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

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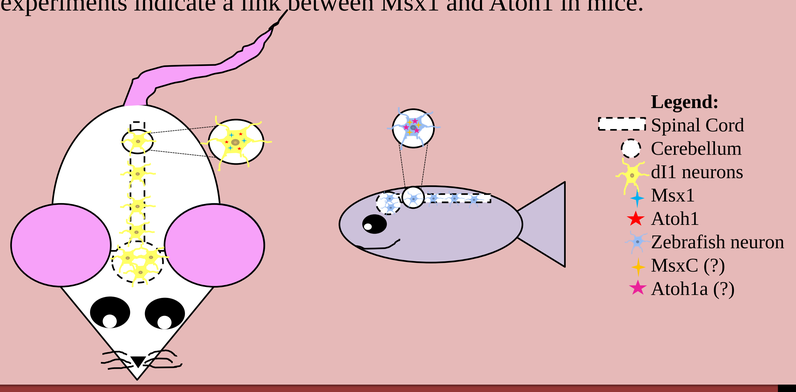
Introduction

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 MsxC 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 spinocerebellar 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

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

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

Primers:

Atoh1a FWD: 5' TTCCCAGGCAAAATATCCGT 3'

Atoh1a REV:5'TAATACGACTCACTATAGGGCCTCCGAACCAG ACTTGCTC3'

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Double In-Situ Hybridization

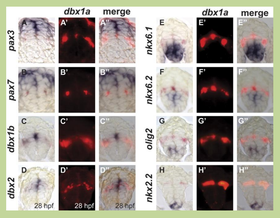


Figure 1. Spinal cord cross-sections showing double in-situ hybridization in zebrafish (Developmental Dynamics, 2007)

Results

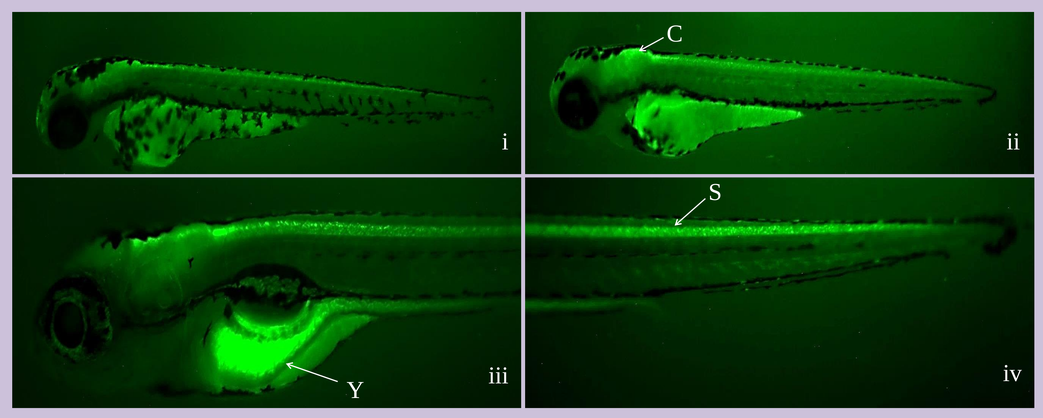
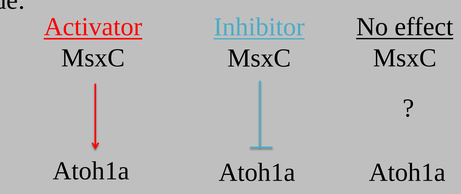


Figure 2. Transgenic Zebrafish (MsxC FragC Bg eGFP) expressing MsxC in different developing areas 2-4 days post-fertilization (dpf). (i) 2dpf at 4.0x (ii) 3dpf at 3.0x (iii) and (iv) 4 dpf at 6.3x. Image taken using an epifluorescence microscope.
(C = cerebellum; Y=Yolk Sac; S=spinal cord)

Discussion

Possible MsxC-Atoh1a interactions include:



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 spinocerebellar 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 spinocerebellar neurons, and mapping the connectivity of spinocerebellar neurons to better understand the circuitry of the spinal cord.

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