Identifying the Role of WBP2NL on Developing Neural Cells

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Introduction

The purpose of this project is to discover whether one of the newly identified maternal mRNAs, named WBP2NL, plays a role in causing blastomere cells to form the nervous system. This maternal mRNA was chosen for study because previous work in the Moody lab showed that when its protein is prevented from being expressed, several neural genes are repressed (Paaqua Grant, Ph.D. dissertation, GWU Dept. Biology, 2013). The project uses different forms of this gene (natural wild-type and mutant versions) to ask by what mechanism WBP2NL influences neural development.

Two domains overlap shown separate here. Sergey, 2014

Material and Methods

Experiment 1: **Does increasing the expression of wild type WBP2NL in dorsal-animal blastomeres alter the development of the embryonic ectoderm?**

- We predicted increasing the level of WBP2NL in this lineage will expand neural genes and reduce epidermal genes.
- We tested this prediction by injecting wild-type WBP2NL mRNA (200 pg/ul then 400 pg/ul) into a dorsal-animal Xenopus Lavius (frog) blastomere and determining changes in expression of neural plate, neural border, and epidermal genes.
- Gene expression analyses were done using in situ hybridization assays to determine whether gene expression domains get larger or smaller.

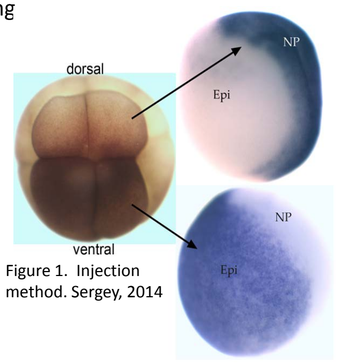


Figure 1. Injection method. Sergey, 2014

Experiment 2: **What regions of the WBP2NL protein are required for the phenotypes found in Experiment 1?**

- I have made five separate mutations to the WBP2NL-WT gene that code for specific binding regions of the protein.
- I will test whether either of these sites are required for the phenotype changes identified in Experiment 1.
- I used polymerase chain reaction techniques to synthesize five complementary DNA (cDNA) strands that are missing each of the five sites
- I will inject this into dorsal-animal blastomeres to test for gene expression as described in Experiment 1.
- I will compare the results from Experiment 2 to those of Experiment 1 to determine the functions of the mutanted regions of the protein and their respective effects on the expression of the various neural and germ layer genes.

Results

Gene Analyzed, Stage Fixed In, Cell Side Injected with WBP2NL-WT (200 ng/ul)

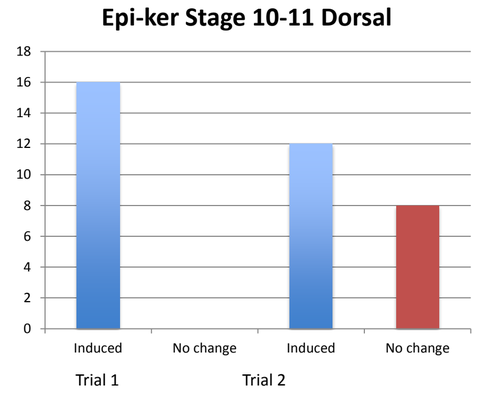


Figure 2.

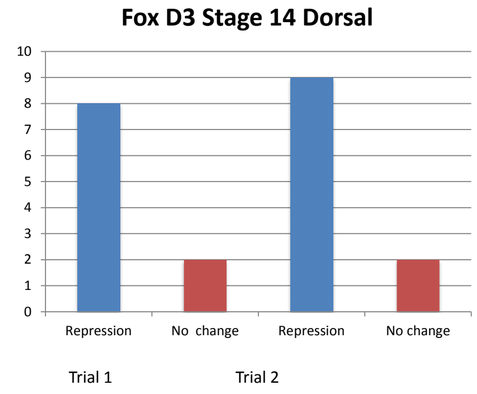


Figure 3.

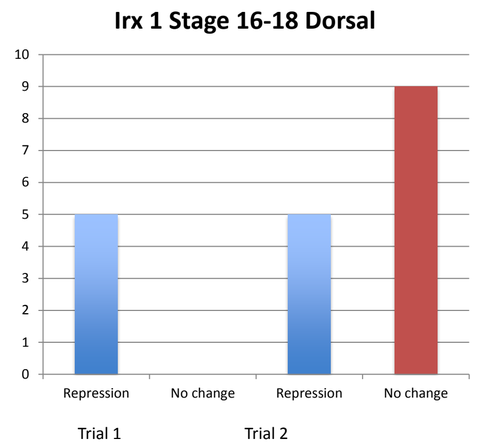


Figure 4.

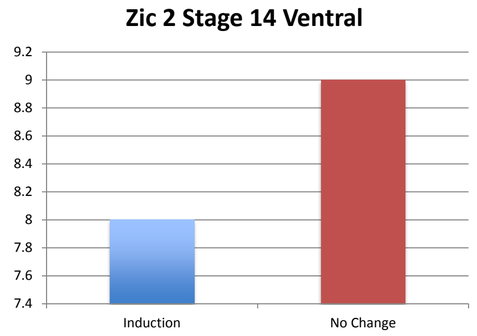


Figure 5.

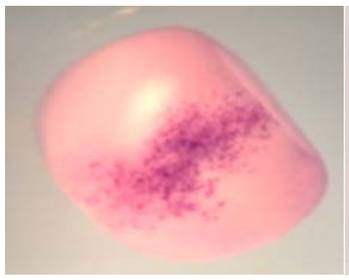


Figure 6. WBP2NL induction of Epi-ker shown by purple probe in pink stained cells

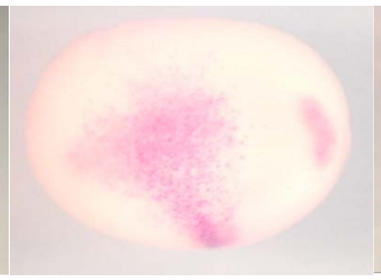


Figure 7. WBP2NL repression of endogenous Fox D3 Domain, while inducing Fox D3 in nonendogenous regions

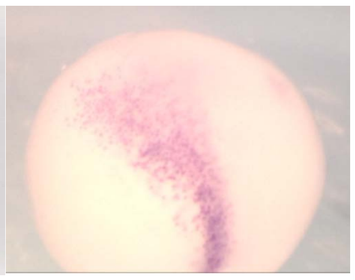
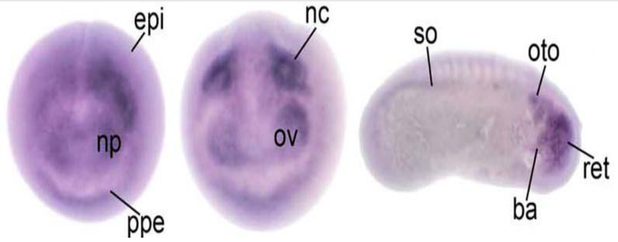
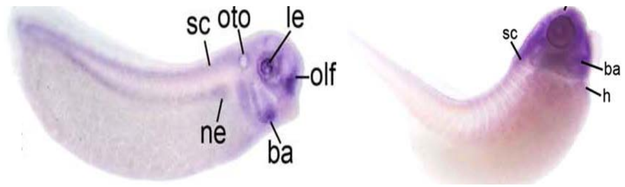


Figure 8. WBP2NL induction of Fox D3 shown by purple probe in pink stained cells

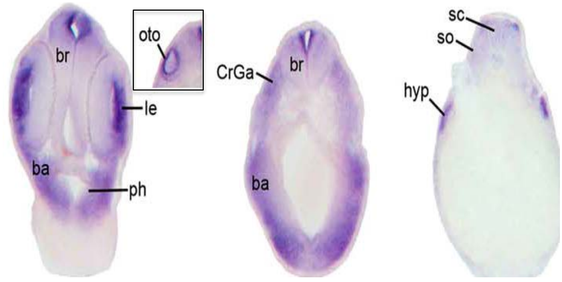
Conclusions



Expression patterns of animal-enriched genes at late larval stages



Expression patterns of animal-enriched genes from neural plate to late tail bud stages (Grant 2014)



- WBP2NL is a part of nervous system development.
- WBP2NL-WT may induce Epi-ker for epidermis development and Zic 2 for Neural Crest development. It also may repress Fox D3 for Neural Crest development and Irx 1 for Placode Development.
- This gene is found in all vertebrates, and therefore the results from this research will be applicable to a number of different animals including humans.

Future Direction

- We will increase concentration to 400 pg/ul to confirm effects.
- We will test the effects of the Five Mutations targeting parts of Amino Acid Sequence shown above (Sergey, 2014)
- This project has a lot of promise in identifying methods to enhance the expression of genes that dictate a cell to become a neural cell and regrow damage parts of the nervous system.

Acknowledgements

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References are available upon request