Expression of the inhibitory receptor subunit $GABA_A$ α2 in neurons and glia of zebrafish embryos

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Introduction

γ-Gamma-aminobutyric acid (GABA) is one of the most crucial neuroinhibitory transmitters within the brain. Just about every thought, feeling, sensation or perception in the brain and nervous system involve this system of inhibitory receptors (Michels et al. 2007). $GABA_A$ receptors are a pentameric ligand-gated ion (Cl^-) channel family. To date, 18 GABAA receptor subunits have been identified and grouped into 7 subunits classes α (1–6), β (1–3), γ (1–3), δ, ε (1–3), θ, π (Jacob et al. 2008). This is crucial because in humans, diseases like addiction, schizophrenia, autism, and epilepsy are highly associated with problems in these genes (Ahn et al. 2011; Michels et al. 2007). To better understand this mechanism we will be using the zebrafish (*Danio rerio*) which, is an excellent model organism for genetic analysis and studying vertebrate nervous system development. In this experiment, we will be able to see both neurons and glia using immunohistochemistry (IHC). We will analyze them for the expression of GABA receptors using RNA *in situ* hybridization. GABA signaling in neurons interact with glial cells to control important functions of the brain such as neuronal activity, differentiation, myelination, and neuroprotection (Vélez-Fort et. al 2012). mRNA is a key factors to understanding the cellular and neuronal expressions within any system. By performing this experiment, we will be able to better understand the $GABA_A$ receptors, learn more about their function, as well as visualize the process using IHC.

Objectives

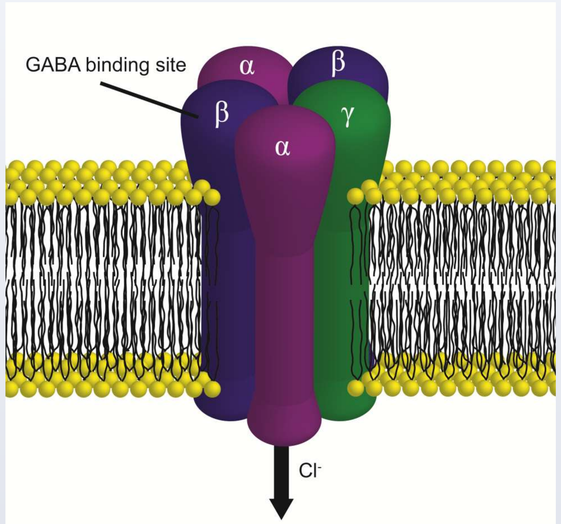
- To examine $GABA_A$ α2 expression in neurons and glia
- Use RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) to create a cDNA template of the zebrafish *gabra2* gene. From the cDNA template, create RNA probes complementary to the *gabra2* sequence and determine expression within the developing zebrafish embryo.
- Hybridize RNA probes *in situ* with native RNA. Label RNA probes with fluorophores, allowing visualization via direct immunohistochemistry.
- Perform indirect immunohistochemistry with NeuroD and GFAP on both neurons and glia cells.
- The ultimate goal of this experiment is to determine exactly where and when the *gabra2* gene is expressed in zebrafish embryos and if these subunits are in both neurons and glia.

Methods

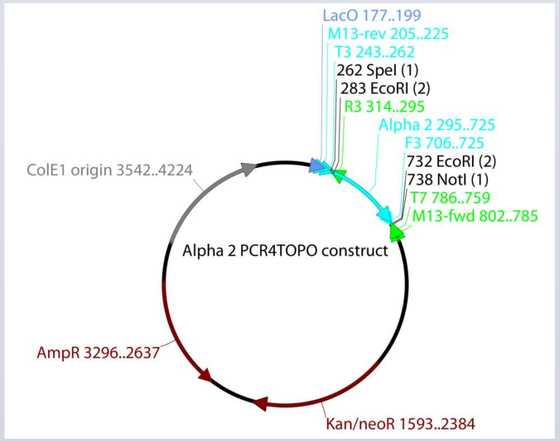
- Primer Design
  - *gabra2* F1- TTGGACCAGTGTCCGATACA (Genelink)
  - *gabra2* R1- CATAGGCCACCTTCGGTAAA (Genelink)
- RNA Isolation
  - SV Total RNA Isolation System (Promega)
- Reverse Transcription
  - GoScript Reverse Transcription System (Promega)
- PCR Amplification
  - GoTaq PCR Core Systems (Promega)
- Troubleshooting
  - Performed different concentration mixes of $MgCl_2$ and thermocycler temperatures ranging from 50-60°C
- Vector Transformation
  - Obtained a designed plasmid vector for our *gabra2* from the University of Massachusetts-Amherst Laboratory of Gerald B. Downes.
- Plasmid Midiprep
  - PureYield Plasmid Midiprep System Kit (Promega)
- Restriction Digest Cleanup – SPE1 enzyme
- Probe Generation
- Troubleshooting
  - Amount of DNA insufficient due to Midiprep yield on first preparation
- Immunohistochemistry
  - Anti-NeuroD and Anti-GFAP antibodies primary antibodies (ANASPEC).
  - Goat Anti-Rabbit IgG secondary antibody (ANASPEC).
- Cryosectioning
- Imaging – Fluorescent Microscopy (Olympus IX73 Microscope system)

Results

Model of a $GABA_A$ Receptor



Plasmid construct



Conclusions

We had issues with the PCR amplification, and we performed multiple attempts to create more cDNA; however, due to failure, we obtained our plasmid construct from University of Massachusetts-Amherst Laboratory of Gerald B. Downes. The Midiprep did not produce a sufficient amount of DNA yield to continue further. Given the amount of time we had to work, we were able to perform a lot of the experiment in one semester that can take years at other laboratories. In addition, the knowledge and hands on skills that we acquired through this class will be able to carry into future work. We were able to come out of this course with a greater knowledge of biological techniques and their application toward the research field.

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