

2016 Summer Research Celebration

INTRODUCTION

Soon after neural tube closure cells inside the neural tube begin to organize and differentiate into motor and interneurons which then project axons to their perspective targets. This process is mediated, in part, by cell to cell contacts. One group of cell adhesion proteins, the cadherins, are known to be involved in organizing motor neurons into motor pools along with aiding axon extension [1, 2]. In the frog *Xenopus laevis*, NF-Protocadherin (NFPC) is expressed in the ventral neural tube in developing motor and interneurons. NFPC has previously been shown to play various developmental roles including involvement in neural tube closure and retinal axon extension [3,4]. Due to its expression in the neural tube we hypothesize that NFPC is playing a functional role in neural organization. I have used immunofluorescence to investigate NFPC expression in the neural tube. To disrupt NFPC function we have used a dominant negative NFPC lacking the extracellular domain (NF Δ E). This construct has previously been shown to inhibit endogenous NFPC function. I have modified NF Δ E so its expression is driven by the beta-tubulin promoter. Beta-tubulin is only expressed in developing neurons so NF Δ E will not interfere with NFPC function outside the neural tube.

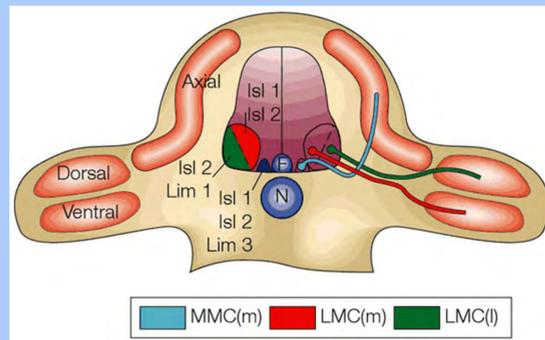


Figure 1. Motor Columns inside the neural tube. Developing motor neurons form clusters termed motor pools in the ventral neural tube. Motor columns have distinctly different muscle targets in which their neurons will innervate. Neurons in the medial motor column will project to axial muscles where neurons in the lateral motor column will project to limb muscles. Taken from [5].

Results

Previous work using *In Situ* Hybridization and Immunohistochemistry shows that NFPC is expressed inside the ventral portion of neural tube by stage 28 in *Xenopus*. To determine the role NFPC may play in the neural tube double *in-situ* hybridizations were performed with NFPC and known neural markers to see which cell types are expressing NFPC. These indicate that NFPC is expressed in neurons, possibly in both motor and ventral interneurons

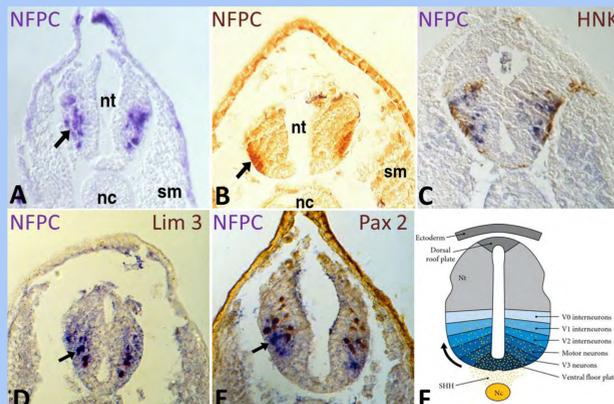


Figure 2. NFPC in the neural tube at stage 28. *Xenopus* embryos were fertilized and allowed to develop till stage 28 before being fixed. NFPC expression is visualized by *in-situ* hybridization (A), which stains mRNA, and by immunohistochemistry (B), where NFPC is visualized. Double *in-situ* hybridizations for NFPC (purple) and various neural markers (brown). These include HNK (C), a general neuronal marker. Lim 3 (D) a marker for motor and V2 interneurons, and Pax2 (E), which stains ventral interneurons. Panel (F) diagrams location on the dorsal-ventral axis that motor and interneurons develop. (nt= neural tube, nc=notochord, sm=somites)

RESULTS

To further investigate NFPC expression inside the neural tube Immunofluorescence was used to double stain for NFPC and motor and interneuron markers. In all embryos NFPC expression was limited to the lateral ventral border of the neural tube. Double stains with motor neuron markers MNR2, and Islet-1 show some overlap of expression with NFPC. The interneuron markers Lim1/Lim2, Lim3 (also motor neuron as well), and Nkx2.2 show much less overlap with the most positive cells being medial and dorsal to the NFPC stain. In addition embryos were stained for neural tubulin and the Ephrin B2 receptor, both of which showed significant overlap with the NFPC on the neural tube border.

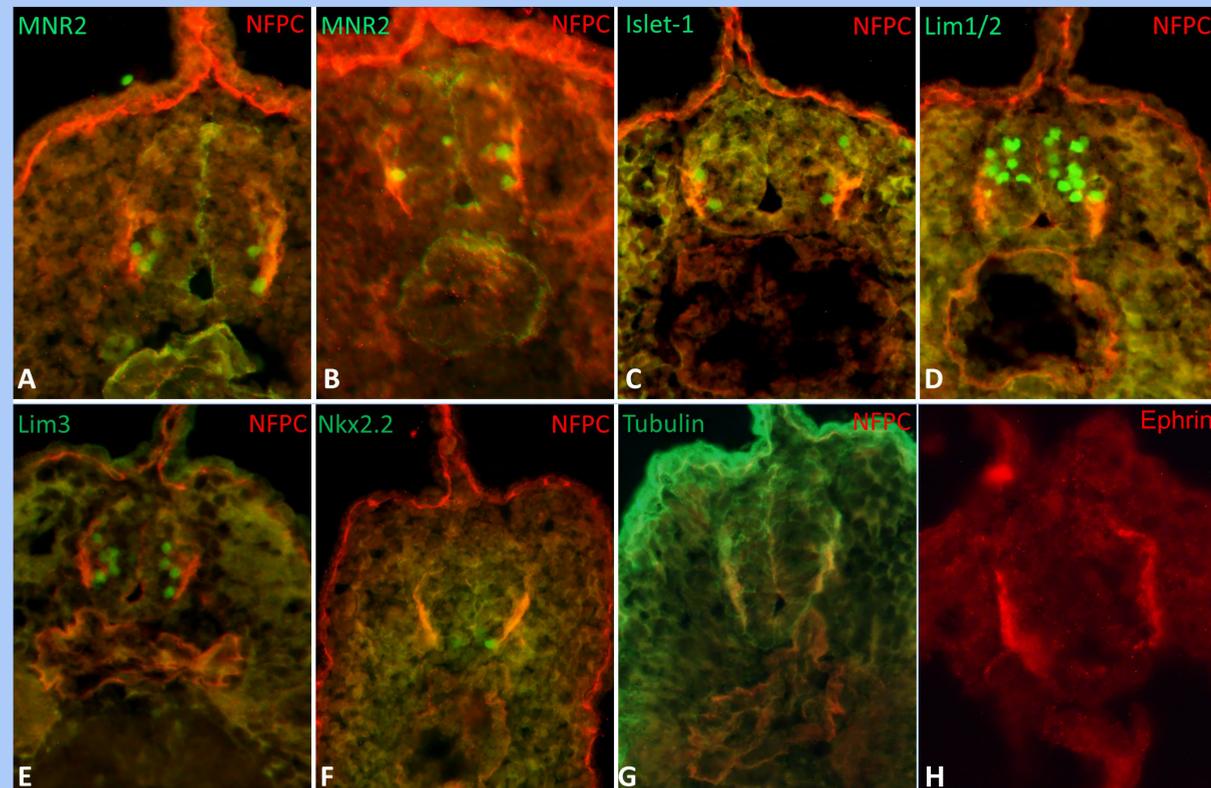


Figure 3. Composites of IF double stains of NFPC (red) and neural markers (green). Embryos were fertilized and allowed to develop to stage 31-32 before being fixed and stained. The embryos were then cryo-sectioned and examined under UV scope. Images of individual stains of the same embryo were then over-layed. Motor neuron markers used are MNR2 (A)& (B), Islet-1 (C). Interneuron markers include Lim1/Lim2 (D), Lim3 (E), and Nkx2.2 (F). Embryos were also stained with tubulin (G) and Ephrin B2 receptor (H).

To investigate the function of NFPC in the neural tube we plan to use a dominant negative version of NFPC, lacking the extracellular domain (NF Δ E) whose expression is driven by the beta-tubulin promoter. This construct is selectively expressed and visualized by HRP staining against the myc- tag fused to the construct (F4, C). The stripped expression pattern along the neural folds observed in the stage 17 embryo below is consistent with beta-tubulin expression during that stage in development.

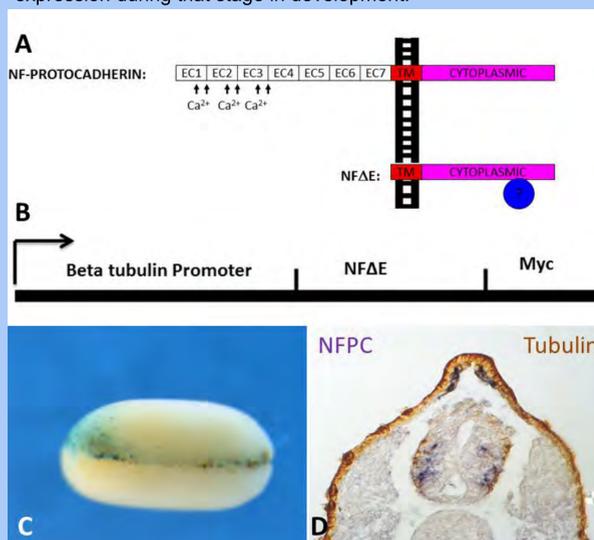


Figure 4. (A) Representation of NF Δ E structure relative to NFPC. (B) Schematic of NF Δ E being driven by beta-tubulin and fused to a myc. (C) Embryos were injected with the b-tubulin/ NF Δ E/myc construct at the 2 cell stage of development and allowed to develop till stage 17. Blue are beta-gal positive cells indicating all cells that received the construct. Myc is visualized in brown by HRP staining and is limited to a line along the neural folds. (D) *in situ* hybridization of NFPC (purple) and tubulin (Brown) in a stage 28 embryo.

The β -tubulin/ NF Δ E/myc construct was injected into a single blastomere at the 2-cell stage of development. Embryos were fixed at stage 31 and processed for immunofluorescence. Antibodies against the myc tag where used in combination with MNR2, Islet-1, Lim-1/2. Positively stained cells were counted and compared on the injected and un-injected side of the embryo.

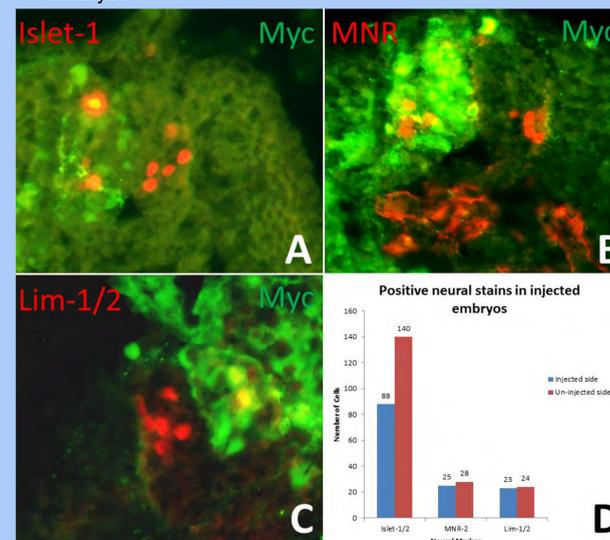


Figure 5. Double stains for the myc tag along with Islet-1 (A), MNR2 (B), and Lim-1/2 (C). Total cell counts are represented in (D) with 3 embryos total for Islet-1 and only a single embryo for both MNR-2 and Lim-1/2.

DISCUSSION/ CONCLUSION

- NFPC is expressed in the ventral neural tube and by stage 31 is limited to the lateral border.
- Results from *in-situ* hybridizations and immunofluorescence staining suggest that NFPC is expressed by developing motor and possibly even some interneurons as well.
- Due to its position on the neural tube border, it is possible that NFPC is expressed in elongating axons. This would be consistent with the overlap of expression with tubulin (Figure 3, F). This would also explain why it has not been obvious to determine exactly which motor and or interneurons are expressing NFPC, as the markers used are transcription factors and will produce nuclear stains which could be distant from NFPC in the axon.
- Further staining for known axonal proteins or *in-situ* hybridizations, which stain mRNA, could be done to confirm specific cell types expressing NFPC.
- While it appears that the β -tubulin/ NF Δ E/myc constructs results in less cells staining, at least for Islet-1, more embryos will need to be tested for all neural markers.

FUTURE WORK

- More double stains for NFPC and axonal markers such as Tuj1 (neural specific tubulin) and Ephrin receptors, as well as how these are affected by β -tubulin/ NF Δ E/myc .
- Double stains for other cadherins such as Cadherin 7, and Cadherin 6B, which are known to be in axons of developing motor neurons, along with NFPC.
- Further injections of beta-tubulin/NF Δ E/myc construct and counting the effect this has on neural and axonal markers.
- Construction of a negative control plasmid. This will consist of the β -tubulin promoter driving the myc tag only.

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ACKNOWLEDGMENTS

I would like to thank Dr. Bradley for mentoring me and giving me this opportunity over the past two years and Dr. Dana Rashid for all of her help in the lab. In addition I would like to thank Dr. Jen Forecki for her assistance with fertilizations.

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health Undergraduate This work was also funded by the Undergraduate Scholars Program here at MSU along with the Hughes scholars program which is funded by the Howard Hughes Medical Institute.