Exploration of sensory hair cell regeneration through the use of light-initiated apoptosis

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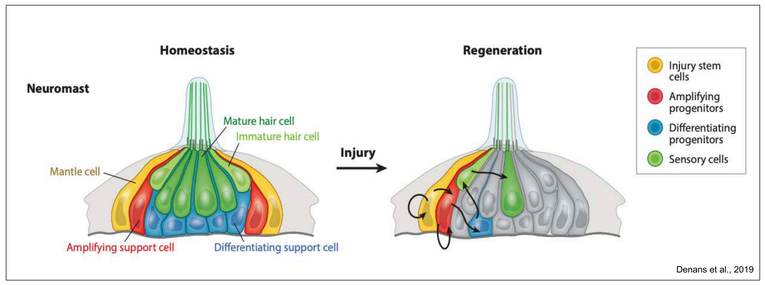
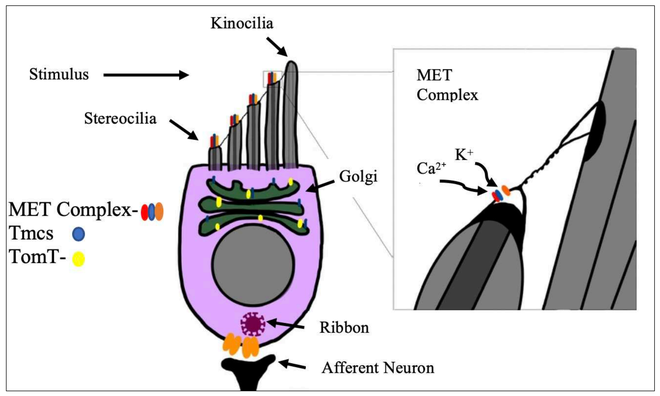
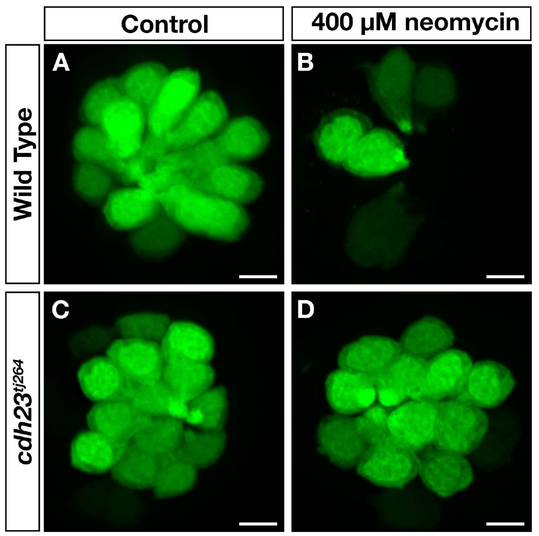
Sensory Hair Cell Death and Regeneration



- In the event of sensory hair cell death, progenitor stem cells begin to differentiate and replace the sensory hair cells.
- This process begins hours after cell death and can be fully replaced within a 72-hour period in Zebrafish.
- To study regeneration in non-mammalian models, apoptosis is triggered by ototoxins that enter the hair cell through functional MET channels.

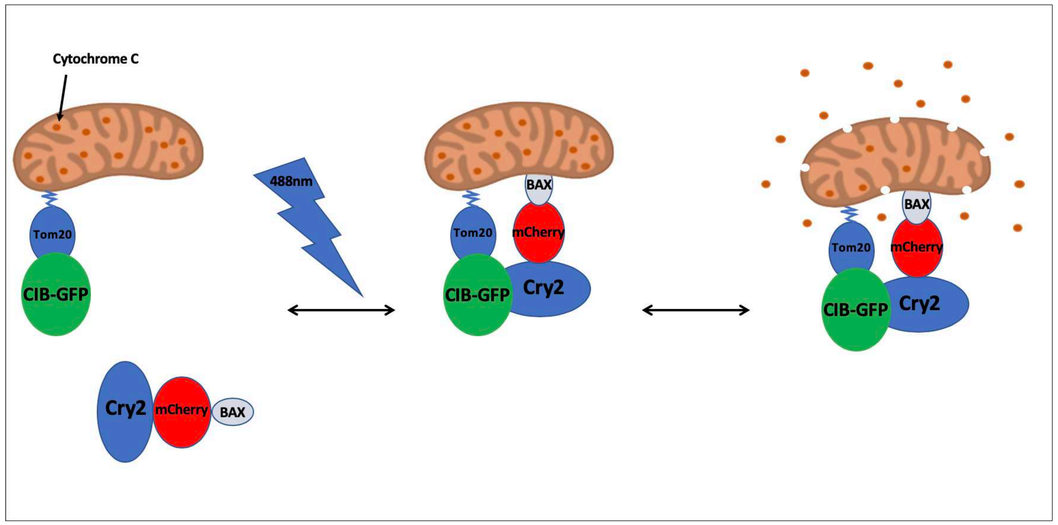


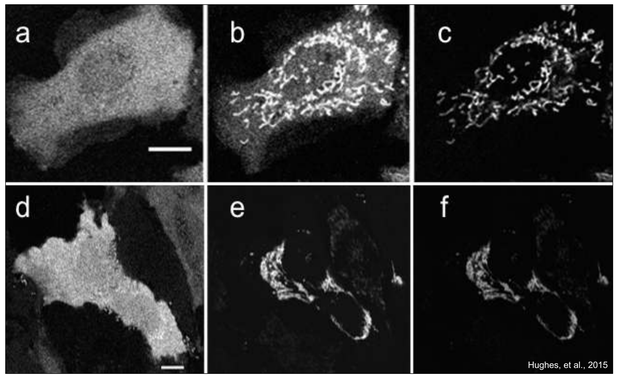
- Sensory hair cells convert mechanical information into electrical signals via a collection of proteins known as the mechanotransduction (MET) complex.
- These proteins make up a mechanically controlled gated- ion channel that allow Ca^{2+} and K^{+} into the cell in order to convert mechanical information into electrical signals transmitted to the brain.



- Mutations in the MET complex make hair cell nonfunctional, and therefore resistant to ototoxin ablation.
- Ototoxins, such as neomycin, are effective in ablating functional hair cells, but we have shown that the traditional use of aminoglycoside antibiotics have no effect on ototoxin resistant hair cells.
- Proposed OptoBAX system could ablate targeted sensory hair cells in zebrafish without causing damage to surrounding cells and creating confounding variables.

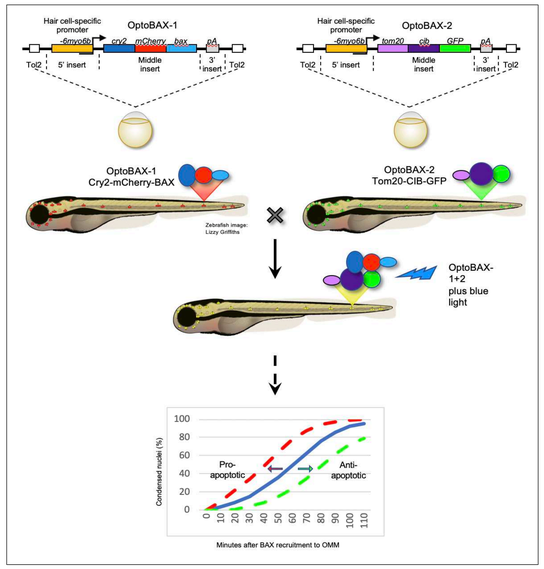
Light- Activated Cell Death Through the use of OptoBAX system



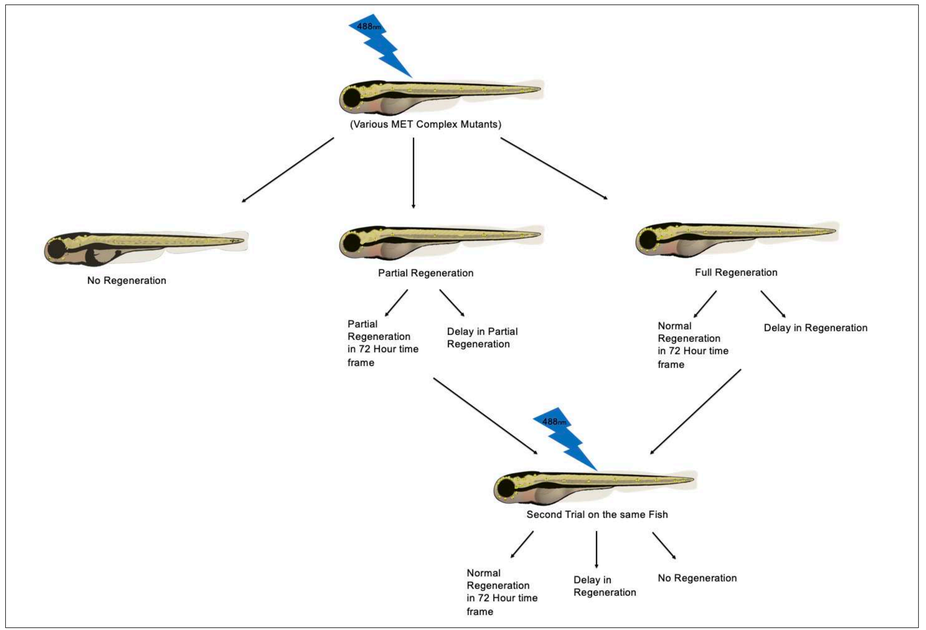


- Previous studies have visualized the recruitment of Cry2-mCherry to the mitochondria in MtLn cells (a-c) as well as HeLa cells (d-f) prior to 488nm illumination (a,d), 1 minute post-illumination (b, e), and the localization of the Tom20-CIB-GFP construct (c,f) in the mitochondria.
- This Optogenetic tool has not been previously attempted in zebrafish.

- In the engineered OptoBAX system, CIB is anchored to the mitochondrial outer membrane via fusion with a mitochondrial-targeting transmembrane domain Tom20.
- The pro-apoptotic protein BAX is expressed as a fusion with Cry2 where it resides harmlessly in the cytoplasm.
- When cells are exposed to blue light, the CIB-Cry2 interaction is activated.
- BAX is rapidly recruited to the mitochondrial outer membrane where it induces morphological changes releasing cytochrome C and the initiates apoptosis.



Expectations



- If MET- deficient hair cells can regenerate, to what compacity these cells regenerate is critical. Confocal analysis and cell count would determine if the hair cells form correctly in the regeneration process.
- The use of various MET complex mutants will determine if each component in the MET complex affects regeneration in a different way.
- Time in which various mutants regenerate (in partial or full compacity) allow indications of MET complexes hinder growth in various stages of regeneration.
- Screen for physiological stresses or treatments that hasten or slow the timing of light-induced apoptosis in wild type hair cells
- If regeneration is possible, experiments with gene therapy techniques to test restoration of MET complex function could be done.
- Current research on gene replacement therapies for human deafness will not work if MET-deficient hair cells have died and cannot be regenerated.