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How the Western Was Won: Evidence for Netrin Signaling Machinery in Tetrahymena thermophila

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Abstract

Netrins are pleiotropic signaling molecules with diverse roles in animal development. Netrin signals through a number of receptors in animals, including the UNC-5 family, neogenin, DSCAM, and DCC. Previous studies have shown that netrin-1-peptide, netrin-3-peptide, and recombinant netrin-4 all act as chemorepellents in *Tetrahymena* (Kuruvilla *et al.,* 2016, Khol *et al.,* 2018; Bradley and Kuruvilla, 2020). In addition, netrin-1 peptide appears to signal through a tyrosine kinase in this organism (Kuruvilla *et al.,* 2016), similar to vertebrate signaling through UNC-5, which uses the tyrosine kinase, src. In light of these data, we hypothesized that ***Tetrahymena thermophila*** **possess netrin signaling machinery, including a tyrosine kinase.** In order to investigate this hypothesis, we searched for various netrin receptors, as well as a src homologue, in *Tetrahymena* using immunofluorescence (Khol *et al.,* 2018). We found that anti-UNC-5 and anti-neogenin antibodies showed fluorescence, while anti-DCC and anti-DSCAM antibodies did not. In addition, an anti-src antibody showed significant fluorescence in *Tetrahymena* (Khol *et al.,* 2018. In our current study, we searched the *Tetrahymena* Genome Database for homologs of UNC-5, neogenin, and src. We also used Western blotting to screen for potential homologues of these proteins. At the present time, there are several proteins of interest which we would like to study further.

Materials and Methods

Database searching was done by entering amino acid sequences from human genes, obtained from the UniProt database, into the *Tetrahymena* Genome Database, and executing a blastp search for homologous proteins (seen in Table 1).

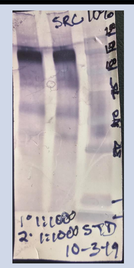
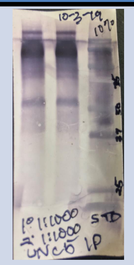
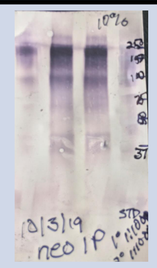
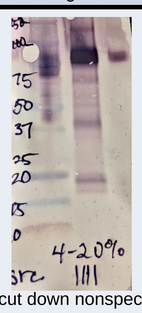
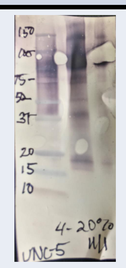
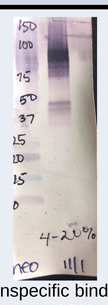
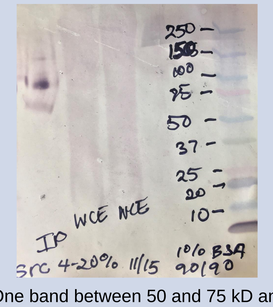
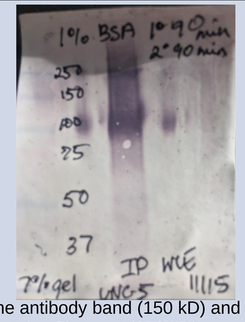
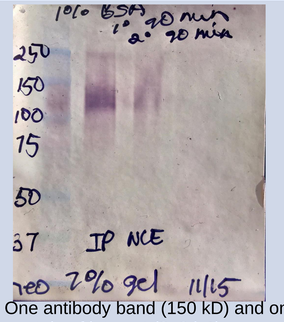
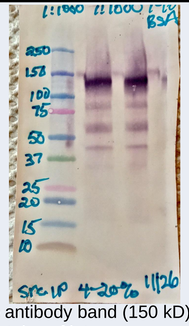
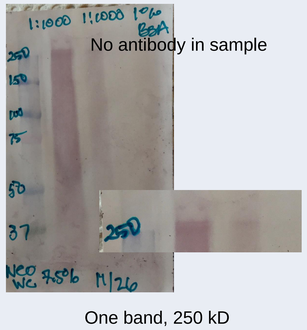
Immunoprecipitation was done on whole cell extract using a Pierce Classical IP kit. Antibodies were used at a 1:1000 concentration in all IP reactions.

Western blots were run on the immunoprecipitation products under various conditions, as laid out in the Western blot table (right).

Results

Table 1: Putative Netrin Signaling Proteins in *Tetrahymena thermophila*

	SRC-1	UNC-5B	Neogenin
Mammalian MW (Da) from UniProt	59,835	103,638	160,017
Top four homologous proteins (TGD)	Tyrosine kinase domain proteins	Zinc finger lsd1 subclass family proteins	Hypothetical proteins
E values (TGD)	4e-30 and higher*	0.006 and higher	0.18 and higher
Shows immunoreactivity in *Tetrahymena thermophila*	Yes	Yes	Yes

	Anti-Src-1	Anti-UNC-5B	Anti-neogenin
10% gel, no BSA, samples from IP, antibodies at 1:1000 in PBS-Tween with no BSA to maximize chances that something would bind	 Need clearer standards and more separation at high MW	 Need to spread out high MW bands	 Need to spread out high MW bands
For more separation at high MW, we ran gradient gel: 4-20% gel, no BSA, samples from IP, antibodies at 1:1000 in PBS-Tween	 Need to cut down nonspecific binding	 Lot of nonspecific binding; need to spread out high MW bands	 Lot of nonspecific binding; need to spread out high MW bands
Added BSA to antibody solution and ran receptors on 7.5% gels: 4-20% (src) or 7.5% (receptor) gel, samples from IP, antibodies at 1:1000 in PBS-Tween + 1% BSA	 One band between 50 and 75 kD and one band between 75 and 100 kD	 One antibody band (150 kD) and one protein of interest (250 kD)	 One antibody band (150 kD) and one protein of interest (250 kD)
Ran receptor gels with WCE to get rid of antibody band. 4-20% gel with samples from IP (src); 7.5% gel with whole cell extract, antibodies at 1:1000 in PBS-Tween + 1% BSA	 One antibody band (150 kD) and 3 proteins of interest	No antibody in sample  One band, 250 kD	 No antibody in sample One band, 250 kD

Conclusions

- Both receptor antibodies (anti-UNC-5B and anti-neogenin) recognize a 250 kD protein. These antibodies have previously immunolocalized to the plasma membrane of *Tetrahymena* as well (Khol *et al.,* 2018). We would like to sequence the 250 kD protein to determine whether it has any similarity to known netrin receptors.
- The anti-src antibody has also been successfully used in immunofluorescence studies in this organism (Khol *et al.,*, 2018) and binds to several *Tetrahymena* proteins on a Western blot. Since the molecular weight of mammalian src is approximately 60 kD, we are particularly interested in sequencing the *Tetrahymena* protein that lies between the 50 and 75 kD markers.

References

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