Apr 11th, 11:00 AM - 2:00 PM

Mapping Netrin Signaling in *Tetrahymena thermophila*

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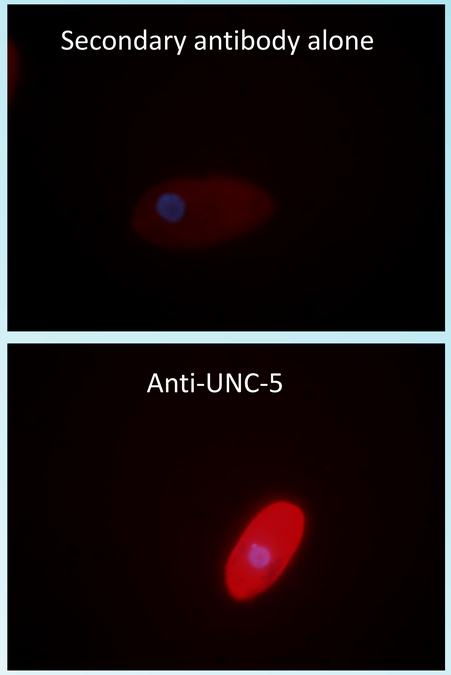
Abstract

The netrin family of proteins, found throughout the animal kingdom, are well known for their roles in developmental signaling. Netrin-1, the best-studied member of this family, signals through four receptor types in vertebrates: the UNC-5 family, DCC, neogenin, and DSCAM. We have previously characterized a netrin-1-like protein in the ciliated protozoan, *Tetrahymena thermophila*. This protein is secreted from *Tetrahymena*, and functions as a chemorepellent. Since a netrin-like protein is produced by this organism, we hypothesized that some components of the vertebrate netrin signaling pathway might also be present in *Tetrahymena*. Through immunolocalization on the plasma membrane of the cell, we have found that *Tetrahymena* appear to have a UNC-5 like protein, as well as proteins that are immunologically similar to neogenin. A homolog of src-1, a tyrosine kinase involved in vertebrate netrin-1, is also present in *Tetrahymena.* Future experiments will allow us to make more comparisons between netrin signaling in *Tetrahymena* with netrin signaling in the animal kingdom, and will allow us to determine the suitability of *Tetrahymena* as a model system for this particular pathway.

Methods

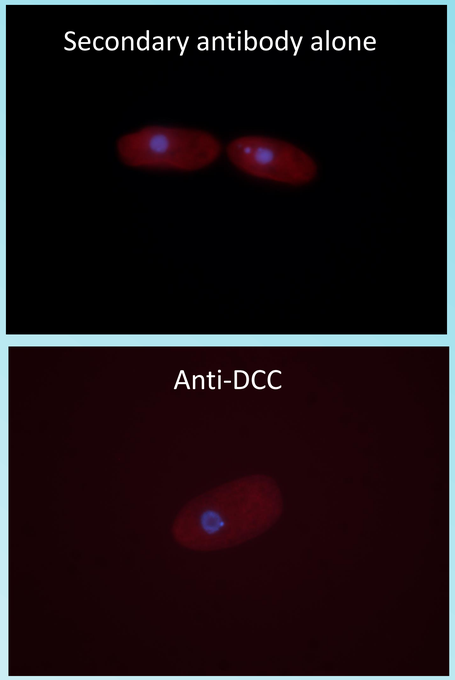
Immunofluorescence was carried out using a modified protocol obtained from cellsignal.com. Cells were fixed in 3.7% formaldehyde for 15 minutes, then rinsed twice in PBS and blocked for an hour in 3% BSA. Cells were then rinsed in PBS and incubated overnight at room temperature in primary antibody at 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 µl of cell suspension was then applied to a slide and mixed with 5 µl 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.

Do *Tetrahymena* have UNC-5?



There is a significant difference between the two groups. ($P < 0.05$)

Do *Tetrahymena* have DCC?

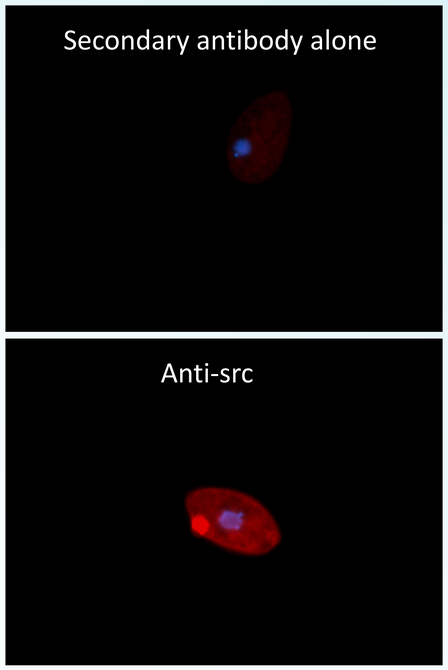


There is no significant difference between the two groups ($P = 0.94$).

Do *Tetrahymena* have DSCAM?

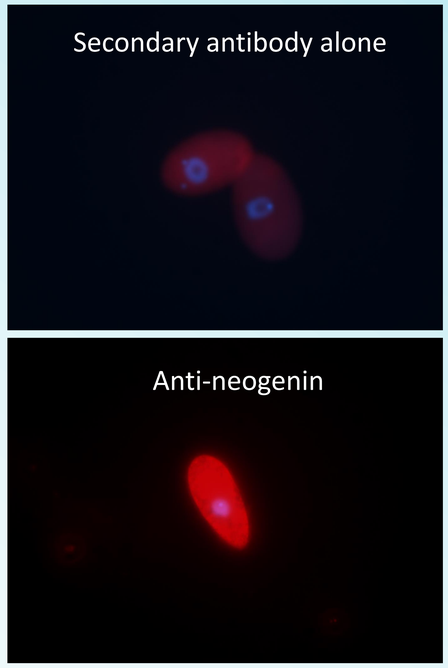
No apparent difference between groups. This is preliminary data; our sample size is not yet large enough to do statistics on.

Do *Tetrahymena* have src-1?



There is a significant difference between the two groups. ($P < 0.05$)

Do *Tetrahymena* have neogenin?



There is a significant difference between the two groups. ($P < 0.05$)

Conclusions

- *Tetrahymena* have UNC-5, src-1, and neogenin; probably because all three are involved in chemorepellent signaling, and netrin-3 is a chemorepellent in *Tetrahymena.*
- Receptors that are more often associated with chemoattraction (DCC and DSCAM) do not show significant immunolocalization in *Tetrahymena.*

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