NF- Protocadherin in the Neural Tube

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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 respective 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 in 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 position on the neural tube border, it is possible that NFPC is expressed by developing motor and possibly even some interneurons as well.

To investigate the functional role of NFPC 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∆E). This construct has previously been shown to inhibit endogenous NFPC function. I have modified NF∆E so its expression is driven by the beta-tubulin promoter. Beta-tubulin is only expressed in developing neurons so NF∆E will not interfere with NFPC function outside the neural tube.

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.

The β-tubulin/ NF∆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 were used in combination with MNR2, Islet-1, Lim-1/2. Positively stained cells were counted on the injected and un-injected side of the embryo.

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.

• 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∆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 Eph receptors, as well as how these are affected by β-tubulin/ NF∆E/myc.

• Double stains for other cadherins such as Cadherin 7, and Takeichi due to its position on the neural tube border.

• Further injections of beta-tubulin/NF∆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.

REFERENCES


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