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Characterizing the Polycation Receptor of Paramecium

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### Characterizing the Polycation Receptor of Paramecium

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**Introduction**

Unicellular eukaryotes are complex systems, performing all the tasks needed for survival within the context of a single cell. Protozoans, such as *Tetrahymena* and *Paramecium*, use chemosensory systems to detect food and to avoid predation.

Both *Tetrahymena* and *Paramecium* have been used as models for studying chemoreceptors. Lysozyme, ATP, and GTP have been found to have chemorepellent activity in both ciliates. In *Tetrahymena*, several PACAP isoforms have been shown to bind to the same receptor as lysozyme, indicating that this receptor may be a more general “polycation receptor” (Keedy et al., 2003). The polycation receptor in *Tetrahymena* appears to be a G-protein linked receptor which activates adenylyl cyclase and phospholipase C (Keedy et al., 2003).

The lysozyme receptors have been affinity purified from both *Paramecium* and *Tetrahymena*. The molecular weight of the *Tetrahymena* protein is approximately 42 kD, while the molecular weight of the *Paramecium* protein is approximately 58 kD (Kuruvilla and Hennessey, 1998).

In our current study, we examined the hypothesis that *Paramecium* have a polycation receptor similar to that of *Tetrahymena*. We found that although both organisms bind similar ligands, the two have multiple differences between the two pathways (see Conclusions).

**Materials and Methods**

*Paramecium tetraurelia*, strain nd6, both generous gifts of T.M. Hennessey (SUNY Buffalo) were used in this study.

Behavioral studies were conducted as described by Hennessey et al., 1995. Briefly, individual cells were transferred from buffer into the polycation of interest, under a dissection microscope. Cells were then observed to determine whether backward swimming occurred. Ten cells were observed for each trial. At least three trials were done for each concentration point.

When inhibitors were used, cells were exposed to the inhibitor for one to two hours prior to exposure to the polycation of interest in order to allow for uptake of the inhibitor.

**Results**

### Polycation Avoidance in Paramecium differs widely from that of Tetrahymena

- **Cross-Adaptation between lysozyme and members of the PACAP family suggest that these ligands use the same receptor and/or second messenger pathway.**
  - **Table 1.** Cells were adapted to either 0.5 μM lysozyme or 50 μM VIP for 15 minutes prior to exposure to 0.5 μM lysozyme, 50 μM VIP, 10 μM PACAP 1-38, or 10 μM PACAP 1-27. Cells were not adapted to any of the PACAP isoforms because prolonged exposure to high concentrations of PACAP is lethal to *Paramecium*. Each trial represents 10 cells. A minimum of three trials was conducted for each concentration. These data are similar to results previously obtained for *Tetrahymena* (Keedy et al., 2003)

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Lysozyme</th>
<th>PACAP 1-38</th>
<th>PACAP 1-27</th>
<th>VIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 μM</td>
<td>20 ± 10%</td>
<td>20 ± 10%</td>
<td>23.3 ± 5.8%</td>
<td>16.6 ± 5.8%</td>
</tr>
<tr>
<td>50 μM</td>
<td>5% ± 20</td>
<td>5% ± 20</td>
<td>6.6 ± 5.8%</td>
<td>10 ± 0%</td>
</tr>
</tbody>
</table>

- **The Pharmacological Profiles of the Receptor Also Differ Between the Two Organisms**
  - **Table 2.** Summary of Pharmacological Data in *Tetrahymena* and *Paramecium*.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th><em>Tetrahymena</em></th>
<th><em>Paramecium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>GTPγS</td>
<td>Inhibits at 1 mM</td>
<td>No effect on avoidance at 1 mM*</td>
</tr>
<tr>
<td>GTP-Antagonist-2 (BOMOL***)</td>
<td>Toxic to cells</td>
<td>No effect on avoidance at 100 μM</td>
</tr>
<tr>
<td>1-Omethyl GTP</td>
<td>Inhibits at 50 μM</td>
<td>No effect on avoidance at 100 μM</td>
</tr>
<tr>
<td>Genistein</td>
<td>Inhibits at 0.1 μM</td>
<td>No effect on avoidance at 100 μg/ml</td>
</tr>
<tr>
<td>Genistein</td>
<td>No effect on avoidance at 0 μg/ml</td>
<td>Toxic to cells</td>
</tr>
<tr>
<td>Genistein</td>
<td>No effect on avoidance at 100 μg/ml</td>
<td>No effect on avoidance at 0 μg/ml</td>
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<td>No effect on avoidance at 0 μg/ml</td>
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</tr>
</tbody>
</table>

*Keedy et al., 2003. **Bartholomew et al., unpublished data** Robinette and Kuruvilla, unpublished data Hennessey et al., 1995

### The Tyrosine Kinase Inhibitor, Genistein, Eliminates Lysozyme Avoidance in Paramecium

- **Figure 2.** Genistein (closed circles) eliminates behavioral avoidance to 0.5 μM lysozyme in *Paramecium*, while daidzein (closed diamonds), a control, has no effect on avoidance. Each point represents the mean ± SD of 2 3 trials. Each trial consisted of ten cells which were individually scored for avoidance.

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**Conclusions**

- The polycation receptor of *Paramecium* appears to have a higher affinity for lysozyme than for PACAP, while the polycation receptor of *Tetrahymena* has a higher affinity for PACAP than for lysozyme, as indicated by behavioral studies.
- Both receptors have a relatively low affinity for VIP, as indicated by behavioral studies.
- Both organisms show cross-adaptation between the various polycations tested (lysozyme, PACAP, VIP), indicating that a single polycation receptor is probably interacting with all of these polycations.
- In *Tetrahymena*, pharmacological studies indicate that the polycation receptor is a G-protein linked receptor. However, in this study, G-protein inhibitors failed to have any effect on lysozyme avoidance in *Paramecium*.
- *Paramecium* avoidance to lysozyme is inhibited by the tyrosine kinase inhibitor, genistein. This inhibitor has no effect on lysozyme/PACAP avoidance in *Tetrahymena*.
- The receptors in the two organisms exhibit very different characteristics, including molecular weight, interaction with ligands, and interaction with second messenger pathways.

**References**