluorescing molecules must absorb light of this spectral range. Assuming that these molecules act as photoreceptor pigments, *Chlamydodon mnemosyne* should, therefore, possess a photoreceptor for near-ultraviolet/blue light. Blue-light photoreceptors have been proposed for, or have already been identified in, several flagellated algae including *Euglena gracilis* (Müller et al., 1987; Kawai, 1988; Galland et al., 1990; Schmidt et al., 1990; Brodhun et al., 1994; Häder and Lebert, 1994; Brodhun and Häder, 1995) and in many plants (Malhorta et al., 1995), but have not been identified in ciliated protozoa. Even though most of the photoreceptors absorbing near-ultraviolet/blue light have not yet been isolated and characterized, there is a general consensus, based mainly on indirect physiological evidence such as action spectra, that flavins (as well as pterins) are among the most likely contenders for the chromophores of these receptors (see

Schmidt et al., 1990, and literature therein). Furthermore, a greenish autofluorescence has been detected in several phototactic flagellates and is thought to originate from the flavin photoreceptors of these organisms (Benedetti and Checcucci, 1975; Müller et al., 1987; Yamano et al., 1996). The autofluorescence in/beneath the plasma membrane of *Chlamydodon mnemosyne* resembles the fluorescence of phototactic flagellates both in colour and in bleaching behaviour. Since flavins and pterins are notoriously unstable under illumination (Holmström and Oster, 1961), it is possible that the rapidly fading fluorescence originates from flavins and/or pterins. Considering that many blue-light-absorbing pigments, such as DNA photolyase and the presumptive human circadian photoreceptors, contain more than one chromophore (Sancar, 1994; Miyamoto and Sancar, 1998), the photoreceptor of *Chlamydodon mnemosyne* might contain both flavin and pterin chromophores simultaneously. There is also indirect physiological evidence that flavin- and pterin-like chromophores are involved in photoreception in *Chlamydodon mnemosyne* from the action spectrum of phototaxis of this ciliate, which has recently been determined. Reactivity was found to be restricted to the blue and near-ultraviolet range, with a sharp peak at 470 nm and similarly high activity at the near- ultraviolet end of the spectrum (363 nm) (Selbach et al., 1999).

Although ultraviolet and blue light are known to induce a variety of responses in microorganisms, fungi and plants (including phototropism, hypocotyl elongation, stomatal opening and expression of specific genes, see Malhorta et al., 1995; Fankhauser and Chory, 1997, and references therein), there is very little biochemical information available on the structure and reaction mechanism of the blue-light photoreceptors. Because *Chlamydodon mnemosyne* is a unicellular organism and complex interactions between cells can therefore be excluded, this ciliate might prove to be a suitable model organism for unravelling the basic principles of the perception of blue light. Furthermore, the fact that starved cells are colourless greatly facilitates spectroscopic investigations.

The rapidly fading autofluorescent structure in the pellicle of *Chlamydodon mnemosyne* was found in all cells examined irrespective of whether the ciliates were well-fed or starved. The localization and pattern of the fluorescent structure were approximately the same in different cells. Nevertheless, cells in different nutritional conditions show qualitatively different phototactic responses and many even be unable to orient themselves with respect to the direction of light (Kuhlmann and Hemmersbach-Krause, 1993a): in slightly starved cells, the stigma-forming granules are thought to play an important accessory role in signal generation and thus could enable positive phototactic movement. Well-fed cells lack a stigma, but show accurate negative phototaxis. Severely starved cells are transparent and obviously cannot sense the direction of light. Since well-fed and severely starved cells, apart from their different size and shape, only differ in the presence or absence of food vacuoles, these organelles may be of importance for the detection of the light direction (Kuhlmann, 1998b).

According to the ‘shading hypothesis’, already suggested by Jennings (1906) and Mast (1911), phototactic orientation in flagellates is brought about by repetitive reorientations of the cell, caused by the stigma, which periodically casts a shadow on the photoreceptor as the cell rotates around its longitudinal axis. More recent investigations have revealed some characteristics of the orientation of *Euglena gracilis* that are not compatible with the shading hypothesis (Nultsch and Häder, 1988; Häder, 1998). An alternative possibility, namely that the stigma could act as a reflector of light, had already been discussed by Buder (1917). From the spacing of the different granule layers in *Chlamydomonas reinhardtii*, Foster and Smyth (1980) suggested that the stigma acts as a ‘quarter-wave interference reflector’, consisting of a stack of alternating layers with high and low refractive indices, the distance between two layers being 0.25λ, where λ is wavelength.

The putative photoreceptive apparatus of *Chlamydodon mnemosyne* obviously shares some common features with the direction-sensitive photoreceptors of *Euglena gracilis* and of *Chlamydomonas reinhardtii*. As in *Euglena gracilis*, the whole globule complex is not membrane-bound, the stigma globules lack close packing, and both globule size and globule number vary. Unlike *Euglena gracilis*, where the photoreceptive structure, the paraxonemal body, is located between the stigma and the longitudinal axis of the cell, the putative photoreceptor of *Chlamydodon mnemosyne* is located in the plasma membrane overlying the stigma. In this respect, the situation in *Chlamydodon mnemosyne* is comparable with that in *Chlamydomonas reinhardtii*, where rhodopsin-like molecules have been detected in the plasma membrane adjacent to the stigma (Zhang, 1994).

To explain the function of the photoreceptive apparatus in the phototactic orientation of *Chlamydodon mnemosyne*, it appears to be of particular importance to establish whether the stigma is a suitable organelle to either absorb or reflect the incident light. Considering the arrangement of the stigma-forming granules, which in living cells appear to be strung out in parallel lines but were never found to form a stack of layers with high and low refractive indices, significant reflection of light apparently does not occur (however, a weak reflection of light can also occur when light enters/leaves a medium of high refractive index; for instance, reflection is observed in eyespots with multiple and single globule layers; Foster and Smyth, 1980; Kreimer, 1994). However, absorption of (blue) light must occur to an unknown extent, taking into account the (orange) colour of the stigma-forming globules. According to the ‘shading hypothesis’, and given that the photoreceptor molecules are located in the plasma membrane overlying the stigma, light becomes modulated during rotation of the cell, which leads to a periodic irradiation and shading of the photoreceptor nearby. In those cells that swim towards or away from the light source, this modulation would be relatively low in contrast to that for cells swimming perpendicular with respect to the light direction. In cells that

are illuminated by two light sources from opposite directions, the periodic irradiation and shading of the photoreceptor should be weak, and this should also be true for cells that swim perpendicular to the light direction. This should result in an increase in the fraction of cells that continue to swim perpendicular to the light, exactly what was observed in the 'two-lights experiments'. Furthermore, the shading function of the stigma-forming globules in unilateral light could be taken over by other light-absorbing cell organelles, predominantly by food vacuoles, so that in well-fed cells, which lack a stigma, a similar modulation of the incident light should occur.

Assuming that irradiation of the photoreceptor produces a signal that triggers the motor response of a cell, e.g. a change in the ciliary beat, the inversion of phototaxis in *Chlamydodon mnemosyne* becomes explicable in accordance with the following model. The putative photoreceptor of a well-fed, ovoid cell becomes continuously illuminated when the cell is swimming towards the light source or is periodically illuminated when the cell is swimming perpendicular to the light. This leads to a signal which causes changes in swimming direction and which continues until, in cells moving away from the light source, the photoreceptor becomes continuously shaded by the food vacuoles. The photoreceptor of a slightly starved cell, which is able to perform positive phototaxis, could become continuously shaded by the stigma-forming globules in those cells that orient towards the light. This appears likely because starved cells have a prominent stigma (see Fig. 1) and typically show a staggering movement when they swim towards the direction of light. Finally, when cells are illuminated by two light beams placed opposite one another, a change in swimming direction is expected to be induced when they swim towards one of the light sources. Because reorientations often occurred in bilaterally illuminated *Chlamydodon mnemosyne* cells, this is compatible with the proposed model of indirect orientation, but is contradictory to a direct light-tracking strategy, which explains phototactic orientation in other ciliates (e.g. in *Ophryoglena*; R. Marangoni, G. Colombetti and H.-W. Kuhlmann, unpublished results).

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