Characterization of a population of spinal neurons in *Danio rerio* expressing MsxC

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**Introduction**

Homeoboxes are sequences that are involved in the regulation of patterns of anatomical developments and function as transcription factors. One such homeobox, Muscle-segment homeobox genes (Msx), has been implicated with muscle development as well as fin development in fish. In zebrafish (*Danio rerio*), there are five different members of the Msx gene family. Msx A to E. Previous work done by Dr. Marie-Andrée Akimenko from the University of Ottawa identified Msx expression in neurons of the spinal cord in zebrafish. One way to characterize populations of neurons is to determine commonly expressed complement of transcription factors. It is known that Atoh1, in mammals, is selectively expressed in dI1 neurons, which have spinalcerebellar activity – spinocerebellar tracts play a role in adapting on-going movements to be more accurate. Furthermore, previous experiments indicate a link between Msx1 and Atoh1 in mice.

**Hypothesis**

This study aims to determine if MsxC expressing neurons are analogous to mammalian dI1 spinal neurons and aid in the fish’s adaptation of movement through communication with the brain.

**Methodology**

**Breed and Screen Zebrafish**

**Extract DNA and Generate RNA Probes for in-situ hybridization of MsxC and Atoh1a**

**Determine the overlap of MsxC and Atoh1a in the spinal cord of embryonic and larval zebrafish**

**Primersex:**

Atoh1a FWD: 5’TCCCCAGGCAAATATTCGT 3’
Atoh1a REV:5’TAAATCATATGATATGGCCCTCGAGCAG ACTTGCCTC3’

**Double In-Situ Hybridization**

**Figure 1.** Spinal cord cross-sections showing double in-situ hybridization in zebrafish (Developmental Dynamics, 2007)

**Results**

Possible MsC-Atoh1a interactions include:

<table>
<thead>
<tr>
<th>Activator</th>
<th>Inhibitor</th>
<th>No effect</th>
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<tbody>
<tr>
<td>MsxC</td>
<td>Atoh1a</td>
<td>MsxC</td>
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There are indications from chicks and mice that the Msx genes are transcriptional repressors and this may be their role in the spinal cord hinting to the fact that their expressions don’t overlap with their targets. In Chick, electroporation of some of the Msx genes has resulted in the expansion of certain neural progenitor domains (dp1 and 3), at the expense of other neural progenitor domains (dp1 and 4) suggesting that normally these Msx genes repress genes like perhaps Ng1 and Lhx1/5. This would allow an expansion of the domains of Atoh1 expressing progenitor domains (dp1) and the Isl1 expression progenitor domains (dp3) (Liu et al. Development, 2004; Alaynick et al., 2011).

**Conclusion**

The ISH probes are still a work-in-progress and once the probes MsxC-DIG, Atoh1a-DIG (positive controls) as well as the MsxC-DNP probe are made, then we will better understand the relationship between MsxC and Atoh1a and their roles in neural connections in zebrafish.

**Future Directions**

Based upon the results of our ISH study, future experiments will seek to compare the molecular identity spinalcerebellar neurons in zebrafish with that in mice, determine the consequences of turning off MsxC on the development of spinocerebellar neurons, performing electrophysiology to study the neural activity of zebrafish spinalcerebellar neurons, and mapping the connectivity of spinocerebellar neurons to better understand the circuitry of the spinal cord.

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[References]


