CEDARVILLE UNIVERSITY

Cedarville University
DigitalCommons@Cedarville

The Research and Scholarship Symposium | The 2018 Symposium

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

Netrin-3 Signals Through Serine Phosphorylation in Tetrahymena thermophila

Cayla C. Eckley
Cedarville University, cekley@cedarville.edu

Rebecca N. Haught
Cedarville University, rhaught@cedarville.edu

Kyle J. Hooper
Cedarville University, kjhooper@cedarville.edu

Jared E. Matz
Cedarville University, jaredmatz@cedarville.edu

Joshua L. Wilson
Cedarville University, joshualwilson@cedarville.edu

See next page for additional authors

Follow this and additional works at: http://digitalcommons.cedarville.edu/research_scholarship_symposium

Part of the Biochemistry Commons, and the Cell Biology Commons

Eckley, Cayla C.; Haught, Rebecca N.; Hooper, Kyle J.; Matz, Jared E.; Wilson, Joshua L.; Khol, Bethany C.; Malik, Katelyn R.; and Kuruvilla, Heather G., "Netrin-3 Signals Through Serine Phosphorylation in Tetrahymena thermophila" (2018). *The Research and Scholarship Symposium*. 4.
http://digitalcommons.cedarville.edu/research_scholarship_symposium/2018/poster_presentations/4

This Poster is brought to you for free and open access by DigitalCommons@Cedarville, a service of the Centennial Library. It has been accepted for inclusion in The Research and Scholarship Symposium by an authorized administrator of DigitalCommons@Cedarville. For more information, please contact digitalcommons@cedarville.edu.

CENTENNIAL LIBRARY
CEDARVILLE UNIVERSITY

Presenters
Cayla C. Eckley, Rebecca N. Haught, Kyle J. Hooper, Jared E. Matz, Joshua L. Wilson, Bethany C. Khol, Katelyn R. Malik, and Heather G. Kuruvilla

This poster is available at DigitalCommons@Cedarville: http://digitalcommons.cedarville.edu/research_scholarship_symposium/2018/poster_presentations/4

Netrin-3 Signals Through Serine Phosphorylation in *Tetrahymena thermophila*

Cayla Eckley, Rebekah Haught, Kyle Hooper, Jared Matz, Joshua Wilson, Bethany Khol, Katelyn Malik and Heather Kuruvilla
Department of Science and Mathematics, Cedarville University, Cedarville, OH 45314

Abstract

The netrin family of proteins are structurally related to laminin and, while first discovered in the nematode *Caenorhabditis elegans*, are now known to be present in species throughout the animal kingdom, including humans. These proteins also have a wide variety of roles that include inhibition of apoptosis, chemorepulsion, and axonal guidance. Due to the results of previous studies involving netrin-1 in vertebrate systems, the current prevailing assumption is that netrins, when acting as chemorepellents, signal using tyrosine kinases. However, data that we gathered through phosphoserine-targeting ELISA assays and immunofluorescence microscopy demonstrates that the netrin-3 peptides signal *Tetrahymena thermophila* through serine phosphorylation instead, causing the ciliate protists to avoid netrin-3 peptides in response. Treatment with netrin-3 peptides also seems to cause mitotic inhibition in *Tetrahymena*, which can be reversed by addition of a serine kinase inhibitor. This new information suggests that netrin-3 may have physiological roles that have previously been unexplored.

Introduction

Netrin-3 is a less-characterized peptide from the netrin family of proteins. In previous research we have shown that netrin-3 is secreted from *Tetrahymena thermophila* and acts as both a chemorepellent and mitotic inhibitor in the unicellular model. In our research, we were seeking to better understand the mechanism for netrin-3 phosphorylation in *T. thermophila.* While netrin-3 has often been assumed to work through the same tyrosine phosphorylation signaling pathway as netrin-1 in vertebrates, recent observations have led us to suggest another mechanism in *Tetrahymena*.

As noted, we have previously shown that netrin-3 produces 100% avoidance behavior in *T. thermophila* at levels of 1 µg/ml. However addition of serine/threonine kinase inhibitor apigenin in conjunction with netrin-3 has been shown to inhibit *T. thermophila* avoidance. This observation led us to hypothesize that we would observe serine phosphorylation in response to netrin-3 exposure in our *T. thermophila* model.

Materials and Methods

ELISA Assay:
For the ELISA assay, phosphoproteins were extracted from *Tetrahymena thermophila*. 0.5% Triton X-100 was used for extraction in the presence of phosphatase and protease inhibitors. Behavioral buffer was used for the control sample. N3C was used at a 5µg/mL concentration, N3N was used at a 5µg/mL concentration, and N3NT was used at 1mM concentration. A phosphoserine control was used at five different concentrations: 1µg/mL, 0.1µg/mL, 0.01µg/mL, 0.001µg/mL, and 0.0001µg/mL. These samples were loaded onto the ELISA plate. The samples were blocked with BSA. Primary anti-phosphoserine antibody and secondary anti-rabbit antibody 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 pink signal appeared and the ELISA was read on the plate reader.

Immunofluorescence:
With immunofluorescence, *Tetrahymena thermophila* exposed to N3NT and N3C were observed. The cells were fixed with formaldehyde at a 1:10 dilution and blocked with blocking buffer. Primary anti-phosphoserine antibody was used at a 1:1000 dilution. After overnight incubation, secondary antibody with a fluorescent tag (either Texas Red or FITC) was used. Excess antibody was rinsed and the cells were observed with the fluorescence microscope.

Results

Table 1. Exposure to Netrin-3 peptides increases serine phosphorylation in *Tetrahymena thermophila.* Four separate sets of *Tetrahymena thermophila* were treated with N3N, N3C, N3NT, or left untreated. After treatment, protein extracts from each experimental group were subjected to ELISA and compared with the untreated controls.

Treatment	Fluorescence Units	Relative Fluorescence	Treatment vs. Control
Control	25,176.67 ± 3060.88	1	----
N3N	51,420.00 ± 560.83	2.04	0.003
N3C	53,233.33 ± 4942.16	2.11	0.003
N3NT	56,723.33 ± 1030.16	2.25	0.001

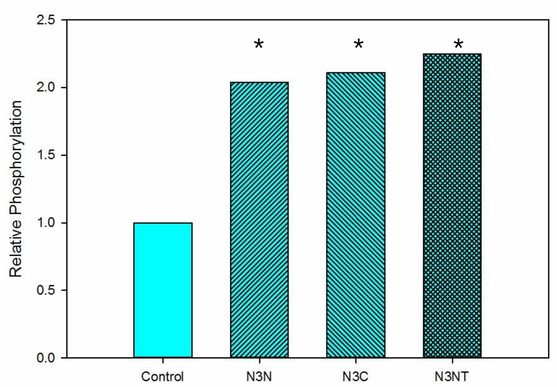

Figure 2. Exposure to netrin-3 peptides significantly increases serine phosphorylation when compared with control cells. *Significance was determined by two-tailed T test. $P \leq 0.003$.

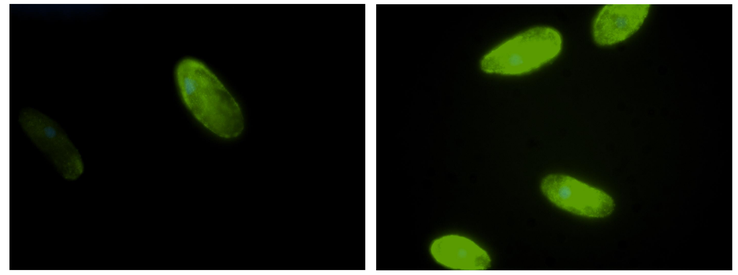

Figure 3. Treatment with 1 µg/ml N3C causes an increase in phosphoserine levels in *Tetrahymena thermophila*. Netrin-3 treated cells (right) show an increased level of phosphoserine levels when compared to control cells (left). Immunofluorescence was conducted using an anti-phosphoserine antibody. Green staining indicates the presence of phosphoserine; blue staining indicates the presence of DAPI.

Figure 4. Treatment with 1 µM N3NT causes an increase in phosphoserine levels in *Tetrahymena thermophila*. Netrin-3 treated cells (right) show an increased level of phosphoserine levels when compared to control cells (left). Immunofluorescence was conducted using an anti-phosphoserine antibody. Red staining indicates the presence of phosphoserine; blue staining indicates the presence of DAPI.

Conclusions

Through our ELISA and immunofluorescence procedures, we were able to determine the following:

1. Phosphorylation of serine kinase was elevated in *T. thermophila* treated with N3NT, N3N, and N3C as compared to the negative control as determined by ELISA. All of these increases in phosphorylation were significant, with P values of 0.003 or less.
2. Immunofluorescence also showed greater levels of fluorescence in Netrin-3 treated cells when compared with controls. Quantititative immunofluorescence studies are currently underway to determine whether these differences are significant.

Contact: Heather Kuruvilla, Ph.D.
heatherkuruvilla@cedarville.edu