Immunolocalization of a Netrin-3 Like Peptide in *Tetrahymena thermophila* Using Antibodies Against the N- and C-terminus of the Protein

Bethany C. Khol  
*Cedarville University*, bkhol@cedarville.edu

Katelyn R. Malik  
*Cedarville University*, kmalik@cedarville.edu

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

Follow this and additional works at: [http://digitalcommons.cedarville.edu/research_scholarship_symposium](http://digitalcommons.cedarville.edu/research_scholarship_symposium)  
Part of the *Cell Biology Commons*

Khol, Bethany C.; Malik, Katelyn R.; and Kuruvilla, Heather G., "Immunolocalization of a Netrin-3 Like Peptide in *Tetrahymena thermophila* Using Antibodies Against the N- and C-terminus of the Protein" (2017). *The Research and Scholarship Symposium*. 34.  
Tetrahymena thermophila are free-living, unicellular, eukaryotic protozoans that live in a variety of aquatic environments. These organisms interact with their environment by responding to chemorepellents and chemoattractants which direct them toward favorable stimuli, such as food, and away from unfavorable stimuli, such as predators. We have previously described two netrin-like proteins, a netrin-1 like protein, and a netrin-3 like protein, which are secreted from Tetrahymena. Both of these proteins act as chemorepellents, and may allow cells to communicate with each other regarding population density, preventing them from outgrowing the available environmental resources. In our current study, we used antibodies against the N- and C-terminal of netrin-3 to show the distribution of this protein throughout the cell. We find that netrin-3 is highly colocalized with the endoplasmic reticulum and colocalizes with tubulin to a lesser extent. This is to be expected for a protein that is secreted from cells and trafficked on microtubules.

**Abstract**

Immunofluorescence was carried out using a modified protocol obtained from cellsignal.com. Briefly, cells were washed twice in PBS, reconstituted in 3.7% formaldehyde in PBS, and allowed to fix for 15 min at room temperature. After fixation, cells were rinsed three times in PBS before being blocked in blocking buffer for 60 minutes. After washing off blocking buffer, cells were incubated overnight at room temperature in primary antibody at a dilution of 1:100. After rinsing three times in PBS, cells were incubated in fluorochrome-containing 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 covered with a coverslip and observed under a fluorescence microscope at 400X.

**Methods and Materials**

**Results**

1. Netrin 3 shows cytosolic staining which colocalizes with ER Tracker™, which is what one would expect for a secreted protein.
2. Anti N3C and anti N3N antibody staining overlaps; however, N3N antibody appears to bind mainly to cytosolic targets, while the N3H antibody binds targets on the plasma membrane as well as in the cytosol.

**Conclusions**

**Contact**

Heather Kuruvilla, Ph.D., Professor of Biology  
Email: heatherkuruvilla@cedarville.edu