USING ZEBRAFISH MODELS OF USHER SYNDROME TYPE 2A TO INVESTIGATE RETINAL CELL FUNCTION AND SURVIVAL

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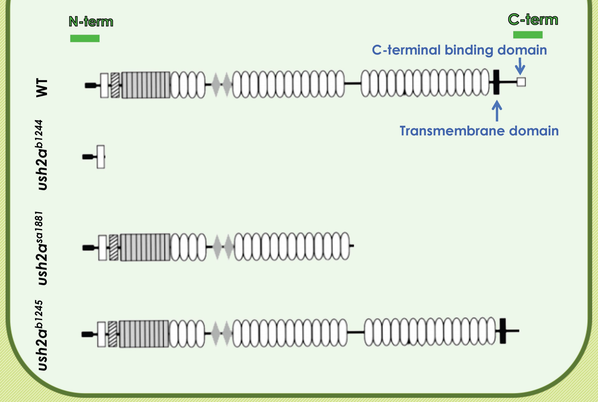
INTRODUCTION

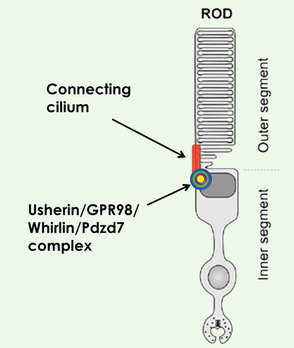
Usher syndrome is the most common cause of hereditary deaf blindness and has no cure. Mutations in the *USH2A* gene, which encodes the protein Usherin, are responsible for Usher syndrome type 2A (USH2A). Patients diagnosed with USH2A have congenital hearing impairment that ranges from moderate to severe and experience progressive vision loss beginning in the second decade of life. This progressive vision loss, called retinitis pigmentosa (RP), is due to degeneration of the photoreceptor cells in the retina, which initially causes night blindness and progresses to tunnel vision. We are investigating retinal symptoms of USH2A using a zebrafish disease model.

Part 1: Mutations in the zebrafish *ush2a* gene:
Truncating mutations in the *ush2a* gene affect protein stability and localization.

Three alleles of *ush2a* affect distinct coding regions of the gene

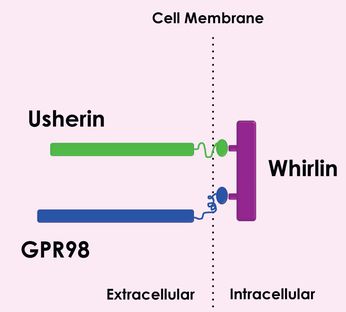


Usherin localizes to the base of the photoreceptor connecting cilium

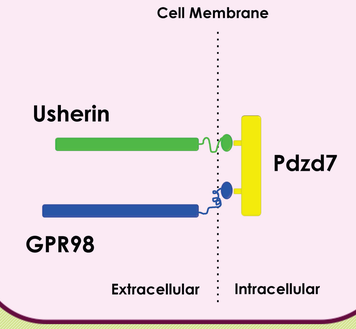


Part 2: The USH2 Protein Complex:
We do not see any evidence that the absence of Usherin disrupts localization of other components of the USH2 protein complex.

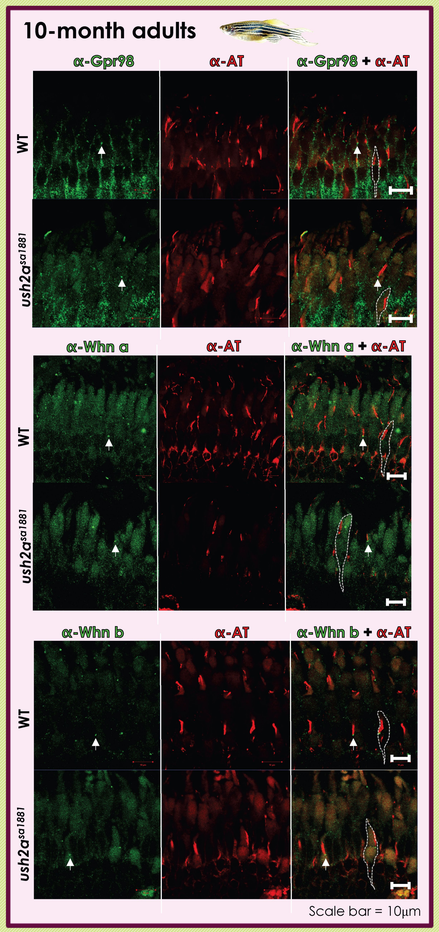
Usherin interacts with other USH2 proteins to form a complex at the base of the connecting cilium



Pdzd7, another USH2 protein, is an alternative binding partner of GPR98 and Usherin.

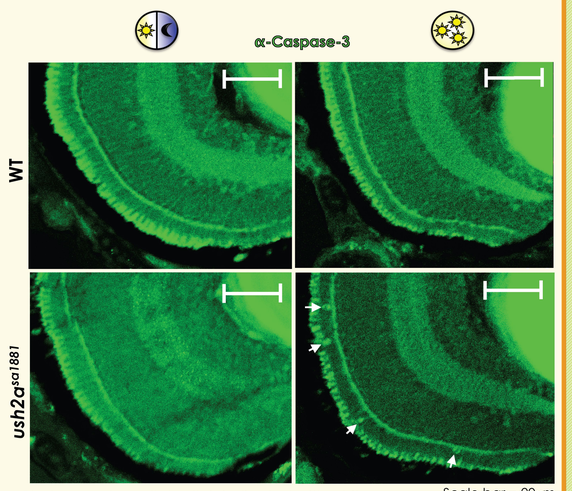


10-month adults



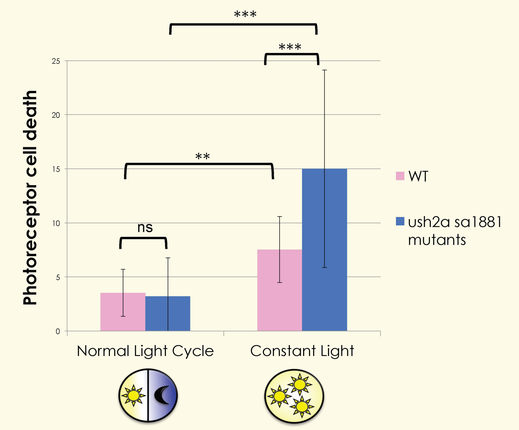
Part 3: Constant light exposure experiment:
We have devised a system that causes light-induced photoreceptor damage and a retinal phenotype similar to what is seen in human USH2A patients.

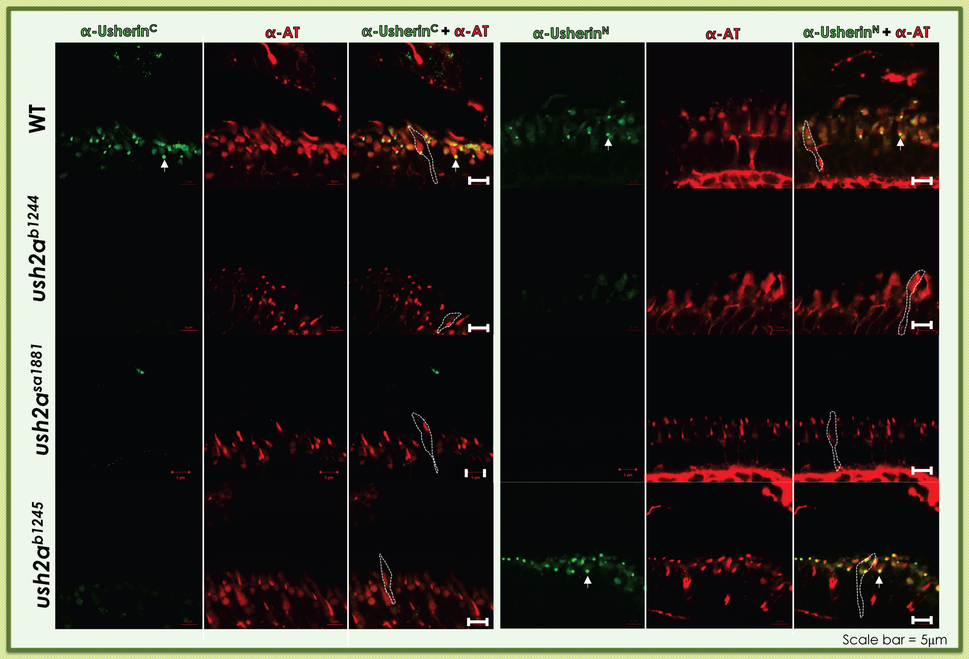
Photoreceptors labeled with α-Caspase-3 were counted in WT and mutant retinas



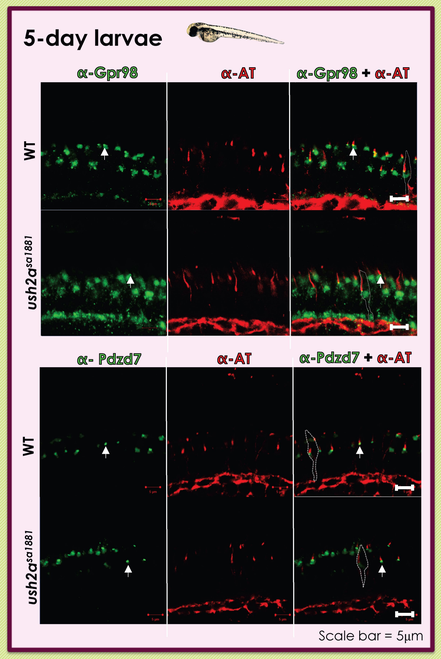
Photoreceptor cell death is indicated with white arrows. Scale bar = 20μm

Photoreceptor death was significantly increased in *ush2a* mutants raised in constant, elevated illumination





5-day larvae



CONCLUSIONS

Part 1: Usherin is not detectable in the *ush2a*^{b1244} and *ush2a*^{sa1881} retinas, indicating that these are complete loss-of-function mutations. The presence of the slightly truncated protein encoded by *ush2a*^{b1245} at the base of connecting cilium demonstrates that C-terminal binding activity is not required for localization. These models can be used to investigate the functional importance of different Usherin protein domains.
Part 2: We have established that localization of other USH2 proteins at the connecting cilium is not affected by the absence of Usherin in zebrafish. This is in contrast to previous studies in mouse. Further studies are needed to understand trafficking, assembly, and function of the USH2 complex.
Part 3: We have observed that short term exposure to constant light accelerates photoreceptor dysfunction in young *ush2a* mutant zebrafish. Being able to reproduce a degeneration phenotype in zebrafish similar to that observed in human USH2A patients gives a clear advantage over mouse models of USH2A in identifying precursors to photoreceptor cell death.

Taken together, the detectable loss of Usherin localization and photoreceptor cell death in *ush2a* mutants exposed to increased light will provide valuable outcome measures for preclinical testing with therapies targeting the retinal degeneration of Usher syndrome type 2A.