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.

**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 IC₅₀ of this compound was 50 μM.

**Intracellular calcium is needed for GTP avoidance**

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**


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