Characterizing the regulatory region of the *parathyroid hormone 2* gene in zebrafish.

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ABSTRACT

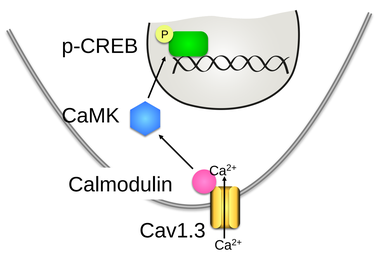
In larval zebrafish, *parathyroid hormone 2* (*pth2*) codes for a peptide hormone that is expressed exclusively in cells near the ventral part of the posterior tuberculum, an area of the forebrain involved in sensori-motor control and social behavior. In areas of the rodent brain, *pth2* is involved in emotional processing and is implicated in behavioral responses to fear, stress, and pain, but it is unknown whether *pth2* plays a similar role in zebrafish. Though *pth2* is only found in one small brain region, the receptors for *pth2* are found throughout the central nervous system of zebrafish. This leaves a number of unanswered questions: (1) How is *pth2* expression regulated? (2) Which areas of the brain do *pth2*-expressing cells connect to?

We identified *pth2* in an RNA-seq experiment looking for genes regulated by voltage-gated calcium channel activity. Relative to wild type larvae, *pth2* was dramatically down-regulated in larvae where the L-type Cav1.3 channel was inactivated either genetically (*cav1.3a* mutants) or pharmacologically (the Ca^{2+} channel blocker isradipine). Using mRNA *in situ* hybridization on *cav1.3a* mutant larval zebrafish and larvae treated with isradipine, we confirmed that *pth2* expression in zebrafish requires Cav1.3a channel activity. This finding led to the question: what are the DNA regulatory elements responsible for the Ca^{2+}-dependent expression of *pth2*?

To characterize the regulatory region from the *pth2* gene, we have cloned a 2 kbp fragment of the zebrafish genome immediately upstream of the *pth2* coding sequence. We will clone the GFP gene into a plasmid with the *pth2* promoter driving its expression and inject it into zebrafish embryos. We predict that, if we have isolated the correct regulatory elements, GFP will be expressed in cells of the posterior tuberculum and be responsive to changes in Cav1.3a activity. If successful, this can open up the possibility for a wide range of experimental manipulations to understand how *pth2* expression is regulated. Furthermore, successful GFP expression will allow us to visualize where *pth2*-positive cells project their axons, giving us some insight into which physiological or behavioral processes may be influenced by *pth2* activity. Using this information, a possible correlation could be drawn between the region of expression of *pth2* in zebrafish and rodents and its behavioral implications.

Background

Through a process called excitation-transcription coupling[1], stimulus-dependent changes in cytoplasmic calcium ion concentration can play a role in the regulation of gene expression. Calcium ions entering a cell through voltage-gated calcium channels can interact with signaling proteins to regulate transcription.



Cav1.3 is an L-type voltage-gated calcium channel that is expressed in the brain, retina, and acoustico-lateralis sensory systems[2].

Sidi et al. 2004

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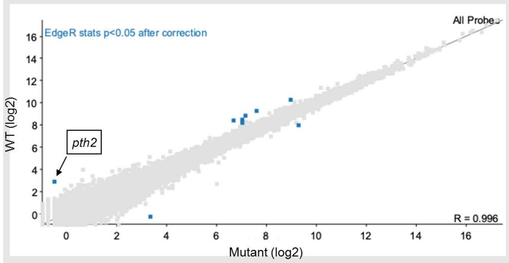
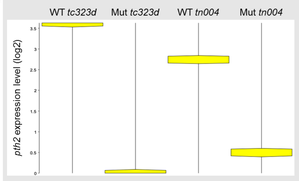
Background

To identify genes that are regulated by Cav1.3 activity, we did RNA-seq to find transcripts that are differentially regulated in *cav1.3a* mutant zebrafish larvae. We found that *parathyroid hormone 2* (*pth2,* a.k.a *tip39*) transcripts were downregulated in two different mutant alleles of *cacna1da* (*tc323d* and *tn004*).





METHODS

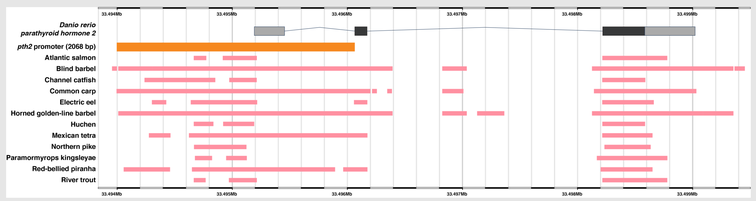
Experiment 1

To confirm the RNA-seq results, we performed mRNA *in-situ* hybridization for *pth2* on wild type and mutant zebrafish larvae. *pth2* transcripts were detected in the brain of wild type larvae (n=51) but were not detected in *cav1.3a* mutants (n=27). (See Figure 1)

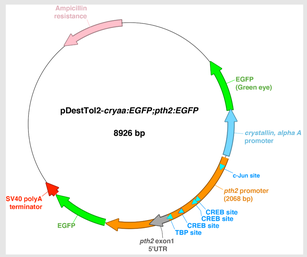
We also used *in-situ* hybridization on larvae treated with the Ca^{2+} channel blocker isradipine. *pth2* was expressed to a large degree in the control and was downregulated in larvae treated with isradipine. These results led to the conclusion that *pth2* is heavily regulated by the Cav1.3 calcium channel.
(See Figure 2)

Experiment 2

To identify the regulatory elements responsible for regulation of *pth2*, we have cloned a part of the zebrafish genome upstream of the coding region for *pth2* into a plasmid with the GFP gene and injected it into embryos.



We predicted that if the correct regulatory elements were isolated, GFP would be expressed in cells where *pth2* is normally expressed, it would be responsive to changes in Cav1.3 activity, and it would allow us to visualize where *pth2*-positive cells project their axons.



RESULTS

Experiment 1: The voltage-gated Ca^{2+} channel Cav1.3a regulates expression of *pth2.*

Figure 1:

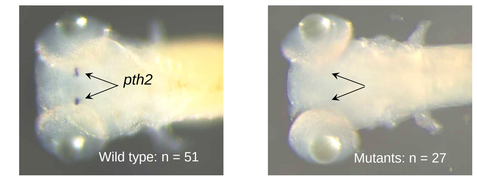
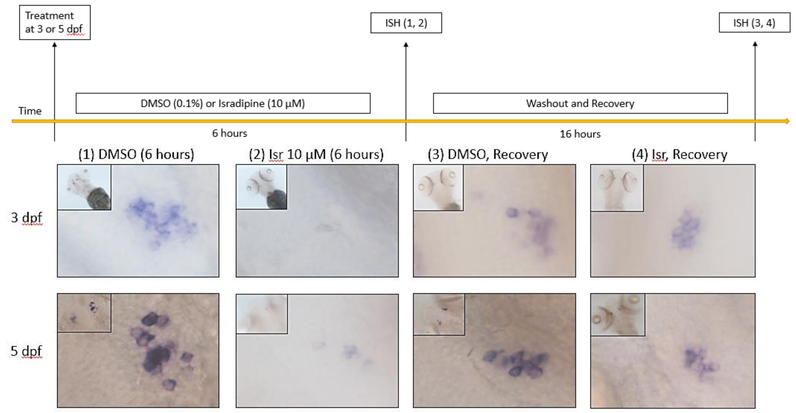
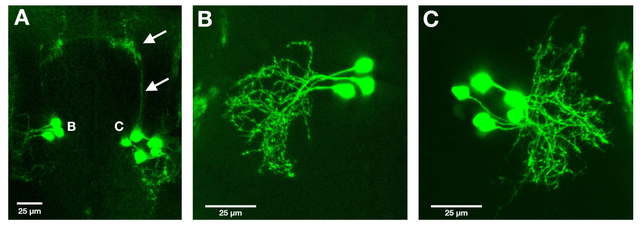


Figure 2:



Experiment 2: The 2 kbp *pth2* regulatory element drives GFP expression in presumptive *pth2*-expressing cells.



DISCUSSION

We used RNA-seq to identify transcripts whose expression was changed in *cav1.3a* mutant zebrafish. We found that *pth2* transcripts were downregulated in mutants. To confirm these results, we performed mRNA in situ hybridization for *pth2* on wild type and mutant zebrafish larvae, as well as larvae treated with isradipine. *pth2* was expressed to a much lower degree in mutants and larvae treated with isradipine than in the control groups. These results suggest that Cav1.3 channel activity plays a crucial role in regulating *pth2* expression.
Expression of GFP was successfully driven by the isolated regulatory elements in cells of the posterior tuberculum and their axons, which project laterally and toward the anterior ventral part of the forebrain. In the future, this knowledge could be used to help determine the many other factors that contribute to expression of the *pth2* gene, and its broader role in the behavior of organisms.

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