Review

Chemosensory Transduction in Paramecium

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This review is dedicated to Dr. Mihoko Takahashi

The behavior of ciliated free living protozoa has fascinated scientists since the nineteenth century. Jennings described in his important treatise on the "Behavior of Lower Organisms" (Jennings, 1906) his observations of *Paramecium* as it is attracted to CO_2 or weak acids and repelled from high amounts of NaCl and other salts. He very astutely described the reaction to stimuli through the "avoiding reaction," a turn in the swimming path that is elicited as a cell leaves an attractant or enters a repellent. He also had the insight that the cell responds to changes in its chemical environment. Given that firm foundation, the study of responses to chemical stimuli was continued by Dryl who screened many compounds for chemoresponses of *P. caudatum* (e.g. Dryl, 1963; see Dryl, 1976 for review). As did Jennings, Dryl found that cells change direction at the boundary of drops when leaving an attractant or moving into relative repellent, leading Dryl to propose that the strength of a repellent is proportional to the frequency of avoiding reactions it elicited.

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Interestingly, Nakatani measured velocity of swimming and found that cells increase the speed of forward movement in an attractant (Nakatani, 1968), but he incorporated Dryl's view of avoiding reactions into his model for chemosensory behavior. Even though Dryl and Nakatani disagreed upon the classification of some attractants and repellents (e.g. ethanol), there was agreement from very early days of the study of chemosensory transduction in *Paramecium* that the key to the behavior of the population (attraction and repulsion) was the modification of the frequency of turning and swimming speed. (See Van Houten and Preston, 1988 for review).

The studies of the physiology of *P. caudatum* and later *P. tetraurelia* made it possible to understand the action potential that underlies the avoiding reaction. As the work of Naitoh, Kaneko, and Eckert demonstrated (reviewed in Machemer, 1988a), the voltage gated calcium channels of the cilia open with a depolarizing receptor potential, bringing Ca^{2+} into the cilia which modify their power stroke to cause the transient change in swimming direction (Machemer, 1988b; Eckert, 1972; Preston and Saimi, 1990; Eckert and Brehm, 1979). As Ca^{2+} is sequestered or removed, the cell's swimming changes to a pivot in place and finally resolves into forward swimming. The re-

sult of the action potential and change in beat form is an abrupt change in swimming direction, a turn in the swimming path which is the avoiding reaction described by Jennings.

Machemer examined the relationship between frequency of ciliary beat and membrane properties, including membrane potential. He found a correlation between a small increase in cilia beat frequency with an increase in negative membrane potential from rest, and a small decrease in ciliary beat upon a small depolarization from rest (Machemer, 1974). A larger depolarization would trigger a calcium action potential and also an increase in the reversed power stroke kind of beating. These changes in ciliary beat frequency directly correlate with increased speed and decreased speed when there are small hyper- and depolarizations of the membrane potential.

As described above, the behavior of cells as they enter relative repellents (i.e. leave attractants or enter repellent areas) is characterized by frequent turns, avoiding reactions, implying that repellents depolarize the cells and attractants hyperpolarize them. Nakatani's observation of faster swimming in attractants is consistent with this, because hyperpolarization in attractants would lead to faster ciliary beating.

Van Houten and colleagues began studies of *P. tetraurelia* chemoresponses in the early 1970's with the development of a simple T-maze assay (Van Houten et al., 1978 and 1982). Unlike Dryl or Nakatani, Van Houten measured both turning frequency and speed in order to assess the possibilities of both a klinokinesis (turning frequency) mechanism as in bacterial chemotaxis and an orthokinesis (speed modulation) mechanism for attraction and repulsion (Van Houten et al., 1978). Van Houten and workers confirmed that organic attractants like acetate and lactate caused cells to swim smoothly and fast, and repellents like quinidine or high salt, caused cells to swim slowly and turn frequently (Van Houten, 1978). Interestingly, if the behaviors were pushed to an extreme, such as extremely fast swimming in high pH or extremely slow net movement due to frequent turns in high $BaCl_2$, the cells would be repelled by fast swimming or attracted by being trapped in a frenzy of turning (Figure 1). A mutant that was repelled by the attractant acetate was found to swim extremely fast in the acetate solution, confirming that cells could be repelled by a swimming speed mechanism (Van Houten, 1977).

Van Houten argued for a chemokinesis mechanism of attraction and repulsion as opposed to a chemotaxis mechanism which implies an orientation and directed movement toward a source. Instead, most organic stimulus attractants, like acetate, attract a population of paramecia by a biased random walk in which cells swim in long mean free paths when swimming up a gradient of attractant, and when a spontaneous turn sends them down or across the gradient, they have a short mean free path before there is another turn. Van Houten and colleagues also took advantage of Pawn mutants of *P. tetraurelia* that cannot turn for lack of functioning ciliary calcium channels (Saimi and Kung, 1982). These mutants became important tools because, despite not being able to turn, they could still be repelled by stimuli that caused extreme behavior (Van Houten, 1978). For example, very high pH caused Pawns to be repelled probably by the very fast swimming it elicited, as it did in wild type cells. Van Houten compared wild type to Pawn swimming behavior and chemoresponse from real data and those from a

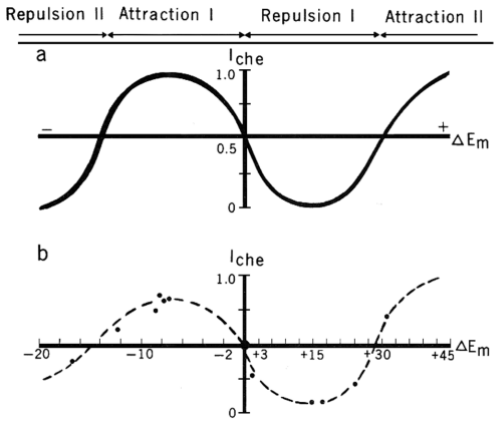

Fig 1. from Van Houten, 1979 (a) Graphical description of membrane potential control of chemokinesis. Change of membrane potential (ΔE_m) from control (at origin) is plotted against the index of chemokinesis; $I_{che} > 0.5$ indicates attraction; < 0.5 indicates repulsion. As chemical stimuli change E_m relative to control, animals will be attracted or repelled, depending on the magnitude and direction of the E_m change. (b) Experimental data plotted as ΔE_m produced by the attractant or repellents versus I_{che}. Scale of ΔE_m is different for depolarizing an hyperpolarizing stimuli.

computer simulation. In this manner Van Houten and Van Houten (1982) identified the components that were essential for attraction and repulsion. The most crucial included the ability to turn, adaptation, and an immediate response upon leaving an area of attractant. When extreme conditions were examined, the ability to modulate speed became very important.

Van Houten and colleagues put forward and experimentally confirmed a hypothesis of membrane potential control of chemokinesis (Van Houten, 1978, 1979). Since that time, the hypothesis has been tested with additional stimuli and mutants, all consistent with small membrane hyperpolarizations in organic attractants, and small depolarizations in repellent stimuli such as quinindine (Van Houten, 1979; Preston and Van Houten, 1987a,b) (Figure 2).

Over time, Van Houten proposed that there are at least three signal transduction pathways in *P. tetraurelia* (Figure 3). Two initiate at receptors (e.g. for folate, cyclic AMP, acetate, glutamate) that all hyperpolarize the cell initially by activating a K conductance, and secondarily by activating a plasma membrane calcium pump (PMCA) to sustain the hyperpolarization. The two signal transduction pathways differ in the second messenger that activates the PMCA: calcium/calmodulin for folate, for example, and cyclic AMP activation of PKA for glutamate (Yang et al., 1997; Wright et al., 1993, 1994; Elwess et al., 1997). The third pathway requires no receptor, but still initiates a K conductance to hyperpolarize the cell. Ammonium chloride crosses the membrane as ammonia and rapidly alkalinizes the cell; each pulse of ammonium chloride onto the cell results in a pulse of increased pH (Davis et al., 1994). More details of the subsequent studies that support this model are included in the review of the field below.

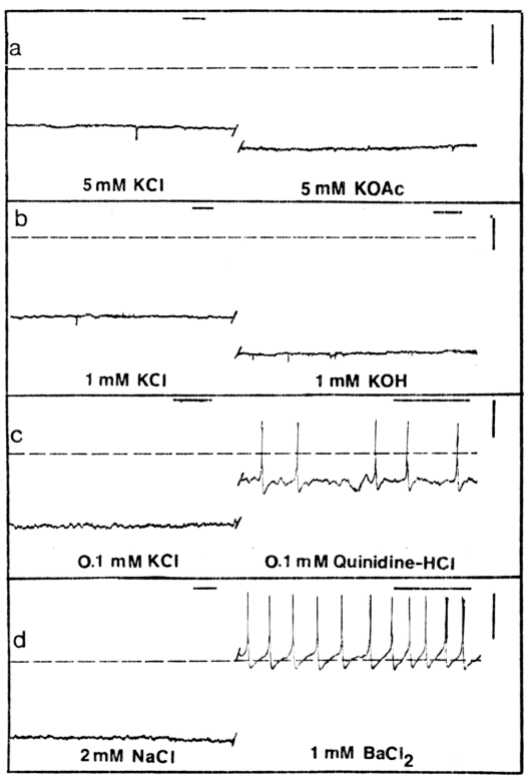

Figure 2 from Van Houten, 1979
Membrane Potentials in: a. Attractant Type I (Acetate) b. Repellent Type II (KOH) c. Repellent Type I (Quinidine-HCl) d. Attractant Type II ($BaCl_2$ relative to KCl)

Since these beginnings, there have been more studies of chemoresponse in *P. tetraurelia* and *P. caudatum*. Oami examined the responses of *P. caudatum* to bitter substances, such as quinine, chloroquine, brucine, and strychnine (1996 a,b). These act as repellents and the application of bitter substances in low concentrations elicited avoidance responses. Oami used a mutant CNR (Takahashi, 1978) that, like the Pawn mutant of *P. tetraurelia,* is defective in the voltage gated calcium conductances of the cilia to examine membrane potential changes in the absence of interfering action potentials (1998). Using this approach, Oami showed that the membrane potential changes elicited were biphasic, that is, an initial depolarization followed by a hyperpolarization (1998). These bitter substances caused measurable changes in membrane potential when applied to either end of the cell, a depolarization upon anterior application and a transient hyperpolarization with posterior application implying that their receptors are not evenly distributed much as the mechano receptors are not evenly distributed (Naitoh and Eckert, 1969). When the chloroquine, strychnine, and brucine were applied in succession or in mixtures, the cells's responses were diminished implying that they have a common receptor or other transduction component. An exception to this was quinine, which appears to have a separate receptor or pathway.

The neurotransmitter GABA has been found to be secreted by *P. primaurelia* (Ramoino et al., 2003). The extensive pharmacology of GABA

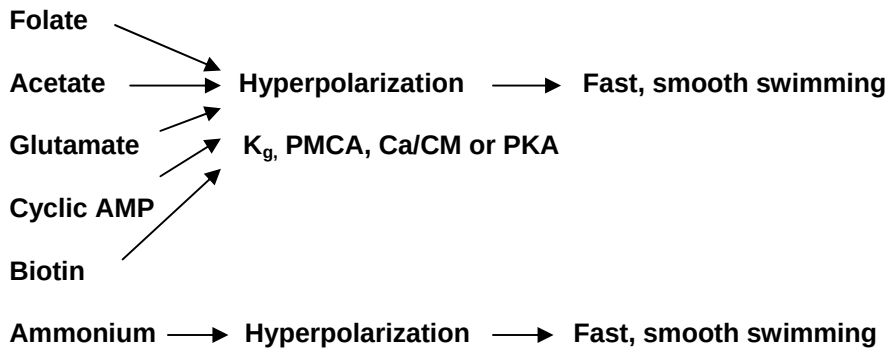

Fig. 3. Chemoresponse Summary
Paramecium tetraurelia show attraction to bacterial metabolites – folate, acetate, glutamate, cyclic AMP, biotin, and ammonium among others.

receptors has allowed for the distinction between $GABA_A$ and $GABA_B$ receptor effect on *P. primaurelia* swimming behavior. Agonists of $GABA_A$ cause a depolarization of *P. primaurelia*, possibly through a Cl^- efflux, and opening of the voltage gated calcium channels that cause action potentials and turns (Bucci et al., 2005). Agonists of $GABA_B$ reduce the backward swimming behavioral response to depolarization through a G protein dependent pathway and inhibition of a dihydropyridine sensitive calcium channel (Ramoino et al., 2003).

Preston and Usherwood (1988 a,b) described attraction to L-glutamate, hyperpolarization in submicromolar amounts of glutamate, and binding of ^{3}H-glutamate to *P. tetraurelia* cilia. More recently, the Van Houten lab identified a candidate gene for an L- glutamate chemoreceptor using RNA interference (RNAi) (Jacobs, 2007). When a gene for a *P. tetraurelia* ortholog of an NMDA-like receptor was down-regulated using RNAi, chemoresponse to L-glutamate was specifically lost, while other chemoresponses were not affected, including that to D-glutamate. Down-regulation of other candidate genes at the level of mRNA did not have any effect on chemoresponse to glutamate. In the L-glutamate chemosensory transduction pathway, the receptor couples by an unknown means to an adenylyl cyclase. Cyclic AMP rises very rapidly when cells are exposed to glutamate, presumably activating protein kinase A (PKA) because kinase inhibitors specifically affect chemoresponse to L-glutamate but not to other stimuli (Yang et al., 1997; Smith et al., 1987). RNAi for PKA catalytic subunit also specifically decreases the response to L-glutamate, suggesting that this is the kinase involved (Pantel, 2007). PKA activation of the plasma membrane calcium pumps (PMCAs) would explain the sustained hyperpolarizing conductance and sustained membrane hyperpolarization that we observe with constant stimulus application (Wright et al., 1993, 1994).

The Van Houten lab has pursued the folate chemoreceptor for many years (e.g. Schulz et al., 1984; Sasner and Van Houten, 1989), and recently, using homology cloning and bioinformatics of the annotated genome, found sequences that could be tested using RNAi (Weeraratne, 2007). Down regulation of the mRNA for the putative folate chemoreceptor sequence led to specific loss of folate chemoresponse. Using an antibody raised against a portion of the receptor, we also demonstrated that RNAi caused a loss of the protein from the cell body membrane and binding sites on the cells. Interestingly, the protein is not in the ciliary membrane, which agrees with the previous binding studies (Schulz et al., 1984).

The folate chemoreceptor is glycosyl-phosphatidyl inositol (GPI) anchored, as predicted by bioinformatics and shown more directly by Western blots using anti-cross reacting determinant antibody against the GPI anchor (Weeraratne, 2007). Many *Paramecium* surface proteins are

GPI anchored, and our studies using RNAi, blocking antibodies against GPI anchored proteins and heterologous folate receptors predicted that the folate chemoreceptor would be a peripheral GPI anchored protein (Paquette et al., 2001; Yano et al., 2003).

Another set of studies that have provided important insights into how chemical stimuli are sensed and result in a motor response come from the experiments with biotin and acetate as attractants (Bell et al., 1998; 2007). Although receptors for biotin and acetate were characterized but not purified, it was still possible to use these two very different stimuli that do not interfere with each other to examine the responses of cells as they enter or leave areas of the stimulus. The key to these studies was the use of various mutants that have defects in a conductance, thanks to the work of Kung and colleagues (Saimi and Kung, 1987; Preston and Saimi, 1990). The results with these mutants showed us that attraction to acetate depends strongly on the on-response and a calcium activated K conductance ($I_{K(Ca,\ h)}$) while attraction to biotin depends on the on-response as well, and the opening of $I_{K(Ca\ h\ or\ d)}$, but also the off-response depolarization, which is initiated by a calcium conductance large enough to open other calcium dependent cation channels (Bell et al., 2007).

Lipid raft microdomains also figure into chemosensory transduction probably as organizing areas for the signaling components (Pike, 2003). Sucrose gradient studies and disruption of sterol content of the membranes (and hence of lipid raft structure which depends on sterols) show that signaling components like the adenylyl cyclase, PMCA2, and the GPI anchored folate receptor are found in lipid rafts and the disruption of rafts with methyl-β-cyclodextrin affects chemoresponse, especially to glutamate and cyclic AMP (Chandran, 2004; Pan, 2007; Ray, 2008). It appears that receptors and PMCAs and second messenger enzymes for chemosensory transduction may be organized into different lipid domains and that microdomains of the cilia also differ from those on the cell body membrane.

There are still other studies of *Paramecium* responses to nucleotide triphosphates that we can only briefly mention here (Clark et al., 1993, 1997 ; Mimikakis et al., 1998; Shering and Plattner, 2003). Likewise, we cannot review the studies of ammonium chloride chemoresponse (Davis et al., 1998) or the evidence for G proteins (De Ondarza et al., 2003). Nonetheless, hopefully this brief review shows that the field of chemosensory transduction in *Paramecium* is characterized by significant studies that are providing important insights into the proteins and signal transduction pathways involved in responses to a large variety of stimuli. What these studies have in common is the inclusion of multiple approaches that *Paramecium* as a model organism makes possible: biochemistry of membranes and second messengers, behavioral analysis, and electrophysiology. Now that the genome is sequenced and annotated, progress in the field begun by Jennings in 1906 will accelerate in this new century.

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