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A Ciliary Sensation: Mapping Components of the GTP Signaling Pathway

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A Ciliary Sensation: Mapping the components of the GTP signaling pathway in *Tetrahymena thermophila*

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Abstract

GTP is a chemorepellent in *Tetrahymena thermophila*, causing cells to exhibit avoidance behavior, characterized by ciliary reversal. Recent work in our laboratory has shown that tyrosine kinase activity is required in order for GTP signaling to take place (Bartholomew *et al.*, submitted for publication). Second messengers which we have found to be important for GTP signaling in *Tetrahymena* include nitric oxide and cGMP. Previous studies by Kim *et al.*, 1999, have shown that a calcium-based depolarization is elicited by the application of extracellular GTP. Currently, our lab is addressing the question of where intracellular calcium is involved in the GTP chemoresponse. Addition of the membrane-permeable calcium chelator, BAPTA-AM, to the extracellular medium abolishes the GTP chemoresponse in *Tetrahymena*. However, addition of this chelator to the extracellular medium does not affect the level of GTP-induced tyrosine phosphorylation, as detected by indirect immunofluorescence. As we continue to pursue the question of where calcium is involved in GTP signaling, we will look at calcium involvement in the nitric oxide/cGMP pathway.

Experimental Design

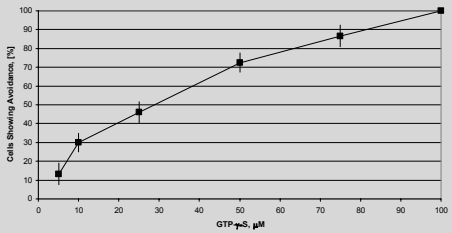
Calcium is an important signaling molecule in many systems, including *Tetrahymena thermophila*. Calcium influx is a component of GTP signaling (Kim *et al.*, 1999). In our current study, we hypothesize that calcium is required in order for ciliary reversal to occur. We use the calcium chelator, BAPTA-AM, as well as the ER calcium ATPase inhibitor, thapsigargin, in order to test this hypothesis. All of our inhibitors, as well as our GTP-γ-S, were obtained from BIOMOL.

Our previous work has shown that a tyrosine kinase is implicated in GTP signaling. In order to determine whether or not kinase activity correlates with calcium levels, we conducted indirect immunofluorescence experiments in the presence and absence of BAPTA-AM.

Finally, as we have sought to ascertain where intracellular calcium reserves are located in *Tetrahymena*, we double-labeled cells using ER Tracker™ and BODIPY-Thapsigargin™ in an attempt to localize intracellular calcium stores. Mitotracker™ was used as a control. All of our dyes were obtained from Invitrogen/Molecular Probes.

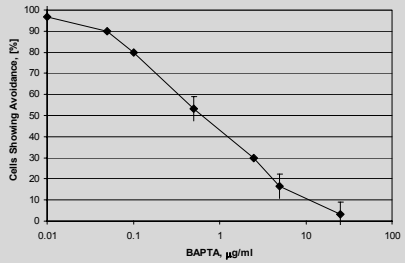
Results

GTP-γ-S is an effective chemorepellent in *Tetrahymena*.



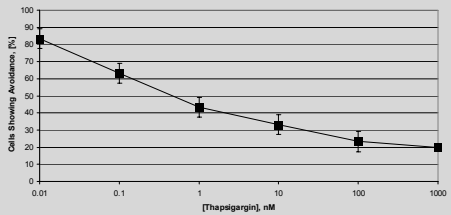
Behavioral assays show that GTP-γ-S is an effective chemorepellent in *Tetrahymena*. Avoidance increases in a concentration-dependent manner, with maximal avoidance being seen at 100 μM. Cells adapt, or lose their responsiveness, after several seconds in GTP-γ-S; a phenomenon which is reversible if cells are washed in buffer and given several minutes to "de-adapt".

Calcium is required for GTP avoidance



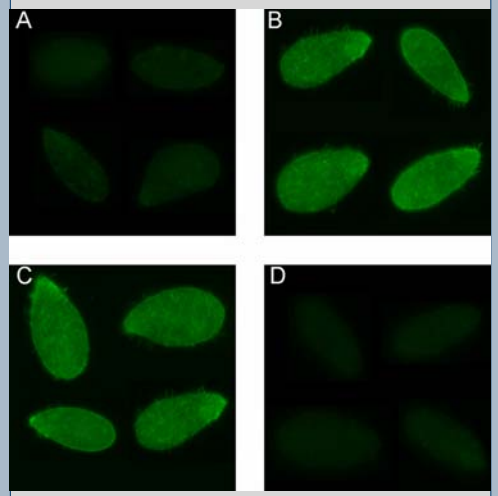
Exposure to the membrane-permeable calcium chelator, BAPTA-AM, effectively eliminates GTP avoidance in Tetrahymena. The IC_{50} of this compound was 50 μg/ml.

Intracellular calcium is needed for GTP avoidance



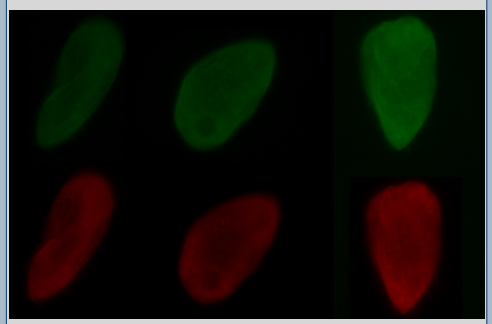
Thapsigargin, an inhibitor of the ER calcium ATPase in many cells, inhibited the GTP chemoresponse in *Tetrahymena*, implicating intracellular calcium in this response. Avoidance was effectively eliminated in cells exposed to 100 nM thapsigargin and higher. The IC_{50} of this compound was approximately 1 nM.

Calcium is not required for tyrosine phosphorylation in *Tetrahymena*



Indirect immunofluorescence labeling with a polyclonal anti-phosphotyrosine antibody shows that tyrosine phosphorylation is not calcium dependent. Control cells (A) showed baseline levels of phosphorylation, similar to cells treated with genistein and GTP (D). GTP-treated cells showed increased levels of phosphorylation (B), which was not reduced when cells were first treated with BAPTA (C). Note ciliary staining in GTP-treated cells.

Thapsigargin localizes to the ER in *Tetrahymena*



Thapsigargin, an inhibitor of the ER calcium ATPase in many cells, and an inhibitor of the GTP chemoresponse in *Tetrahymena*, colocalized with ER Tracker™ in *Tetrahymena*. Cells were double-labeled with BODIPY™ thapsigargin (green) and ER Tracker™ (red). Staining patterns seen were very similar. Similar results were seen in cells double-labeled with BODIPY™ ryanodine and ER Tracker™ (not shown), but not in cells double labeled with thapsigargin and Mitotracker™ (not shown).

Discussion

- GTP is an effective chemorepellent in *Tetrahymena*.
- Both extracellular and intracellular calcium are needed for GTP avoidance, as seen from previous electrophysiological studies, along with our current study.
- Calcium is not required for tyrosine phosphorylation, but may be involved further down the GTP signaling pathway, possibly with motor proteins such as inner arm dynein 1(Hennessey *et. al*, 2002).
- Thapsigargin appears to localize to the ER in *Tetrahymena*, as would be expected from our knowledge of other cell types.
- Further roles for calcium signaling in *Tetrahymena* remain to be explored.

References

1. Bartholomew J, Abraham H, Black A, Hamilton T, Reichart J, Mundy R, Recktenwald J, Kuruvilla H. 2005. GTP signaling in *Tetrahymena thermophila* involves a tyrosine kinase pathway coupled to NO and cGMP. *Acta Protozoologica,* in press.
2. Hennessey, T. M., Kim, D. Y., Oberski, D. J., Hard, R., Rankin, S. A., Pennock, D. G. 2002. Inner arm dynein 1 is essential for Ca++-dependent ciliary reversal in *Tetrahymena thermophila*. *Cell Motil. Cytoskel.*, 53: 281-288.
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