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Netrin-3 and Netrin-4-Like Proteins are Secreted from *Tetrahymena thermophila*

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Presenters
Leah C. Anderton, Nicholas Bradley, Sorrel Paris, Aaron T. VanGeest, Reese Watkins, Kassie E. Young, and Heather G. Kuruvilla

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Netrin-3 and Netrin-4-Like Proteins are Secreted from *Tetrahymena thermophila*

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Abstract

Netrins are signaling proteins, acting as chemorepellents or chemoattractants, and their role is especially important in vertebrate development. In studies involving *Tetrahymena thermophila*, netrin proteins often act as chemorepellents, so research centered around verifying if this was also true for netrin-4 protein. Since netrin-1 and netrin-3 have been shown to influence neurological and developmental growth in organisms, the implications for discovering the cellular effects of netrin-4 are significant for human health and research. Through behavioral assays, we were able to confirm that netrin-4 does act as a chemorepellent. In addition, our ELISA and Western blots also helped substantiate the idea that *Tetrahymena* secrete netrin-3 and netrin-4-like proteins. The function of netrin-4 in this organism is unknown; further testing is needed to determine the physiological functions of netrin-like proteins in this organism.

Introduction

Netrins are a group of laminin-related proteins that are secreted from a variety of cell types in human tissues. Netrins have been implicated in various biological functions including lymphangiogenesis, angiogenesis, cell division and neuronal cell guidance. Netrins have been shown to function through 4 different receptors utilizing various cellular pathways. Previously, research has been done to show that netrin-1 and netrin-3 are chemorepellents in *Tetrahymena* and that *Tetrahymena* synthesize proteins that are immunologically similar to netrins. For these reasons, we hypothesized that *Tetrahymena* may also be responsive to netrin-4 protein.

Methodology

ELISA Assay:
For the ELISA assay, total cell proteins were extracted from *Tetrahymena thermophila*. 0.5% Triton X-100 was used for extraction in the presence of protease inhibitors. Primary anti-netrin-4 antibody and secondary anti-rabbit antibody, HRP conjugate, were used at a 1:1000 dilution. The substrate (2mL stable peroxide, 2mL enhancer solution, 40mL of ADHP concentrate) was added. Stop solution was added when the signal appeared and the results were quantitated on a microplate reader.

SDS-PAGE
SDS-PAGE was run using 10% precast acrylamide gels from BIO-RAD and stained with Coomassie Blue.

Western Blotting
Protein extracts were prepared from 2-day old *Tetrahymena* cultures and run on a 10% SDS-PAGE. Proteins were transferred to a nitrocellulose membrane, and Western blots were performed using a 1:1,000 dilution of goat anti-netrin-3 IgG as the primary antibody, and a 1:10,000 dilution of rabbit-anti-goat IgG, HRP-conjugated antibody as the secondary antibody. Chemiluminescence was used to detect HRP activity.

Results

Table 1. Netrin-4 ELISA. A netrin-4-like protein is present in significantly higher amounts in secreted protein obtained from *Tetrahymena thermophila* than in our negative control or conditioned media. P-values were obtained via two-tailed T test.

Sample	Mean Fluorescence	P value
Negative control	5.16E+04	--
Conditioned media	5.94E+04	0.137691
Secreted protein	6.51E+04	0.022652
Positive control	7.58E+04	0.00829

Results

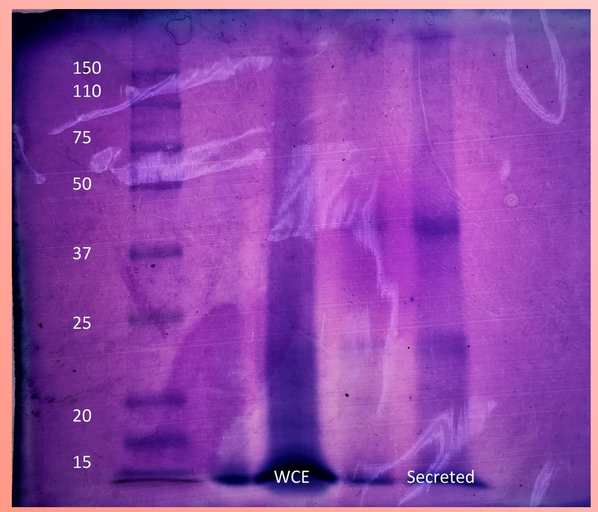

Figure 1. SDS-PAGE . SDS-PAGE shows the molecular weights of whole cell extract proteins compared to secreted proteins of *Tetrahymena* in 2 day old cultures.

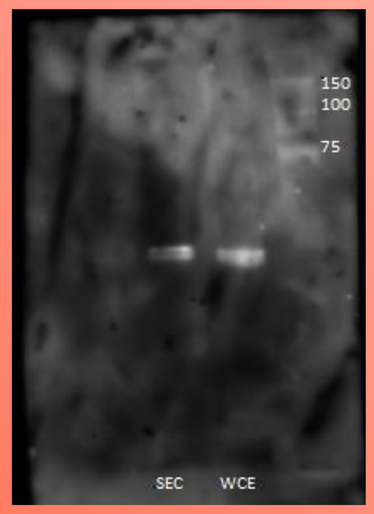

Figure 2. Netrin-3 Western Blot. Western blot displays evidence of Netrin-3-like protein in both whole cell extract and secreted proteins obtained from *Tetrahymena thermophila*.

Conclusions

- A netrin-4-like protein is secreted from Tetrahymena as determined by ELISA.
- Western blot shows evidence of a netrin-3-protein in whole cell extract and secreted proteins.
- We plan to repeat Western blotting with an anti-netrin-4 antibody to determine the molecular weight of the netrin-4-like protein.

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