Immunolocalization of Kinetodesmal Fibers with an Anti-Laminin Antibody in Tetrahymena thermophila

Heather G. Kuruvilla
Cedarville University, heatherkuruvilla@cedarville.edu

Anna Ward
Cedarville University, annajward@cedarville.edu

Stephen Vinczi
Cedarville University, svincziii@cedarville.edu

Immunolocalization of a PIGR-like Protein in *Tetrahymena thermophila*

Emily Fitts, Fabio Herrera, Georgia VonLehmden, Heather Kuruvilla
Department of Science and Mathematics, Cedarville University, 251 North Main St., Cedarville, OH, 45314

Introduction

Netrins are pleiotropic signaling molecules which guide axonal development and help regulate processes such as angiogenesis. Netrins can act as chemorepellents for developing axons, and our previous work has shown that several netrins, including netrin-1, netrin-3, and netrin-4, are chemorepellents in *Tetrahymena thermophila.* In vertebrates, netrin-1 signals through several receptors, including those in the UNC-5 family. UNC-5 family proteins often signal through the *src* family of tyrosine kinases. We have previously characterized UNC-5 and *src*-like proteins in *Tetrahymena*, by immunolocalization and Western blotting. Sequencing of our *src*-like proteins gave a number of homologous sequences, including the sequence for polymeric immunoglobulin-like receptor (PIGR). With all of these findings in mind, we hypothesized that *Tetrahymena* might possess a receptor similar to PIGR, which would localize either to the plasma membrane or cilia of *Tetrahymena.*

Materials

Immunofluorescence was carried out using a modified protocol obtained from cellsignal.com. Cells were fixed in 3.7% formaldehyde for 15 minutes, then rinsed twice in PBS and blocked for an hour in 3% BSA. Cells were then rinsed in PBS and incubated overnight at room temperature in primary antibody at a dilution of 1:100 in the presence of BSA. After rinsing three times in PBS, cells were incubated in a 1:100 dilution of secondary antibody for 1–2 hours at room temperature in the dark. Cells were then rinsed three times in PBS. 5 ml of cell suspension was then applied to a slide and mixed with 5 ml of DAPI. Cell suspension was then observed under a fluorescence microscope at 400X. Mean fluorescence of each group (approximately 10 cells) was compared using a two-tailed T test.

Results

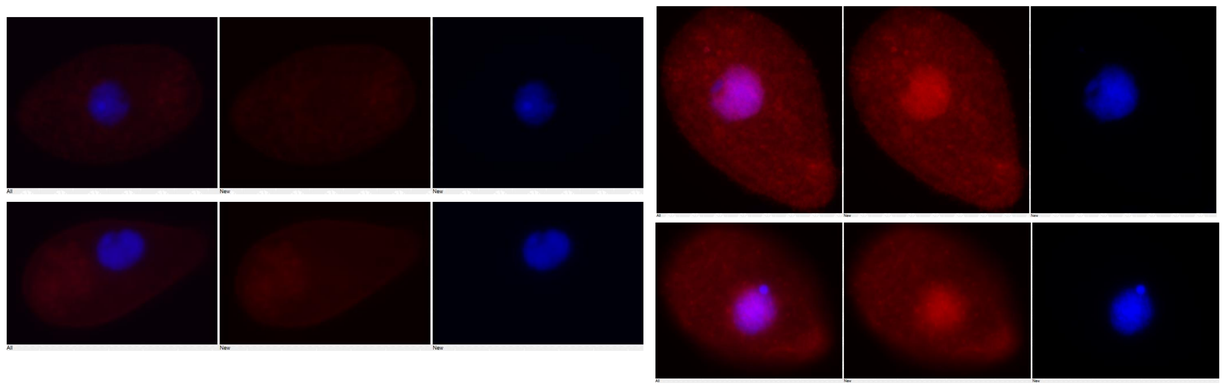

Figure 1. Antibodies against PIGR bind to the cilia and nucleus of *Tetrahymena thermophila.* Binding of the anti-PIGR antibody (right) to *Tetrahymena* was significantly higher than binding of secondary antibody alone, as determined by two-tailed T-test ($P < 0.00000001$). Secondary antibody alone did not localize to the cilia or the nucleus of *Tetrahymena.*

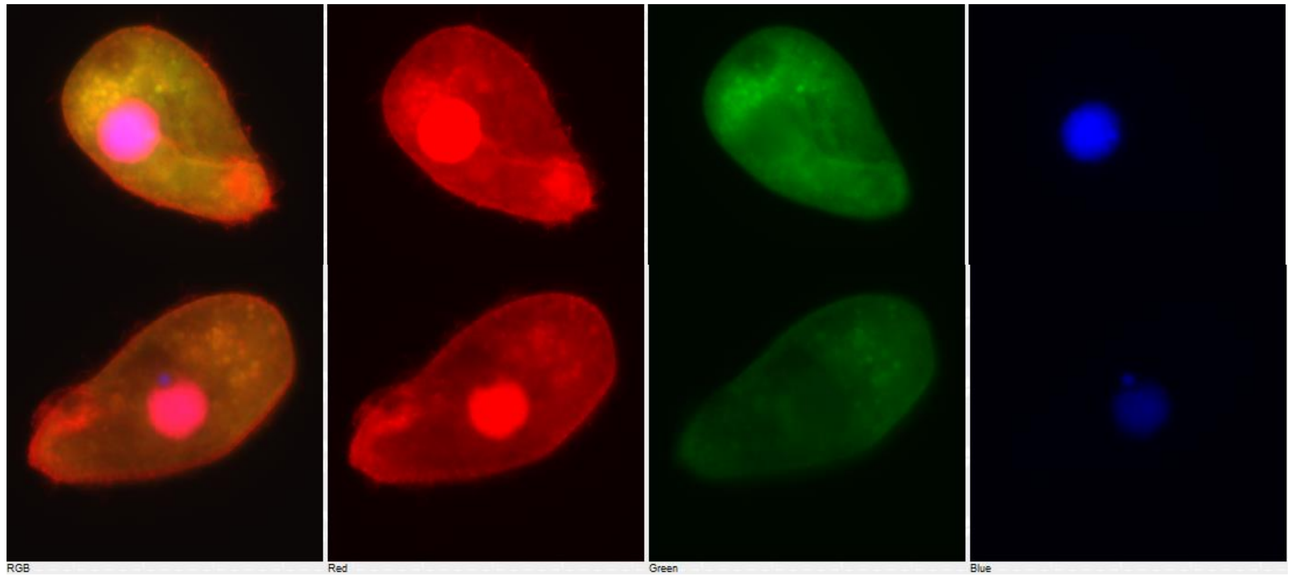

Figure 2. The PIGR-like protein appears to be trafficked to the cilia via the ER secretory pathway. There is some colocalization between the anti-PIGR antibody (red) and ER Tracker™ (green), indicating that the PIGR-like protein is synthesized and trafficked via the ER trafficking pathway. Staining of cilia can be seen in the PIGR (red) panels. Also, the anti-PIGR antibody appears to bind specifically to the macronucleus, but not the micronucleus, as seen in the far-left panel.

Conclusions

- Staining with anti-PIGR antibody is significantly higher than staining with secondary antibody alone. Anti-PIGR antibody localizes to the cilia, the nucleus, and some cytosolic vesicles.
- There is some co-localization of anti-PIGR antibody with ER Tracker™, which likely indicates that the PIGR-like protein is trafficked to cilia via the secretory pathway.
- Further analysis of PIGR-like proteins will be conducted via Western blotting. A preliminary Western blot showed low levels of signal (data not shown) so we plan to concentrate our protein by immunoprecipitation prior to performing additional Western blotting.

Contact Information

Heather G. Kuruvilla, Ph.D., Senior Professor of Biology
heatherkuruvilla@cedarville.edu

Fabio Herrera, Research Assistant
fabiomcaballero@cedarville.edu

Emily Fitts, Research Assistant
emilygfitts@cedarville.edu

Georgia Vonlehmden, Research Assistant
gvonlehmden@cedarville.edu