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REVIEW

Chemotaxis in the Green Flagellate Alga *Chlamydomonas*

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Abstract—Behavior of the green flagellate alga *Chlamydomonas* changes in response to a number of chemical stimuli. Specific sensitivity of the cells to different substances might appear only at certain stages of the life cycle. The heterogamous species *C. allensworthii* demonstrates chemotaxis of male gametes towards pheromones excreted by female gametes. In *C. reinhardtii* chemotaxis towards tryptone occurs only in gametes, whereas chemotaxis towards ammonium, on the contrary, only in vegetative cells. Chemotaxis to different chemical stimuli might involve different mechanisms of reception and signal transduction, elucidation of which has only recently begun. Indirect evidences show that the cells likely respond to tryptone with changes in the membrane electrical conductance. The recently completed project of sequencing the whole nuclear genome of *C. reinhardtii* provides the basis for future identification of molecular elements of the chemosensory cascade in this alga.

Key words: chemoreception, signal transduction, photoreceptor current, ammonium, tryptone, gametogenesis

The ability to respond to chemical stimuli is among the key sensory functions found in almost all living organisms. Green flagellate algae including *Chlamydomonas* that are model objects in many fields of biological research use chemical stimuli to adapt to their environment by specific chemoinduced behavior. Although the first observations of such responses in *Chlamydomonas* date back to the 19th century [1], our knowledge of them is still at the level of phenomenology. On the contrary, significant progress has been achieved in investigation of chemoregulation of behavior in other cells. Best of all has been characterized chemotaxis in motile cells in humans and animals [2, 3], as well as chemotaxis in some prokaryotes (mainly enterobacteria [4, 5]). Also, much is known about chemotaxis in the lower eukaryotes: the myxamoeba *Dictyostelium* [6, 7] and the ciliate *Paramecium* [8, 9].

With the exception of *Paramecium*, success in investigation of chemoregulation of motility in these particular objects was due to their being amenable for molecular genetic analyses. Recently completed sequencing of the whole nuclear genome of *Chlamydomonas reinhardtii* [10] created a possibility for rapid progress in research into chemoreception in this microorganism as well. This optimism is supported with the remarkable progress in investigation of other physiological functions of this alga achieved since it became the object for establishment of nucleotide sequence databases. In particular, this facilitated the identification of rhodopsin receptors for phototaxis and the photophobic response in *C. reinhardtii* [11, 12].

The nuclear genome of *C. reinhardtii* contains 100 to 110 million base pairs combined into 17 linkage groups (chromosomes). Its sequencing has been undertaken by the US Department of Energy Joint Genome Institute. By the time of this review, initial assembly of the information obtained by sequencing overlapping short fragments of the genome has been completed. The results of this work, as well as their preliminary analysis, can be accessed at the JGI web page: http://genome.jgi-psf.org/chlre2/chlre2.home. html.

One of the reasons for *Paramecium* being a popular experimental organism is that it is suitable for electrophysiological measurements. It has been found that mechanisms for chemical signaling in *Paramecium* involve bioelectrical processes in the cell membrane, and detailed studies on these processes have been carried out [9]. Although *Chlamydomonas* is too small for reliable microelectrode recording widely used in *Paramecium*, methods for extracellular measurement of asymmetrically localized transmembrane currents in this and related algae have been developed [13-15]. It has been shown that photoexcitation of rhodopsins leads to generation of such currents, which play a key role in photoregulation of motility in these microorganisms [16]. The possibility of photoelectric recording prompted investigation of photoreception and photomovement in *Chlamydomonas* [17].

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According to indirect data (see below) and accepting an analogy with *Paramecium*, it can be suggested that reception and transduction of chemical stimuli in *Chlamydomonas* might also involve changes in the membrane electrical conductance, although no direct evidence for this has been obtained so far. On the other hand, the involvement of some molecular components of prokaryotic chemosensory cascades in *Chlamydomonas* chemoreception cannot be completely ruled out yet. The basis for this hypothesis is the similarity between primary sequences of recently identified *Chlamydomonas* rhodopsins and those from prokaryotes (type I rhodopsins [18]).

A separate problem of *Chlamydomonas* chemotaxis research is to establish how the cells detect chemical gradients and orient along them. Most work published so far has been carried out by measuring chemotaxis as a net response of a cell population (accumulation of the cells in a capillary filled with a solution of the test substance). Technical feasibility is an obvious advantage of this method, but solving the above problem requires tracking individual cells responding to a chemical stimulus.

In this review, we attempted to systematically narrate and analyze the available literature on chemical regulation of *Chlamydomonas* motility.

CHEMORECEPTION AND SIGNAL TRANSDUCTION

Chlamydomonas behavior changes in response to a number of organic, as well as inorganic, substances. Although mechanisms for reception and transduction of chemical stimuli are only little known in green flagellate algae, one can assume different signaling pathways for different active substances. Such heterogeneity of the chemosensory system is well known in other microorganisms. For instance, *Paramecium* uses at least three different chemosensory cascades depending on the chemical nature of the stimulus [9]. Chemical stimuli can be divided into several groups, reception and transduction of which are likely to involve different mechanisms in *Chlamydomonas*.

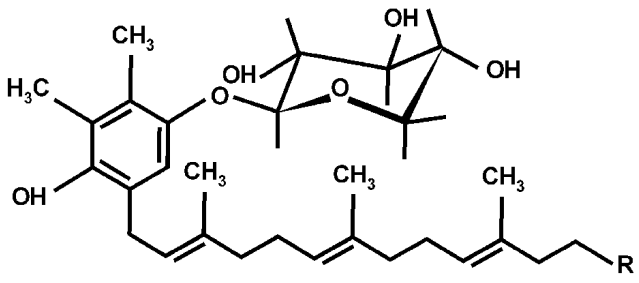

Fig. 1. Chemical structures of lurlenes, sexual pheromones of *C. allensworthii*. Lurlenol: R = CH_2OH; lurlenic acid: R = COOH (after [24]).

Pheromones. Gamete mating in the two best studied isogamous *Chlamydomonas* species, *C. reinhardtii* and *C. eugametos*, is generally accepted to only depend on random collision chances without help of any diffusable chemical intermediate [19, 20]. Early literature reports observations of accumulation of male gametes around a female gamete in some hetero- and oogamous species of this genus, which is explained by excretion of specific chemical agents by female gametes (for review see [21]). The chemical nature of such agents has been, however, established only in the case of the heterogamous *C. allensworthii* [22]. This species has even been originally described as the microorganism characterized by a pronounced accumulation of male gametes around female gametes.

Active molecules, named lurlenes, have been first isolated from culture medium of female gametes and subsequently chemically synthesized [23]. They are plastoquinone derivatives attached to a D-xylose residue (Fig. 1) [22, 24]. It has been shown that the structure of the sugar residue determines the biological efficiency of these compounds [25]. Attraction of male gametes by chemical agents excreted by female gametes is well known in brown algae [26, 27]. It is noteworthy that despite a similar function, the chemical nature of lurlenes of *C. allensworthii* is totally different from that of the sexual pheromones of brown algae, which are polyunsaturated hydrocarbons [28].

The pheromone excretion by the female gamete of *C. allensworthii* stops after fusion with the male gamete. Different geographic isolates of *C. allensworthii* form two groups, each of which uses lurlenic acid or the corresponding alcohol, lurlenol, as the sexual pheromone [24]. Besides, all isolates fall into five mating subgroups, and members of different subgroups may use the same chemical form of the lure [29]. In this case the accumulation of male gametes around a female gamete persists for a long time, because no mating and hence no cessation of the pheromone production occurs.

The biological activity of lurlenes, including synthetic ones, has been confirmed by soaking polymer granules in their solutions and placing these granules in suspensions of mature male gametes. The male gametes form a cloud around such granules, but not around granules soaked in control buffer. Chemically pure lurlenes are efficient in concentrations not exceeding 1 pM [22]. Chemotaxis towards lurlenes is inhibited by the removal of calcium from the external medium, or by the addition of its antagonists [24]. Therefore it has been concluded that this ion is involved in chemical signaling, as it is known for phototaxis in *Chlamydomonas*.

Peptone, tryptone, and amino acids. Peptone and tryptone are products of pancreatic digest of casein and are widely used as supplements to growth media for cultivation of microorganisms, including photosynthesis-defi-

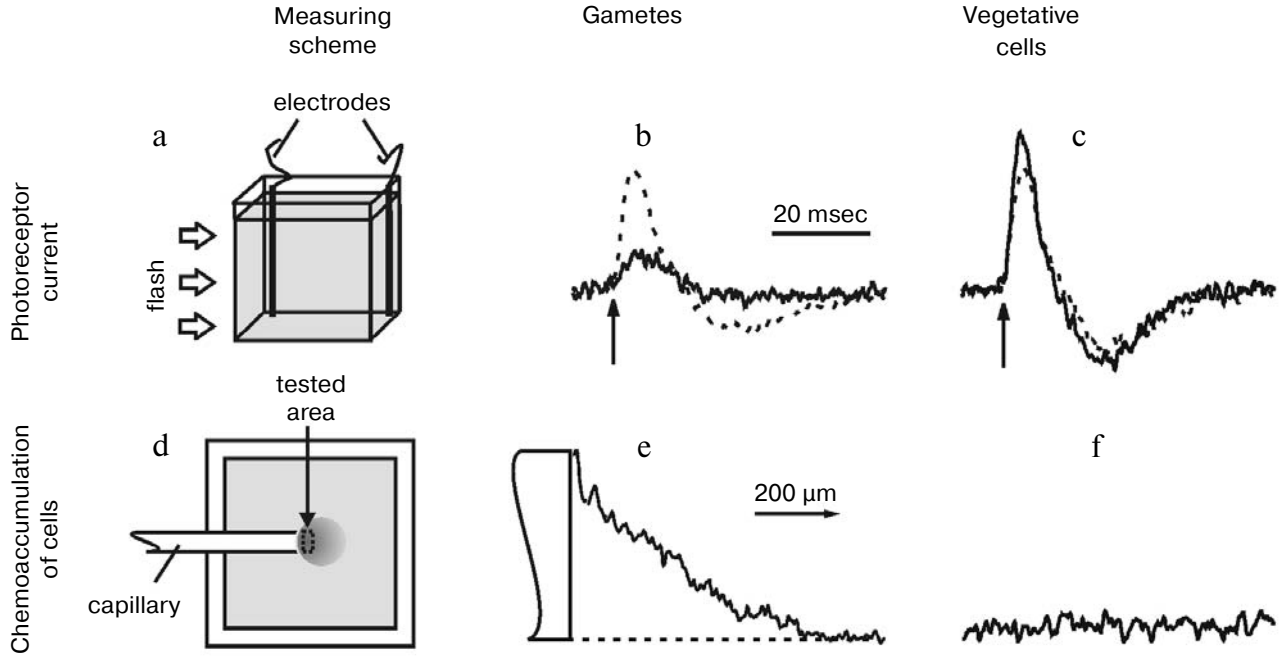

Fig. 2. Photoelectric (b, c) and behavioral (e, f) responses in gametes and vegetative cells of *C. reinhardtii* upon the addition of tryptone (0.1%), and schemes for their measurement (a, d). b, c) Dashed lines show the normalized photoelectric signals in suspensions of *C. reinhardtii* before the addition of tryptone; solid lines, after the addition of tryptone; the arrows show the time of the excitation flash. e, f) Solid lines show the density of the digitized image of the test area at the capillary opening.

cient *C. reinhardtii* mutants. Peptone and tryptone composition comprises oligopeptides, individual amino acids, as well as traces of carbohydrates, vitamins, and other substances.

An early study [30] revealed that peptone attracts or repels, depending on the concentration, *C. eugametos* gametes. Similar data were also obtained for tryptone in *C. reinhardtii* [31]. Moreover, it has been established that vegetative cells of *C. reinhardtii*, unlike gametes, are insensitive to tryptone.

The addition of tryptone to a suspension of chemotactic gametes of *C. reinhardtii* induces transient inhibition of photoreceptor currents (Fig. 2, b and e). The fast photoreceptor current is the earliest so far detected event in the signal transduction chain for phototaxis and the photophobic response in green flagellate algae [32]. On the other hand, the amplitude of photoreceptor currents in vegetative cells, which display no chemotaxis towards tryptone, slightly increases after the addition of tryptone (Fig. 2, c and f). Correlation between the time courses and concentration dependencies of chemotaxis towards tryptone and the suppression of photoreceptor currents by this agent has been found [31]. The data show that gamete-specific inhibition of photoreceptor currents by tryptone is due to the activation of the signaling cascade involved in chemotaxis towards this agent, and that initial steps of transduction of photic and chemical stimuli involve common elements (Fig. 3).

Inhibition of photoreceptor currents is already observed a few seconds after the addition of tryptone to a gamete suspension. Therefore, it can be concluded that the initial steps of chemosensory transduction are coupled to a change in the membrane electrical conductance, as it happens in *Chlamydomonas* phototaxis and *Paramecium* chemotaxis. The dependence of the inhibition of photoreceptor currents by tryptone on the external potassium concentration indicates that chemotaxis towards tryptone might involve activation of potassium channels in the plasma membrane. The results of inhibitory analysis show that these channels might be reg-

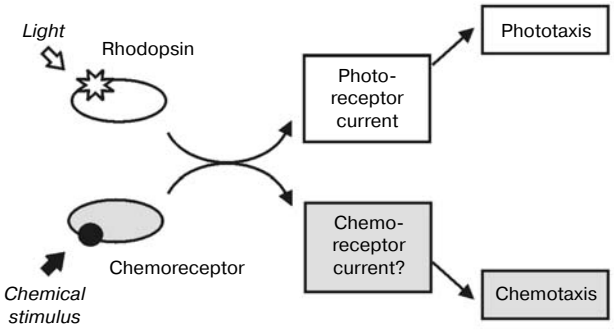

Fig. 3. A hypothetical scheme for the coupling of signal transduction chains for photo- and chemotaxis in *C. reinhardtii*.

ulated by the intracellular concentrations of cyclic nucleotides.

In the *C. reinhardtii* genome several gene models have been identified that correspond to subunits of potassium channels and cation channels regulated by cyclic nucleotides. The same source also contains multiple gene models corresponding to various components of enzymatic cascades involved in chemosignaling in other cell types (in particular, G protein-coupled membrane receptors, adenylyl and guanylyl cyclases, phosphodiesterases of cyclic nucleotides, etc.). It seems that probing for a possible role of the products of some of these genes in *C. reinhardtii* chemotaxis could be undertaken in the future by knocking down their expression by methods of genetic engineering and subsequent functional testing of the resultant clones.

Several studies have probed *Chlamydomonas* behavioral responses to individual amino acids. D,L-Ala caused weak accumulation of gametes in *C. eugametos* [30]. In a study [33], all common amino acids, except Trp, Asp, and Glu, were tested in vegetative cells of *C. reinhardtii* in the presence of ammonium in the medium. Only L-Arg induced a chemotactic response, acting as a strong repellent in a concentration range from 10^{-6} to 10^{-3} M without affecting the mean swimming velocity of the cells. D-Arg was not efficient. None of the 20 common amino acids (L-forms) changed the behavior of vegetative cells of *C. reinhardtii* 2 h after the removal of ammonium from the medium [34].

Analysis of the influence of individual amino acids on photoreceptor currents in *C. reinhardtii* gametes revealed that none of them is responsible for the inhibition of these currents by tryptone, although only a low molecular weight fraction of tryptone is biologically active. Two possible explanations of this result can be suggested: 1) the active substances are not amino acids, but rather di- or tripeptides; 2) a specific combination of individual amino acids acts as an attractant, as it is known, for instance, for chemotaxis of zoospores in the lower fungus *Allomyces* [35].

The behavior in some other green flagellate algae is sensitive to a wider range of amino acids than that in *Chlamydomonas*. For example, chemotaxis of zoospores in the related alga *Chlorococcum minutum* is induced not only by peptone, but also by L-Ser, L-Thr, and L-Glu [36]. L-Ala and L-Lys, and to a lesser extent Gly, are attractants for *Dunaliella salina*, whereas their homooligopeptides are less efficient compared to the individual amino acids [37]. Finally, in the colonial flagellate alga *Astrephomene gubernaculifera* Phe causes accumulation, whereas Arg, Lys, and His cause weak dispersal of colonies [38].

Ammonium. Ammonium is a strong attractant for *C. reinhardtii* [34] and *C. eugametos* [30]. However, vegetative cells in the presence of ammonium in the growth medium are not sensitive to it. Removal of ammonium stimulates the appearance of chemotaxis towards this substance [34].

Removal of nitrogen (which is usually present in the form of ammonium in most commonly used growth media) is known to serve as the signal for the beginning of gametogenesis in *Chlamydomonas* [20]. In most *C. reinhardtii* strains in the dark gametogenesis only proceeds to the formation of so-called pregametes, which are not able to fuse with gametes of the opposite mating type. Illumination of pregametes leads to their conversion to mature gametes. Phototropin has been identified as the photoreceptor responsible for this process [39].

Pregametes maintain the ability to chemotax towards ammonium, which is induced by removal of this substance from the medium [40], whereas mature gametes completely loose it [41]. Thus, chemotactic sensitivity of *C. reinhardtii* is tightly related to its life cycle: gametogenesis is accompanied with the loss of chemotaxis to ammonium and the appearance of chemotaxis to tryptone (Fig. 4).

The *C. reinhardtii* mutants *lrg1*, *lrg3*, and *lrg4*, which are able to form mature gametes in the dark [42], also loose chemotaxis to ammonium in the dark [40]. Furthermore, photoinduced conversion of pregametes to mature gametes and the loss of chemotaxis to ammonium are suppressed in *C. reinhardtii* transformants with a reduced content of phototropin [39, 43]. These results point to a common mechanism for light regulation of gametogenesis and the loss of chemotaxis toward ammonium in *C. reinhardtii*.

The influence of amino acids on the loss of chemotaxis towards ammonium in mature gametes has been studied [44]. In the presence of Gln, Ala, Ser, or Phe in the medium cells do not loose chemotaxis to ammonium if the medium contains acetate. In the presence of Arg, chemotaxis to ammonium is maintained regardless of the presence or absence of acetate. Finally, Thr, Pro, or Glu do not affect the loss of chemotaxis to ammonium in mature gametes.

Arginine is the only amino acid for which *C. reinhardtii* has a specific membrane transporter [45]. Nevertheless, this microorganism can also use other amino acids as the only nitrogen source by means of extracellular desamination [46]. The induction of biosynthesis of an enzyme responsible for this process—L-amino acid oxidase—is observed after the removal of ammonium from the medium in the presence of acetate [47, 48]. The specific activity of this enzyme is significantly higher for Gln, Ala, Ser, and Phe than for Thr, Pro, and Glu, the activity for Arg being intermediate [48]. Therefore, the efficiency of individual amino acids in the block of the loss of chemotaxis to ammonium [44] correlates with the efficiency of their extracellular desamination.

The presence of amino acids (substrates for extracellular desamination) in the medium does not prevent

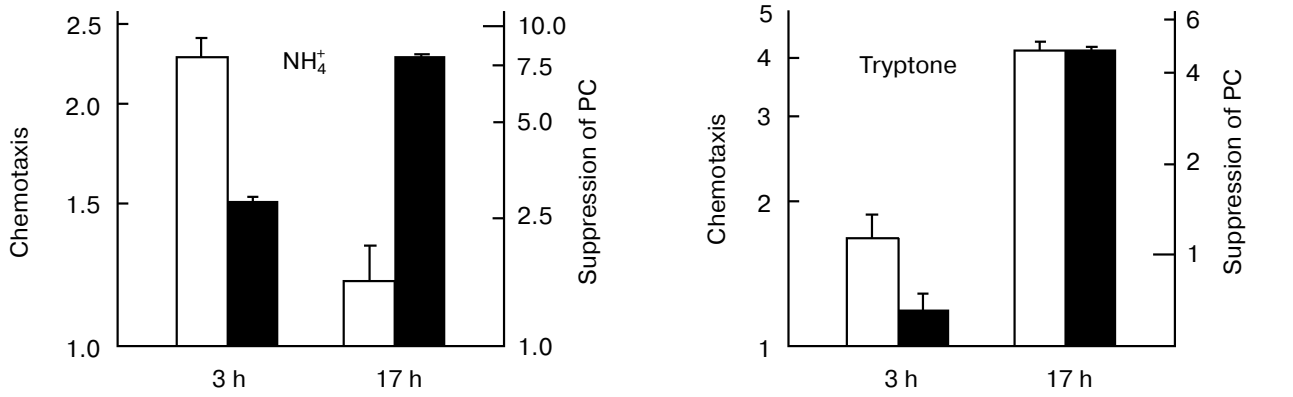

Fig. 4. Chemotaxis and the suppression of photoreceptor currents (PC) after the addition of ammonium (30 mM) or tryptone (0.1%) at two characteristic times in the course of gametogenesis in *C. reinhardtii*. The abscissa shows the time after the removal of nitrogen from the medium under illumination. The cells after 3 h of this treatment are still vegetative, and after 17 h they are mature gametes capable of fusion with gametes of the opposite sex. Chemotaxis (left axes, open bars) was measured as the ratio of the degree of accumulation of the cells at the opening of a capillary filled with a tryptone solution to the degree of accumulation of the cells at the opening of a capillary filled with control buffer. The suppression of PC (right axes, filled bars) was measured as the ratio of the PC amplitude after the addition of buffer to the PC amplitude after the addition of the test substance. The data are the mean values ± SEM of three independent experiments.

gametogenesis [20]. It is assumed that during a long (several hours) lag-period of the induction of L-amino acid oxidase the cells experience nitrogen deprivation and perceive it as the signal for the beginning of gametogenesis [20]. Preservation of chemotaxis to ammonium under these conditions gave rise to the conclusion that its switching off is regulated by genes activated at the late stages of gametogenesis in the absence of ammonium [44].

The intracellular concentration of ammonium has been suggested to regulate the sensitivity of chemotaxis towards this substance [44]. This hypothesis, however, cannot explain preservation of chemotaxis to ammonium in the presence of Arg in the medium. The absence of acetate influence on this process [44] indicates its independence of extracellular desamination of Arg, whereas ulilization of Arg transported into the cell by the membrane carrier most likely occurs without release of free ammonium [20].

The dependence of the degree of chemoaccumulation on the concentration of ammonium in a capillary has a bell-shaped form with the maximum at 10^{-1} M [34]. The curves for the dependence of chemoaccumulation on the external concentrations of Ca^{2+} and H^+ ions have similar shapes; maximal accumulation is observed at 10^{-3} M Ca^{2+} and pH 7 [34]. On the contrary, cell motility is practically independent of Ca^{2+} and pH over the tested ranges (from 10^{-5} to 10^{-2} M, and from 3 to 9, respectively).

Chemotaxis towards ammonium undergoes a circadian rhythm with the maximum in the middle of the dark period [41]. The uptake of radioactive methylammonium used as the tracer for measurement of ammonium uptake shows a rhythm with the same period length, but phase-shifted 6 h compared to the rhythm of chemotaxis to ammonium. The authors explain this shift by the necessity of light for ammonium uptake [41].

Among other protists, *Paramecium* displays pronounced chemotaxis towards ammonium [9]. The mechanism of this process is relatively well studied. Using ion-selective microelectrodes [49] or a permeable fluorescent pH-indicator [50] it has been shown that the addition of ammonium induces a rapid basification of the cytoplasm in *Paramecium*. This can be explained by proton binding by NH_3 that penetrates across the lipid bilayer. Furthermore, it has been established that ammonium causes hyperpolarization of the cell membrane, which in *Paramecium* induces an increase in the ciliary beating frequency leading to an increase in the linear swimming velocity, and a decrease in the frequency of spontaneous reversals of beating direction leading to an increase in the duration of forward swimming [51]. These two changes bring about a movement of the cell up the chemical gradient (more about mechanism of chemotaxis see below). The reason for membrane hyperpolarization after the addition of ammonium is not yet clear. It has been suggested that the membrane might be hyperpolarized by a cation efflux via channels in the plasma membrane, which are activated by an increase in the intracellular pH [9].

Chemotaxis towards ammonium in *Chlamydomonas* might also involve a similar mechanism operating without any specific receptors for this substance. Genes homologous to *amt*-genes, the products of which serve as transmembrane NH_3 transporters in bacteria [52], have been also found in *C. reinhardtii*, although it is not known whether they play any role in chemotaxis to ammonium.

On the other hand, it cannot be ruled out that, in contrast to *Paramecium*, in *Chlamydomonas* ammonium

is transported across the membrane in the form of NH_4^+ ions. The addition of ammonium to *C. reinhardtii* suspensions leads to suppression of photoreceptor currents (Fig. 4). Changes in the amplitude of photoreceptor currents likely indicate changes in the membrane potential, direct measurement of which with intracellular microelectrodes is difficult in small *Chlamydomonas* cells. This hypothesis is corroborated by the results of experiments with red background illumination, which induces hyperpolarization of the membrane [53] and an increase in the amplitude of photoreceptor currents [32] in the green flagellate alga *Haematococcus pluvialis*.

A decrease in the amplitude of photoreceptor currents in *Chlamydomonas* upon the addition of ammonium should reflect de-, rather than hyperpolarization of the membrane, which has been measured in *Paramecium*. In *Chara* internodal cells the addition of ammonium at pH < 9 also results in depolarization of the membrane, which has been interpreted as the transport of NH_4^+, rather than NH_3 [54]. The membrane depolarization upon the addition of ammonium has also been observed in higher plant cells [55, 56].

The degree of the suppression of photoreceptor currents by ammonium increases in the course of gametogenesis in *Chlamydomonas*, while chemotaxis toward ammonium disappears (Fig. 4). The opposite directions of changes in these parameters in the case of ammonium shows that the signal transduction mechanism for chemotaxis towards this substance differs from that for chemotaxis towards tryptone, when the degree of the suppression of photoreceptor currents increase in parallel with the increase in the magnitude of chemotaxis in the course of gametogenesis (Fig. 4).

Sugars. Maltose, sucrose, xylose, and mannitol have been shown to attract *C. reinhardtii*, whereas glucose does not elicit a behavioral response in this microorganism [57]. Both vegetative cells in the logarithmic phase of growth and gametes 20 to 30 h after the beginning of gametogenesis show the sensitivity to these substances [57]. The curves for the concentration dependence for maltose and sucrose have bell shapes with the maxima at 10^{-3} and 10^{-2} M, respectively [58].

After UV or chemical mutagenesis the mutants *che1-che10* were selected, which show a decreased sensitivity of chemotaxis to one or several tested sugars, but have normal chemotaxis towards ammonium and normal photoinduced behavioral responses [57, 58]. Another group of mutants has been obtained by insertional mutagenesis: the strains *ctx2* and *ctx3* show a decreased chemotaxis only to xylose; the strain *ctx1* is not sensitive to xylose, maltose, and mannitol, but displays normal chemotaxis to sucrose; the strains *ctx4* and *ctx5* are not sensitive to all four tested attractant sugars [59].

The strain *ctx4* has been studied in more detail. It shows normal phototaxis and the photophobic response, as well as the normal deflagellation upon a pH-shock. The mutants of the *che* group and the mutants *ctx1-ctx3* have been suggested as receptor mutants, because they are deficient in chemotaxis only to some of the four attractant sugars; on the other hand, the mutants *ctx4* and *ctx5*, which are insensitive to all four tested sugars, presumably carry a lesion in some downstream element(s) of the signal transduction chain [59].

Other chemicals. *Chlamydomonas* belongs to a group of the so-called "acetate" flagellates, i.e., it is capable of utilization of acetate, which is therefore used as a component of standard growth media for cultivation of this alga [60]. Acetate does not, however, induce chemotaxis in *C. reinhardtii* [33, 34], whereas *C. eugametos* shows a dispersal only at high concentrations of acetate [30]. Pronounced chemotaxis to this substance has been found in the colonial green alga *Astrephomene gubernaculifera* [38]. In this alga, the mean linear swimming velocity and the frequency of reversals are not affected by acetate after 10 min incubation.

Organic acids that contain four or more carbon atoms in their structure induce chemoaccumulation in *C. reinhardtii*, but this effect is accompanied with a significant decrease in the average swimming velocity [33]. *C. reinhardtii* accumulation in response to Co^{2+} and Mn^{2+} has also been reported [33].

DETECTION OF THE CONCENTRATION GRADIENT AND ORIENTATION

Theoretically, accumulation of cells in a capillary filled with a solution of a test substance might result from negative chemokinesis, i.e., a decrease in the average swimming velocity in the cells accidentally entering the capillary. However, a relatively high speed of accumulation observed in *Chlamydomonas* argues against this explanation. Furthermore, it has been directly measured that the average swimming velocity of *Chlamydomonas* does not change in homogenous solutions of some attractants and repellents, as compared to control buffer [33]. Therefore, it can be concluded that accumulation (or dispersal) of *Chlamydomonas* cells in a capillary reflects their ability to move up a gradient of the concentration of the test substance.

A question arises, how can *Chlamydomonas* cells detect a gradient of a chemical stimulus? There are two different basic strategies for this: 1) direct measurement of a spatial gradient by comparing the stimulus intensities at the opposite ends of the cell at the same instant of time; 2) conversion of the spatial gradient to the temporal gradient, i.e., comparing the stimulus intensities with the same sensor in different (consecutive) instances of time, between which the cell travels a certain distance. According to theoretical models, relative efficiencies of these two strategies depend on the steepness of the stimulus gradient, the size and shape of the cell, the type of

locomotion (movement on a solid surface, or in liquid medium), and the speed [61, 62].

Leukocytes and the mixamoebae *Dictyostelium* use the first strategy for chemotaxis [63], whereas chemotaxis in the enterobacteria *Escherichia coli* and *Salmonella typhimurium* is based on the second strategy [64]. Calculations show that physical parameters of bacterial cells do not rule out their using the first strategy under certain conditions [62]. Experimental evidence to support this conclusion has been recently obtained in a study of chemotaxis in certain marine bacteria [65].

Regardless of the strategy used for its detection, orientation of an object along the gradient may be achieved by different mechanisms. One can distinguish: 1) "real" orientation, when the direction of progressive movement of the cell coincides with the direction of the stimulus gradient; 2) so-called "orientation by trial and error" (biased random walk). In microorganisms that freely swim in liquid medium along helical paths "real" orientation can be achieved by so-called "helical klinotaxis", which was first suggested as a theoretical model [66], and subsequently observed experimentally during chemotaxis in *Paramecium* [67], the ciliate *Strombidium* [68], and in the bacterium *Thiovulum* [69].

On the contrary, the best studied chemotaxis in enterobacteria is based on the second mechanism of orientation (for review see [70]). In these bacteria, the movement is characterized by spontaneous changes in the swimming direction, the probability of which is proportional to the change in the stimulus intensity in the course of the movement. In the other words, when the attraction concentration increases (or the repellent concentration decreases) in the course of the movement, the bacterium swims progressively forward for a longer time than in the opposite situation. As the net result, the position of the bacterium shifts along the stimulus gradient, although no "active" orientation occurs.

A similar mechanism of chemotaxis (biased random walk) is also known in eukaryotes. In the ciliate *Paramecium*, attractants not only reduce the frequency of spontaneous directional changes, as it occurs in bacteria, but also increase the linear velocity, whereas repellents induce the opposite effects [71]. The influence of chemical stimuli on the frequency of spontaneous directional changes has been also demonstrated in the ciliate *Tetrahymena* [72]. Finally, the same model applies to chemotaxis in leukocytes, although they crawl upon the solid surface and directly sense a spatial gradient of the stimulus concentration [73]. It has to be noted that sometimes such behavior in eukaryotes is called not "chemotaxis" but "chemokinesis" [51], or "oriented chemokinesis" [72], whereas the term "chemotaxis" is reserved only for the behavior based on the "real" orientation. Some eukaryotic cells are likely to combine both mechanisms of orientation up a gradient of a chemical stimulus. For instance, in male gametes of the brown alga *Laminaria* the frequency of spontaneous directional changes increases when the cell moves down the gradient of the concentration of a pheromone released from the female gametes, but, in contrast to bacteria, the new direction of movement after a stop is not random, but points to the pheromone source [74].

Chemotaxis in *Chlamydomonas* is not sufficiently studied to draw final conclusions about the strategy of detecting the stimulus gradient and mechanism of orientation inherent in this microorganism. Nevertheless, it can be proposed that flagellate algae are likely to use the "trial and error" mechanism of orientation, because their movement is characterized by spontaneous directional changes, as it occurs in other microorganisms. A system of intracellular oscillators has been suggested to control the frequency of these changes in green flagellate algae [75]. The existence of such system has been first recognized in the green flagellate alga *Haematococcus pluvialis* by recording periodic electrical activity of the cell membrane [76-78] and periodic micromovements of the protoplast [79]. On the other hand, a possibility of "active" orientation in *Chlamydomonas* chemotaxis can also be considered.

According to preliminary data, the *C. reinhardtii* mutant *ptx1* displays normal chemotaxis to ammonium [80], but lacks chemotaxis to sugars [81]. The *ptx1* mutation affects the characteristic for the wild type differential sensitivity of the axonemes of the two flagella of the cell to Ca^{2+}, which is necessary for photoorientation [82]. If these data are confirmed, one may conclude that chemotaxis towards ammonium and chemotaxis towards sugars are based on different mechanisms of orientation.

PHYSIOLOGICAL ROLE OF CHEMOTAXIS

The attraction of male gametes by sexual pheromones excreted by female gametes, as it occurs in *C. allensworthii*, is frequently observed in eukaryotic protists, as well as in higher animals and plants [2, 21]. In most cases the pheromone-releasing eggs are non-motile, which, in the first place, provides the efficiency of chemical communication between gametes of the opposite sexes. Female gametes of *C. allensworthii*, however, have flagella and actively swim, as do male gametes [22]. It is not yet clear how chemotaxis can contribute to gamete fusion in this situation.

As it has been already mentioned above, tryptone is used as the growth medium supplement for heterotrophic strains of *C. reinhardtii*; therefore it can be suggested that chemotaxis towards tryptone might guide the cells to the food source. A similar role has been considered for chemotaxis in *Paramecium*, because in this case the chemotaxis stimuli are the products of metabolism of bacteria upon which this ciliate feeds [83]. On the other hand, the existence of chemotaxis to tryptone only in

gametes, but not in vegetative cells of *C. reinhardtii* (even after nitrogen deprivation under conditions not leading to gamete formation), implies its possible significance for communication between gametes of the two opposite sexes. But, both chemotaxis and the inhibition of photoreceptor currents by tryptone are observed in gametes of both plus and minus strains of *C. reinhardtii* (gametes in this isogamous species are not referred to as male and female, but rather as plus and minus gametes, respectively), although the mean sensitivity of photoreceptor currents to tryptone is slightly higher in the tested plus strains than in the minus strains. It is not yet known if this difference has any biological relevance.

The adaptation significance of chemotaxis is probably not confined to the guidance to the food source, and, for gametes, to the attraction of gametes of the other sex. In particular, *Chlamydomonas* cannot metabolize sugars and mannitol [60]; therefore, chemotaxis to these compounds cannot be related to the search for the carbon source. Attraction to non-metabolizable substances is well known, for instance, in enterobacteria [84]. On the other hand, it has been suggested that chemotaxis towards sugars in *C. reinhardtii* might facilitate attraction of the gametes of the other sex, since the structure of pheromones in *C. allensworthii* comprises a xylose residue [59]. But, the presence of this type of chemotaxis not only in gametes, but also in vegetative cells argues against this hypothesis. Therefore, a possible physiological role of chemotaxis to sugars has yet to be established.

To conclude this review, we would like to note that the presently available, however limited, data on chemotaxis in *Chlamydomonas* unequivocally show that this process is an interesting example of regulatory motile responses, and that elucidation of molecular mechanisms for reception and transduction of chemical stimuli in this eukaryotic microorganism might contribute to the expansion of our conception for the evolution of chemosensory systems.

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