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Role of RNA-binding proteins Rbfox1l and Rbfox2 in neuronal development and behavior in zebrafish

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Abstract

Rbfox proteins are RNA-binding proteins that play a significant role in the alternative splicing of neuronal transcripts in the central nervous system (CNS). Rbfox proteins are required for proper brain development and function. In humans, RBFOX1 has been implicated in a variety of neurological disorders, including autism, anxiety, epilepsy, and schizophrenia. Rbfox2 is involved in cerebellar development in mammals. The zebrafish is used as a model system for studies in neurobiology given their neuroanatomical conservation with mammals, and remarkable capability to regenerate parts of their CNS. Rbfox1l (Rbfox1-like) and Rbfox2 have been identified in neurons of the adult zebrafish brain. Rbfox1l was found in a restricted population of dorsal telencephalic neurons, and Rbfox2 was found broadly throughout the brain. Both genes have been found in Purkinje cells of the cerebellum. We will use *rbfox1l* and *rbfox2* mutant zebrafish (in collaboration with Ohio State University) to better understand the role of *rbfox1l* in behavior and determine whether *rbfox2* is necessary for regeneration of the cerebellum. Understanding the role of the Rbfox proteins in neural development, regeneration, and behavior may lead to substantial advancement in the research field and health care.

Background

The Rbfox family (Rbfox1, Rbfox2, and Rbfox3) are RNA-binding proteins involved in alternative splicing of neuronal transcripts - an important mechanism in gene regulation, especially in mammalian nervous systems (Gehman et. al. 2012). Defects in alternative splicing can cause neurological and neuromuscular diseases (Lipscombe 2005; Licatalosi and Darnell 2006; Li et al. 2007).

In humans, mutation of the RBFOX1 gene has been linked to neurological disorders such as epilepsy, autism spectrum disorder, anxiety, ataxia, and mental retardation. Rbfox1 has been found in neurons and muscle cells. Rbfox1l knockout mice have seizures and display an increase in neuronal excitability (Fig. 1; Gehman et al., 2011).

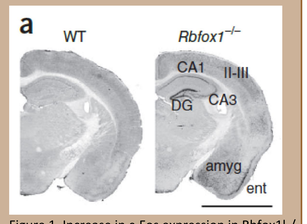


Figure 1. Increase in c-Fos expression in Rbfox1l-/- (Gehman et al., 2011).

Rbfox2 is expressed in brain and muscle as well as in embryonic stem cells and hematopoietic cells, indicating it may play a role in embryonic growth and development. In addition, Rbfox2 is important in cerebellar development in mammals.

Gehman et. al (2012) bred mice with a CNS-specific deletion of the Rbfox2 gene using a knockout technique. The mutant mice had a smaller cerebellum and an abnormal cerebellar cortex (Fig. 2). Rbfox2 lacking mice also had a different outward appearance and abnormal posture and locomotion (Fig. 2), indicating significant motor impairment. Rbfox1 knockout mice do not display defects in the cerebellum suggesting that Rbfox1 may be expressed later in development.

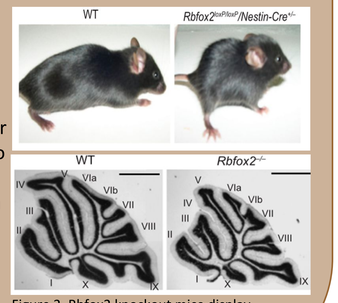


Figure 2. Rbfox2 knockout mice display differences in posture and locomotion and a smaller cerebellum (Gehman et al., 2012).

Role of Rbfox1l in telencephalic development and behavior

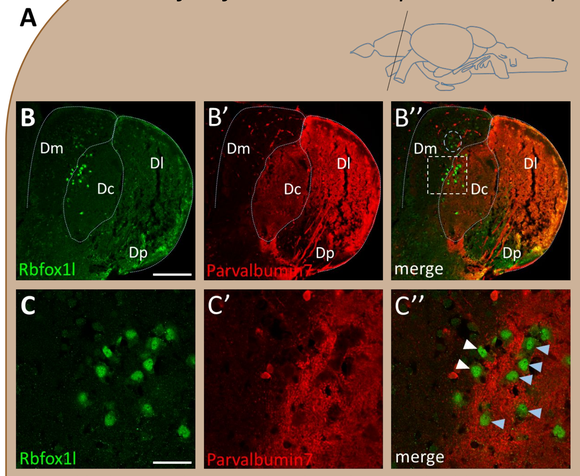
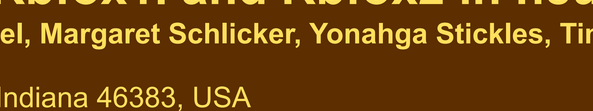


Figure 3: Rbfox1l is expressed in a restricted population of neurons in the adult zebrafish dorsal telencephalon spanning Dm and Dc regions. Ma, F., Dong, Z., and Berberoglu, M.A.* (2019). Expression of RNA-binding protein Rbfox1l demarcates a restricted population of dorsal telencephalic neurons within the adult zebrafish brain. *Gene Expr. Patterns* 31: 32-41. (*Corresponding author)

Expression of Rbfox1l (Rbfox1-like) has been identified in the adult zebrafish brain in a restricted population of neurons within the dorsal telencephalon, spanning Dm and Dc regions (Fig. 3; Ma et al., 2019).

When is Rbfox1l first expressed in the zebrafish telencephalon during development?



3 days post-fertilization (dpf) (larval zebrafish)

Preliminary studies indicate that Rbfox1l is not expressed in the brain at 3 days post-fertilization (dpf). However, Rbfox1l is expressed in the adult brain and within the adult telencephalon (Ma et al., 2019). We will look at late-larval time-points during zebrafish development to determine when Rbfox1l is first expressed.

Is *rbfox1l* necessary for telencephalic development and in regulating anxiety and/or autistic-like behaviors?

CRISPR technology has been used to generate *rbfox1l* and *rbfox2* mutant zebrafish which we will use for this study (Dr. Sharon Amacher, The Ohio State University, in collaboration).

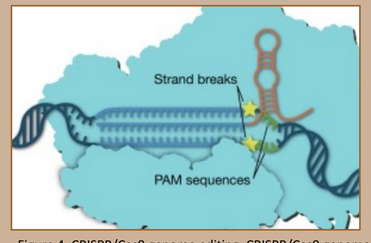


Figure 4: CRISPR/Cas9 genome editing. CRISPR/Cas9 genome editing requires a single guide (sg) RNA that directs the Cas9 endonuclease to a specific region of the genomic DNA resulting in a double strand break (Costa et al., 2017).

Previously established assays can be utilized to assess the behavior of zebrafish. One specific example of measuring anxiety in zebrafish is via a novel tank diving assay. In this assay, the zebrafish is transferred from a pre-treatment beaker to a novel tank. Naturally, the zebrafish will model anxiety due to being surrounded in a new environment. However, once the zebrafish has acclimated to the novel tank, it will slowly start swimming to the top of the tank. The amount of time it takes for the zebrafish to swim freely to the top of the novel tank is measured, and the longer it takes, the higher the anxiety levels of the zebrafish. There are several other similar assays that can be used to assess the behavior of zebrafish. We would also like to extend our work to understand whether *rbfox1l* regulates autistic-like behaviors in the zebrafish, and will assess shoaling behavior as a measure of social interaction.

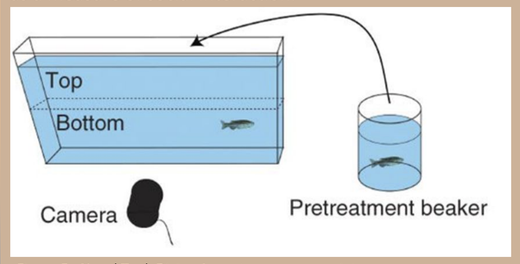


Figure 5: Novel Tank Diving Assay to measure anxiety.

Role of Rbfox2 in cerebellar development and adult regeneration

Expression of Rbfox2 has been identified in the adult zebrafish brain and within the cerebellum, including expression in cerebellar Purkinje cells (Fig. 6; Ma et al., 2019).

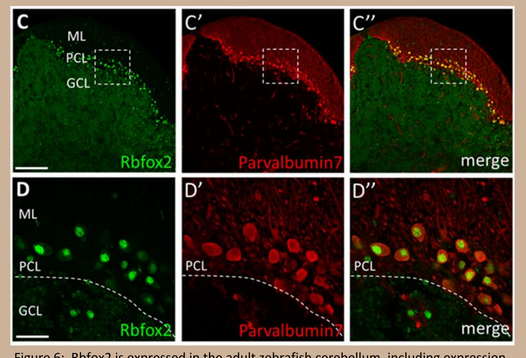


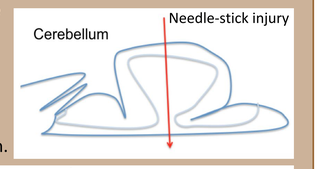
Figure 6: Rbfox2 is expressed in the adult zebrafish cerebellum, including expression in cerebellar Purkinje cells (Ma et al., 2019).

We are interested in better understanding Rbfox2 expression during development of the zebrafish brain. We will also analyze *rbfox2* mutant zebrafish (in collaboration with Dr. Sharon Amacher at The Ohio State University) to determine whether *rbfox2* is required for development of the zebrafish cerebellum as in mammals.

Is *rbfox2* necessary for regeneration of the adult zebrafish cerebellum?

To determine whether Rbfox2 is required for cerebellar regeneration, we will be conducting an EdU pulse-chase experiment. First, we will injure the cerebellum and inject EdU intraperitoneally (I.P.) which labels cells in "S" phase of the cell cycle, which may include neural progenitor cells of the cerebellum. Then, conduct the pulse-chase experiment to assess the production of new neurons after injury. We will perform immunohistochemistry for HuC/D (neuronal marker) on brain tissue sections and visualize together with EdU. Cells that exhibit both these markers are likely to be adult-born neurons.

The number of double-positive cells will be quantified and compared between wild-type and *rbfox2* mutant zebrafish, which would indicate the level of adult neurogenesis during regeneration of the cerebellum.



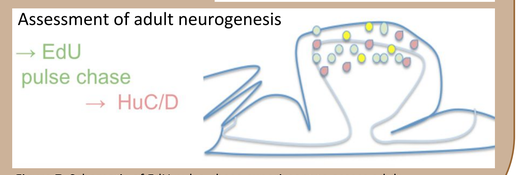


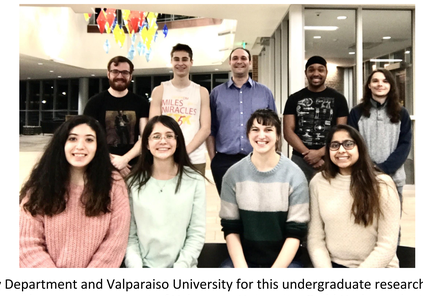
Figure 7: Schematic of EdU pulse-chase experiment to assess adult neurogenesis after injury to the cerebellum. Double-positive cells in yellow indicate potential new neurons.

Conclusions

- Rbfox1l is expressed in a restricted population of neurons within the adult zebrafish dorsal telencephalon spanning Dm and Dc regions (Ma et al., 2019).
- Rbfox2 is expressed in the adult zebrafish cerebellum, including expression in cerebellar Purkinje cells (Ma et al., 2019).
- Our work aims to better understand expression of Rbfox1l and Rbfox2 in brain development, neurogenesis, and behavior.

Future Directions

- Determine the time point in which *rbfox1l* is expressed in the brain during development and assess the expression of *rbfox2* in the brain during development.
- Determine whether *rbfox1l* is required for proper telencephalic development and whether *rbfox1l* mutants show differences in anxiety and/or autistic-like behaviors.
- Determine whether *rbfox2* is necessary for cerebellar regeneration.



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