UNCHARACTERIZED PROTEIN MKRN2OS.2 FOUND TO LOCALIZE AT THE TIPS OF STEREOCILIA, IMPLYING POTENTIAL ROLE IN SENSORY HAIR CELL FUNCTION.

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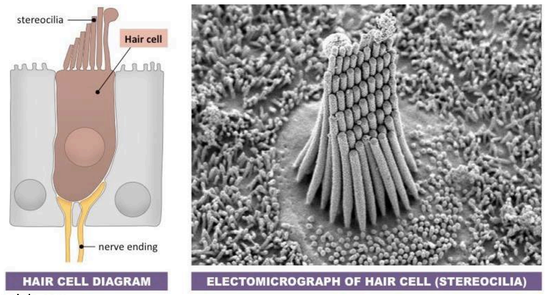
INTRODUCTION

Hair cells are the receptors for the auditory, vestibular, and lateral line sensory systems of vertebrates. Through a process called mechanotransduction, hair cells convert mechanical stimuli into electrical activity that allows the brain to perceive the sensation of hearing. Mechanotransduction occurs in a sensory organelle called the hair bundle, which is a specialized collection of actin-filled microvilli (stereocilia) on the apical surface of the hair cell.



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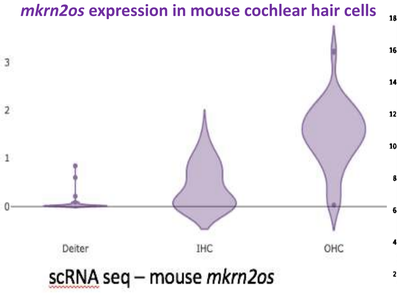
BACKGROUND

We performed an analysis of the hair cell transcriptome in larval zebrafish via Thiouracil RNA tagging. From this analysis, it was found that transcripts from an uncharacterized gene called MKRN2 opposite strand, tandem duplicate 2 (mkrn2os.2) were enriched in the hair cells. We expressed GFP-tagged versions of Mkrn2os.1 and Mkrn2os.2 and found they localized at the tips of the stereocilia, the site where mechanotransduction occurs.

GFP-tagged Mkrn2os.2 localizes to the tips of the stereocilia

***mkrn2os* expression in mouse cochlear hair cells**

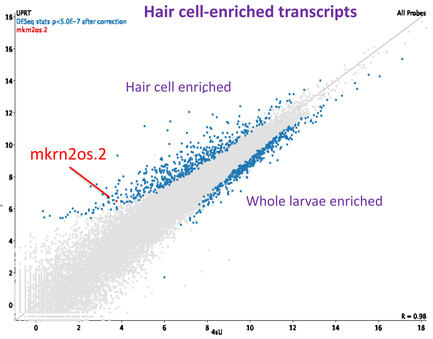


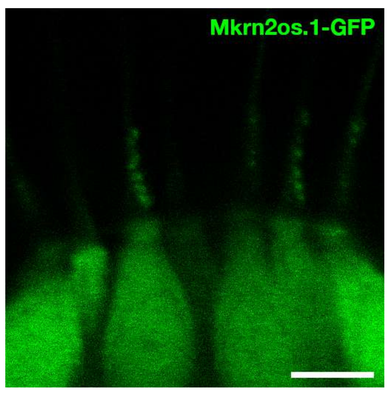
scRNA seq – mouse *mkrn2os*

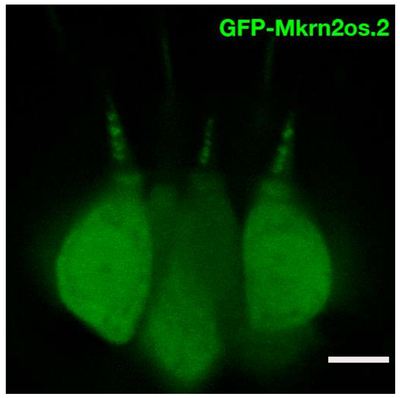
Paul T Ranum, Cell Reports

***mkrn2os* is expressed in the cochlear cells of multiple species**

Hair cell-enriched transcripts







HYPOTHESIS

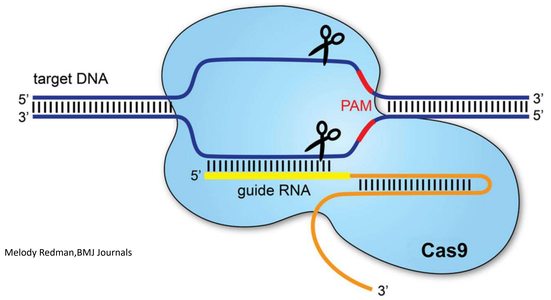
Based on the localization of Mkrn2os.1 and Mkrn2os.2 at the tip links of the stereocilia, we hypothesize that Mkrn2os.2 may play a role in hair bundle function and /or morphology.

EXPERIMENTAL METHODS

To test our hypothesis, we are using CRISPR-Cas9 gene editing tools to create mkrn2os.1 and mkrn2os.2 mutant zebrafish. We will then observe if the mkrn2os mutants exhibit any defects in mechanotransduction and / or structural variations in the hair bundles.

CRISPR-cas9

CRISPR-cas9 is a gene editing tool that employs a guide RNA attached to a cas9 enzyme that cuts DNA strands to cut DNA at specific sequences. It can be used to disrupt sequence or introduce new DNA fragments.



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CREATION OF THE *mkrn2os.* MUTANTS

A CRISPR-cas9 gene edit was performed on the *mkrn2os.1* site in wild type zebrafish larvae.

. >mkrn2os.1_sgRNA1 >mkrn2os.1_sgRNA2 >mkrn2os.1_sgRNA3

```
>XM_001922864.6:137-805 PREDICTED: Danio rerio MKRN2 opposite strand, tandem
duplicate 1 (mkrn2os.1), mRNA
ATGGAGGAAACCGTCATCAAGTACATACATTGTGGAAGAAGCATTTACAGTTTCTCGTGTCCGAACGGCG
GATATTCTGAGAGACCTGGAGGAGCCGCTCGTCAGTGTCCGATCTGTCTTCAGATTCTCACTTTCGGGCT
TTTAGACGCCCCGGTGTGTGTTCCCTGTCCGCTTAGGAATGGACACCAAGTTTCCTGCGCGTTTCTGATA
GGATCTGCGCACGGGCCCTCACATCTGGGAGAGTGGCAGGACTCAGAGATCCATGTGGGCCTGTCAGATT
CAGCAGGTCTGGTCTACAACTACACACTGTCCGGGGTCCAGCGGGATGATCGCGGCTGGGAACAGTGTGT
GTGTGTGCAGCTCGTCCCTCCGTCGAGACCTGAGCTCCGGGAATTATGGGATACACACCTCCAGCTGTTT
GCCCTGCTGCCAGAATGGGCTTCAGAGCGGTTTGAAGAGGAGCGTGAGTTCGGCTCCTGCTGCTATGGCT
TCGCTCTGACCTTCATAAACCGCATGCGTTCACTGGACAATAAAGACTGTCTGAGCAGAGATGAGTTCAC
TGGACGGTACATCTTACCCCGCATGAAGACCGTGTCCCTTTACATCAGCATCTACCAGACGATATTACAA
CACGGCTTTCACATAGCTAATACAGCGGATGACGAGTGA
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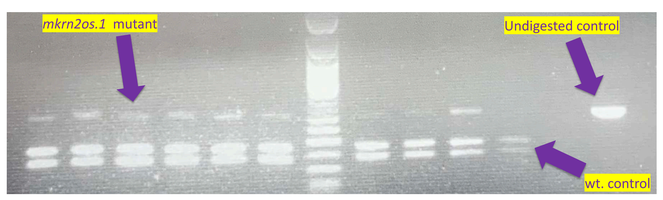
A restriction digest of the *mkrn2os.1* site performed on a few of the genetically modified larvae showed evidence of the disruption of the mkrn2os.1 site.



A CRISPR-cas9 gene edit was performed at the mkrn2os.2 site in wild type zebrafish larvae. This resulted in a four bp deletion, causing a frameshift mutation in *mkrn2os.2.*

>mkrn2os.2_sgRNA2

```
>XM_001920173.7:549-1214 PREDICTED: Danio rerio MKRN2 opposite strand, tandem
duplicate 2 (mkrn2os.2), mRNA
ATGGAGAAGAGCGTGATCAAATTCAGCCACTGCCACAGGGACATCTTCTGCTTCTTCGTGCCGGATCAGT
GTCCCGAATGTGGGGAGAGTTTGAGCGGGAAGCGGCTGGAGGAGGCGCCCGTCTCCATCCCAAACCCCTT
CAGCAACGGACACAAAACCCCATGTGCTTTTCTGGTGGCGTCAGCTGAAGACCGGCTGCTCAGGGACTTT
GATGGACAATCTGATCTGCATACAGGAATCACCAACACAAATGGCGTGGTGTATAATTACACATGTGCGG
GTGTGCAGAGGGAGACTCAGGGCTGGGAGCGCTGCATCTGTGTGCCGCTGGTGCAGCCGGATATGTTCAG
TCTGATCAGCCAATGGGATCAATATCTGGAGAAATTCTCCACCGCTCAGATGTGGGACCCGCTCTGGCAG
AGCTTTAATGAAGAGAGCCACAACTGCTTCAGCTTCACACTGATGTTCATCAACTGTGTTCTGGCCACGC
AGTCCAAGCGCGCGCTCAGCAAAGATGAGTTCACACACTCGTTCGTGCTCCCTCGCATTAAAAGAGCCTC
CAAATACATGATGCTGTGCAGCCAGATAACCCAGAACCACTTCTACATCGTCAACAACCCGAGGAGAAAC
TCTCAAGACGACCCAATGGAGGAAAACCACAACTGA
```

***mkrnos.2* Sequence Alignment**

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mkrn2os.2_WT      81 TGGGGAGAGTTTGAGCGGGAAGCGGCTGGAGGAGGCGCCCGT
                     ||||||||||||||||||||||||||||||||||||||||||
c.106_109del      81 TGGGGAGAGTTTGAGCGGGAAGCG----AGGAGGCGCCCGT
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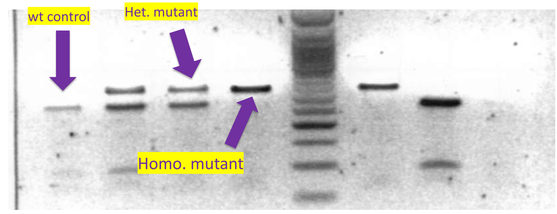
We have successfully created a line of Homozygous *mkrn2os.2* mutants by in-crossing Heterozygous *mkrn2os.2* mutants.
This was confirmed by a restriction digest of the *mkrn2os.2* site in a few of the resultant larvae from this in-cross.

Restriction digest gel



CONCLUSION & FUTURE DIRECTIONS

mkrn2os.2 is enriched in hair cells and the tagged protein localizes to the tips of stereocilia. To determine the role of the *mkrn2os* genes we successfully induced lesions into the genome of zebrafish using CRISPR-cas9 to disrupt gene function.

Moving forward, we will perform a CRISPR-cas9 gene edit of the *mkrn2os.1* site if the *mkrn2os.2* homozygous mutants, hopefully resulting in *mkrn2os.1* and *mkrn2os.2* double mutants. We will then observe if the *mkrn2os* mutants exhibit any defects in mechanotransduction and / or structural variations in the hair bundles.