Online Proceedings

SESSION 1J

MECHANISMS OF CELLULAR REGULATION

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 251
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

The Impact of miRNAs on Postnatal Retinal Progenitor Cell Development
Ellen Riddle, Senior, Biochemistry
Mentor: Thomas Reh, Biological Structure
Mentor: Stefanie Wohl, Biological Structure

Small RNA molecules called microRNAs (miRNAs) are translational repressors and are involved in a variety of processes important for normal cell development and function. Previous studies in our lab showed that miRNAs are required in early (embryonic) stages of retinal development. After depletion of miRNAs in early retinal progenitor cells (RPCs), late RPCs and their progeny, such as Müller glia (MG) which are the primary glial in the retina, are not generated. Instead, only early born neurons are produced. The depletion of miRNAs can be obtained through deletion of an enzyme that generates mature miRNAs called Dicer. Since we know that miRNAs are crucial for early retinal development, this study investigated if miRNAs are also important for proper late (postnatal) retinal development. We used a Sox2 reporter mouse which allows visualizing late retinal progenitors (Sox2 is a gene expressed in RPCs) and selectively deleted Dicer in RPCs at postnatal day 2-6. The tissue was analyzed 2, 4 and 7.5 weeks after Dicer deletion and compared to normal retinas using immunofluorescent staining of retinal cross sections and confocal microscopy. We found massive disruptions in the retinal architecture such as the formation of rosette/ bubble-like structures, indicating that miRNAs are required for proper formation of the retinal layers. Since MG play a role in maintaining the retinal structure, in a second series of experiments we induced Dicer deletion specifically in MG at postnatal day 11 –14, when the retina is almost completely developed. The tissue was analyzed 8 and 20 weeks after Dicer-deletion and compared to normal tissue. We found an atypical location of MG and disruptions in the layered retinal structure, indicating that miRNAs are also required for accurate MG location in the retina, which is essential for normal retinal architecture.

POSTER SESSION 2

MGH 206, Easel 165
1:00 PM to 2:30 PM

CRISPR Based Behavioral Screening for Genes Affecting Zebrafish Nociception
Nicolas P. Germanos, Senior, Neurobiology
Mary Gates Scholar, Innovations in Pain Research Scholar, UW Honors Program
Mentor: Ajay Dhaka, Biological Structure
Mentor: Andrew Curtright, Biological Structure

Chronic pain affects millions of people worldwide, and current treatments and ineffective and come with unwanted side effects. Modern research focuses on studying the mechanisms of pain sensation in the nervous system, with the goal of using our improved knowledge to develop more efficient analgesics. Our research focuses on neurons located in the trigeminal and dorsal ganglia, as they are known to play an important role in the sensation of touch and pain; we also study the genes that regulate the development and function of these neurons. Recent advances in gene editing methods, particularly the CRISPR/Cas9 system, allow us to mutate specific genes of interest in a living animal and monitor the effect of such mutations within a matter of days. To this end, we have developed a behavioral assay which determines the effect of CRISPR induced mutations on pain sensation in zebrafish larvae. CRISPR injected larvae are exposed to noxious stimuli such as AITC (mustard oil) and extreme temperatures. The CRISPR treated larvae's response to the painful stimuli is compared to wild type larvae's response to determine the impact that the gene we targeted had on their nociception. Through this assay we have identified a gene, zbtb7b, as potentially important to the sensation of noxious stimuli. Further experiments are now required to determine the precise mechanisms through which this genes plays a role in the sensation of pain, including other behavioral assays, RNA hybridizations, and imaging techniques. Several improvements can also be made to this assay to increase the efficiency of future runs.

POSTER SESSION 3

Commons East, Easel 66

2:30 PM to 4:00 PM

Notch Signaling and Sox2 Expression in Mice Cochlear Cells

Isabel Y. Lee, Senior, Neurobiology
Erica Airu Lee, Senior, Biochemistry
Mentor: Olivia Bermingham-McDonogh, Biological Structure
Mentor: Brent Wilkerson, Biological Structure

Studies have shown that both Notch signaling and the transcription factor Sox2 are involved in development of hair cells and support cells in the inner ear, which are essential for hearing and balance. To determine when and where the Notch signaling pathway and Sox2 interact in cells of the cochlea, we observed location of Sox2 and the Notch pathway proteins, Rbpj (transcription factor) and Jagged 1 (ligand of Notch receptors), in mice cochlea at different developmental stages using immunofluorescence labeling. We have observed that Rbpj levels are possibly higher in the floor of the cochlear duct–where the prosensory cells (Jag1+/Sox2+) are–than in the roof of the cochlear duct. This could suggest activation of the Rbpj gene by Sox2 activity or vice versa. To determine whether Sox2 could activate the Rbpj gene or Rbpj could activate the Sox2 gene, we will predict Rbpj- and Sox2-DNA-binding motifs in the open chromatin near Sox2 and Rbpj genes using informatics and our lab's ATAC-seq data. If Sox2 binding sites are found near the Rbpj gene, then that could suggest activation of Rbpj by Sox2. If Rbpj binding sites are found near the Sox2 gene, then that could suggest activation of Sox2 by Rbpj. These findings will help us understand how Sox2 and the Notch pathway interact to influence development of hair cells and support cells.

POSTER SESSION 3

Commons West, Easel 22

2:30 PM to 4:00 PM

Measuring Endoplasmic Reticulum Calcium Concentrations in Hair Cells

Jenni Logue, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: David Raible, Biological Structure
Mentor: Andrea McQuate, Biological Structure

Degeneration of mechanosensory hair cells in the inner ear leads to complications in hearing and balance. Calcium plays an important role in hair cell function and failure to maintain calcium homeostasis is an underlying cause of hair cell death. We used hair cells in the zebrafish lateral line as our model, to explore the role of endoplasmic reticulum (ER)-mitochondrial calcium flow in hair cell damage. We utilized ER-GCaMP-150 for these studies, a new low-affinity calcium indicator made distinctly to look at calcium concentrations in the ER. Injection of zebrafish embryos with ER-GCaMP-150 expressed under a hair cell specific promoter allowed for visualization of ER Ca^2+concentrations under a high-resolution microscope. To date we have confirmed expression of ER-GCaMP in our zebrafish models and quantified Ca^2+ transfer between the ER and mitochondria. Examining changes in calcium concentrations of hair cell organelles provides the opportuninty to gain more insight into the intracellular mechanisms underlying hearing loss.