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Symposium: "Protistan Autotrophic/Heterotrophic Interactions" Introductory Remarks

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THIS afternoon symposium is trisponsored by ASLO (American Society of Limnology and Oceanography), PSA (Phycological Society of America), and SP (Society of Protozoologists). A number of those attending belong to two of these societies; very few belong to all three. Yet there is a commonality of interest which we hope will be stimulated by these papers. The autotrophic and heterotrophic organisms dealt with here attract attention from a variety of disciplines and specializations, from taxonomists and ecologists to behaviorists and molecular biologists, though we sometimes seem unsure what to call the subjects of our research (protozoans? protists? protoctists? algae?).

Each of the three societies suggested possible speakers; the final choice was mine. In these presentations I aimed for as diverse a melange as possible without straying too far from the main theme. Thus, we are treated to summaries and reports starting with protistan responses to molecular chemical stimuli and theoretical considerations of their tactic responses. These are followed by a look at how heterotrophs consume autotrophs, how mixotrophic protists combine these nutritional modes, and the impact protists have on carbon and nitrogen cycling. Finally, since some of the problems presented will surely get each of us thinking about what we would like to do with protists, there will be a discussion of a source of research cultures (primarily autotrophic) which, up to now, has perhaps been less well known to ASLO and SP members than to PSA.

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Chemoresponse Mechanisms: Toward the Molecular Level[1]

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I will begin this review by stating the obvious: unicellular organisms detect and respond to chemical stimuli in their environment. The stimuli signify the presence of food, mates, toxic conditions, hosts, among other things (19), and the cells respond by moving to accumulate in or disperse from the stimuli. These movements are part of taxic mechanisms in which the cells orient and move directly up or down the gradient of stimulus (3) or kinetic mechanisms in which there is no orientation but nonetheless, there is a progression toward or away from stimuli by a biased random walk or speed modulation (3). Taxes are limited to amoeboid motility, as exemplified by *Entamoeba* (1). Kineses are not so limited and come in at least two forms: klinokinesis, which requires the modulation of turning frequency, or orthokinesis, which requires the organism to modulate speed of movement (3).

The identities of at least some chemical cues are known for many protists: *Paramecium* detects folate, lactate, acetate, and cyclic AMP, which are secreted products of bacterial metabolism and probably indicate the presence of food to paramecia (15). *Tetrahymena* responds to peptides and amino acids, which likewise might indicate food (6). *Blepharisma* responds to gamones to become mating reactive, and gamone II also serves as an attractant of complementary mating type cells (8). *Entamoeba* migrates toward polysaccharides and other products that might simulate the host lumen where the trophozoites will establish themselves (1). While the identification of cues is not complete and we have more to learn about the behavioral mech-

[1] This article is based on a presentation in the symposium "Protistan Autotrophic/Heterotrophic Interactions" cosponsored by the Society of Protozoologists, Phycological Society of America, and the American Society of Limnology and Oceanography at the University of Rhode Island on June 26, 1986.

anisms by which cells respond, it is time to move on and to ask: what are the chemical messages transduced into? What are the second and third messengers of transduction? It is time to bring state-of-the-art immunological techniques to the identification of pathway components and to clone genes for receptors and other components in order to study and manipulate them.

The need to turn to molecular techniques is not unique to protists; chemoreception science in general needs to move to more molecular levels. There are advantages, however, to using protists in dissecting chemosensory transduction pathways. For example, *Tetrahymena* and *Paramecium* can be grown in mass culture to provide not only large amounts of material, but also homogeneous cell populations. The large sizes of ciliates make them amenable to electrophysiological studies. Short generation times make genetic manipulation feasible in order to provide mutants that are so useful in a dissection, because, in theory, no pharmacological approach to the study of a complex pathway can approach the possibilities of a genetic approach.

In order to illustrate the progress and possibilities of studying chemoreception using protists, I will use an example from my laboratory's work on *P. tetraurelia* and folate chemoresponse. Paramecia are attracted to folate, which probably signifies food. The cells respond by a kinetic mechanism that results in fast, smooth swimming up gradients of attractant (15). Through the use of mutants, we have shown that paramecia modulate both frequency of turning and speed, which add up to the net population response of attraction or dispersal (18). From the elegant work of Jennings, Eckert, Naitoh, Kung, Machemer, and others (4), we know that turning depends upon the transient reversal of cilia caused by a calcium action potential and that speed depends upon frequency and angle of ciliary beating, which are controlled by membrane potential (2). The understanding of these individual aspects of *Paramecium* physiology, when put together, allowed us to make predictions about the effects of chemoattractants and repellents on membrane potential, and these predictions were borne out by direct electrophysiological testing (16). It was very satisfying to realize that even a complex behavior makes sense in terms of the components of basic *Paramecium* physiology.

How then does an external chemical stimulus eventually affect membrane potential, which in turn, controls ciliary motility? The accepted paradigm is that receptors bind the stimulus, and this binding is transduced into internal information, that is, second and third messengers. Receptors are identified by first examining the number and affinities of surface-binding sites, among which should be the receptor. Studies of ^{3}H-folate binding indicated that there are saturable, specific binding sites on the *Paramecium* cell and that the binding to these sites is greatly reduced in a chemoreception mutant (12). Fluorescein-folate was also used to investigate binding of folate to whole cells (20). Normal cells show fluorescence that is specific for folate while mutant cells show little discernable fluorescence (20).

The next step in receptor characterization is the identification of binding proteins from the cell membrane. The folate-binding proteins of interest on *Paramecium* were not to be found on the cilia, which comprise about 50% of the surface membrane, but on the cell body membrane (10, 12), and these binding sites were not evenly distributed down the cell as indicated by local perfusion during electrophysiological recording (10). We have identified folate-binding proteins from the cell body membrane by affinity chromatography and determined that five of these are surface exposed (unpubl. results), as expected for a receptor. Like many other external chemoreceptor systems, the binding proteins involved in chemoreception should be of relatively low affinity. Therefore, to circumvent problems of affinity chromatography of weakly binding ligand, we turned to the sensitive method of immunodetection to identify folate-binding proteins. The rationale was that the chemoreceptor would be among the membrane proteins to which we could crosslink folate because crosslinking folate onto whole cells specifically inhibited attraction to folate, but not to acetate (17). Membrane proteins crosslinked with folate were identified by electroblotting proteins from polyacrylamide gels onto nitrocellulose and immunodetection of the nitrocellulose with anti-folate antibodies. Currently, we are cataloging crosslinked proteins, which compare well with affinity chromatography proteins, and are examining crosslinked proteins from chemoreception mutants (unpubl. results).

The receptor binding should elicit a second and perhaps third messenger, which in the case of *Paramecium* must account for the change in membrane potential, change in ciliary beating, and adaptation. The first messengers to come to mind are the following: internal calcium, internal pH, cyclic nucleotides, IP_3. There is indirect evidence of a role for calcium in chemoreception and we are examining internal calcium movements using not only electrophysiology but also calcium-sensitive fluorescent dyes (13). These dyes can be loaded into the cells and give fluorescence signals large enough to be useful without causing extreme buffering of internal calcium with the consequent destruction of chemoresponse. Likewise, internal pH can be examined with permeant fluorescent dyes. Cyclic nucleotides have been implicated in ciliary beating control (2, 5, 7, 14) and levels of cyclic nucleotides (M. Gustin, unpubl. results, 6, 10) can be examined by HPLC (Van Houten, unpubl. results) and radioimmunoassay (M. Gustin, unpubl. results), among other methods. There is a recently renewed appreciation for the role of inositol phospholipids in receptor function (9). Phosphoinositol lipids can be labeled and quantified in protozoa and, indeed, at this meeting there was a report of a mechanical stimulation possibly involving IP_3 release of internal calcium stores and diacyl glycerol stimulation of protein kinase C in *Heliophrya*.

In summary, the descriptions of the chemoreception pathways in protozoa are incomplete, but the technical means are available to study these pathways at the molecular level, and mutants will be a particular advantage to the use of protozoa in the study of chemoresponse.

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Feeding and Swimming Behavior in Grazing Microzooplankton[1,2]

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ABSTRACT. A random-walk model of food-searching behavior is considered for the microzooplankton. It is suggested that in still waters a random walk of the conventional sort, modeled by a Wiener process, is less efficient than a Levy walk (a random walk whose excursions follow a Levy distribution) with Levy parameter less than two. For Levy parameter less than one, however, little advantage is gained by further reduction. In turbulent water, on the other hand, dispersion due to a random walk is dominated by the turbulent diffusion of the medium so that the Levy parameter appears to be less important. The effect of chemosensory responses is considered. It is suggested that these are most useful in still water, whereas in turbulent water their value would be less, and a non-specific filtering behavior might be more plausible.

ECOLOGISTS are beginning to realize that the microzooplankton—ciliates and phagotrophic flagellates—are in some cases a critical component in marine and freshwater planktonic foodwebs (19). For this reason, the feeding behavior of such organisms is of interest but we have little actual information about this in the natural setting. The purpose of this paper is to explore the consequences of alternative types of behavior and to offer some predictions about adaptations to various hydrodynamic regimes. The argument will be abstract in the absence of data, involving a certain family of probability distributions, the general properties of turbulent flow, and spatio-temporal scales of swimming activity of the microzooplankton.

We shall start by assuming that these organisms swim about at random, grazing on either bacteria or other eukaryotes, and that the random swimming is of the general nature of a random walk: approximately straight paths, of varying lengths, punctuated by (random) changes in direction. This picture conforms roughly to what has been observed in the laboratory with certain well-studied species (e.g. 10, 12, 21).

FENCHEL'S MODEL: CAN MICROPHAGOTROPHS SURVIVE IN OFFSHORE WATERS?

Several years ago Fenchel (8, 9) calculated the likelihood that a bacterivorous suspension-feeding ciliate would find enough food to survive at various prey densities and concluded that there would not be enough food to support ciliates in the oligotrophic open oceans. He assumed that the ciliates were continually filtering the water as they swam. By estimating the maximum pressure drop that could be sustained across a filter consisting of a row of closely spaced cilia, a maximum rate of flow through the filter was calculated. This, multiplied by the cross-sectional area filtered, gave a filtering rate and multiplying this by the number-density of food organisms gave a feeding rate. Given the number-densities of food bacteria reported in oligotrophic waters, Fenchel concluded that the feeding rate would be too low for ciliates to survive.

Implicit in this argument was the assumption that filtering is a continual process; the grazer is a sort of animated vacuum cleaner permanently switched on. This may have been a reasonable assumption in the absence of other information, but some recent experiments with a few species suggest that swimming behavior of some microzooplankters may be more complex than this. In particular, reports of changes in swimming behavior in response to chemical signals (2, 12, 15; Stoecker,

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