These abstracts led to an "inchworm model" of foveal cone nuclei in primates. Initially, sections through the foveas of humans, macaques, and marmosets reveal a sinusoidal arrangement of nuclei.

**Purpose:** Our understanding of how light exposure affects rhodopsin synthesis, trafficking, and rod outer segment (ROS) formation in the mammalian rod cell is still incomplete. We expressed a fluorescently tagged rhodopsin, Rho-Timer, to visualize these processes in intact mouse rods.

**Methods:** Timer was attached to rhodopsin’s C-terminus, followed by an additional 11 a.a. of rhodopsin C-terminus repeated at Timer’s C-terminus. Mice expressing Rho-Timer were raised in standard mouse room conditions (12 h light:12 h dark) and at 1 month of age they were treated as follows for 10 days: complete darkness (n=12), diurnal (12h light, 12h dark, n=10), constant light (n=18), and 5d dark/5d light (n=11). Dissociated ROS were imaged using a Leica SP8 microscope. ROS lengths were measured with LAS Lite software, and fluorescent intensity profiles and number of fluorescent bands were measured using ImageJ. Northern blots for endogenous rhodopsin and fluorescent intensity profiles and number of fluorescent bands were measured using ImageJ.

**Results:** One-way ANOVA detected a statistically significant difference in ROS length between treatment groups (p<0.001). ROS length decreased in both 10d light (14.1±3.9 μm) and 5d dark/5d light (10.7±1.9 μm) when compared to dark (23.4±3.3 μm) or cyclic light (21.1±1.4 μm). A post-hoc t-test showed that length differed significantly (p<0.001) in dark, light, and 5 d dark/light conditions from diurnal light conditions. An ANOVA on fluorescent banding pattern yielded significant variation among the treatments (p<0.001). Clear patterns of alternating intense and dim fluorescent bands were observed in the ROS of both constant dark and diurnal treatment groups, whereas 10d light and 5d dark/5d light caused a complete or partial loss of fluorescent banding pattern. The number of fluorescent bands are: 10d dark (10.2±1.1), 10d light (5.9±1.2), 5d dark/5d light (5.6±1.6), and cyclic light (9.3±1.2). Finally, no significant difference was found in the RNA expression of rhodopsin and Rho-Timer under light or dark conditions.

**Conclusions:** Rho-Timer mRNA tracks endogenous rho mRNA. Rho-Timer incorporation into the ROS is regulated at the post-transcription level. Pattern of Rho-Timer distribution in discs appeared to mirror an internal circular rhythm. Excess light disrupted rho incorporation and ultimately changed disc rho composition.

**Support:** NIH Grant EY12155
Genetic influences on the nuclear architecture of photoreceptors

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Purpose: Each cell has an unambiguously organized nuclear architecture involving the two major types of chromatin, heterochromatin and euchromatin. Only rod photoreceptors from nocturnal animals have an “inverted” pattern that features a single, large heterochromatin chromocenter localized to the nuclear interior. Every other cell type, including the closely related cone photoreceptors, exhibit a “conventional” nuclei pattern that features several heterochromatin chromocenters scattered throughout the nuclei. This study investigates genes that may play a role in regulating these nuclear architecture patterns in photoreceptor cells.

Methods: An expansive genetic screen was performed to identify abnormal phenotypes concerning nuclear architecture within rod photoreceptors. A candidate list of 720 genes was established to have differential expression between rod and cone photoreceptors, and encephalopathy disorders. STXBP1/Munc-18 is expressed by all photoreceptors. This study aims to characterize the physical characteristics of rod nuclei architecture involving the two major types of chromatin, heterochromatin and euchromatin. Only rod photoreceptors from nocturnal animals have an “inverted” pattern that features a single, large heterochromatin chromocenter localized to the nuclear interior. Every other cell type, including the closely related cone photoreceptors, exhibit a “conventional” nuclei pattern that features several heterochromatin chromocenters scattered throughout the nuclei. This study investigates genes that may play a role in regulating these nuclear architecture patterns in photoreceptor cells.

Results: Approximately 2.5% of the genes from the screen altered the physical characteristics of rod nuclei architecture. Knockdown of these genes increased the number of chromocenters within the rod nuclei and increased euchromatin area distributed throughout the cell body as compared to the control groups. Confirmation that the observed phenotype can be attributed to the knockdown of these genes was achieved by analyzing multiple retinas injected with the original shRNA. Additional validation was achieved by injecting and imaging a second knockdown construct of each gene and by performing rescue experiments.

Conclusions: Through this extensive and ongoing screen, it has been shown that some target genes affect chromatin organization in rod photoreceptor cells, moving away from the inverted pattern. Further characterization is currently underway in order to understand the mechanistic properties of the nuclear arrangement caused by these genes.

Commercial Relationships: Hannah Fann, None; Dustin T. Whitaker, None; Matthew Brooks, None; Anand Swaroop, None

Support: NIH Intramural Research Program

Program Number: 344 Poster Board Number: A0186

Presentation Time: 1:30 PM–3:15 PM

Syntaxin binding protein 1b is essential for photoreceptor morphogenesis and survival

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Purpose: Retinal photoreceptor cells are vulnerable to mutations in genes essential for cilia formation, outer segment (OS) morphogenesis, and phototransduction. In a forward genetic screen of zebrafish larvae, we previously identified a mutation at the doughnut (dou) locus characterized by rapid degeneration of rod and cone photoreceptors. This study aims to characterize the dou locus and identify a cellular mechanism responsible for the photoreceptor cell phenotype.

Methods: Homozygous dou mutant larvae were identified in crosses of heterozygous adults by immunolabeling for photoreceptor markers or lack of an OKR. To identify the molecular lesion, DNA was pooled from 89 dou mutants and subjected to shotgun Illumina sequencing. Genetic mosaic animals were generated by blastula-stage cell transplantation. The photoreceptor structure was analyzed by immunolabeling and electron microscopy.

Results: Whole genome sequence analysis of DNA isolated from dou mutants revealed a single base change of A>T that introduced a premature stop codon (K213*) of stxbp1b. truncating ~600 amino acid protein in domain 2. STXBP1/MUNC 18-1 facilitates synaptic vesicle docking and fusion, and haploinsufficiency causes epilepsy and encephalopathy disorders. Stxbp1/Munc-18 is expressed by rods and cones, but its specific function in photoreceptors remains unknown. In genetic chimeras photoreceptor degeneration was cell autonomous and survival of inner retinal neurons was unaffected. Ultrastructural analysis showed that photoreceptor terminals in mutant larvae lack invaginating synapses and contain floating ribbons consistent with a role for stxbp1b in synaptic function. The photoreceptor OSs formed in dou mutants, but were shorter in length compared to those of wildtype siblings. The inner segments were characterized by swollen Golgi and endoplasmic reticulum. Confocal images of embryos and larvae immunolabeled for acetylated-tubulin and intraflagellar transport proteins revealed shortened photoreceptor connecting cilium in developing photoreceptors, however no defects were observed in cilia of the nasal epithelium or sensory hair cells of the lateral line neuromasts.

Conclusions: These data indicate that in addition to synaptic function, stxbp1b is essential for photoreceptor survival and likely
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plays a role in vesicular trafficking in the photoreceptor inner segment.

Commercial Relationships: Mailin Sotolongo-Lopez, None; Kathleen E. Kyle, None; Daniel L. Vera, None; James M. Fadool, None
Support: NIH R01 EY017753

Program Number: 345 Poster Board Number: A0187
Presentation Time: 1:30 PM–3:15 PM
Impaired Rod and Cone Photoreceptor Function in Mice Lacking Type II Iodothyronine Deiodinase
Fan Yang, Hongwei Ma, Michael Butler, Xi-Qin Ding. Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Purpose: Thyroid hormone (TH) signaling regulates numerous physiological functions, including cell growth, differentiation, and metabolic homeostasis. In the retina, TH signaling is known to regulate cone opsin expression and affect cone viability. Although TH levels in the circulation are fairly stable, the intracellular TH signaling is regulated by the type II iodothyronine deiodinase (DIO2) which converts the prohormone thyroxine (T4) to the active hormone triiodothyronine (T3). This work investigates the necessity of DIO2 for the retinal function and survival using DIO2-deficient mice.

Methods: The retinal function in Dio2−/− mice was measured by electroretinogram (ERG). Rod and cone survival was evaluated by analyzing the thickness of the outer nuclear layer (ONL) and cone density. The expression levels of the rod and cone phototransduction components were examined by q-RT-PCR and immunoblotting. To further clarify the role of TH signaling, we conducted a T3 supplementation experiment (0.1 μg/ml and 0.3 μg/ml in drinking water) in Dio2−/− mice.

Results: The scotopic and photopic ERG responses in 1-month-old and 5-month-old Dio2−/− mice were reduced by about 50% compared to wild-type (WT) mice. No differences in ONL thickness and cone density were observed between Dio2−/− and WT mice. Although we did not observe a reduction in cone opsin expression, the expression levels of G protein subunit alpha transducin 2 (GNAT2) and cone arrestin (CAR) were significantly reduced in Dio2−/− mice compared to WT. However, we did not detect any alteration in the expression levels of the rod phototransduction proteins, including rhodopsin, rod transducin and rod arrestin. The ERG responses and the expression levels of GNAT2 and CAR were rescued in Dio2−/− mice after T3 treatment.

Conclusions: Dio2-deficient mice show an impaired retinal function without a significant loss of photoreceptors. The phenotypes were rescued by supplementation of T3, indicating a TH-dependent retinal functionality and the necessity of DIO2 for retinal functional development. The reduced expression levels of the cone phototransduction proteins may explain the cone defects in Dio2−/− mice. However, how rod function is affected remains to be elucidated.

Commercial Relationships: Fan Yang, None; Hongwei Ma, None; Michael Butler, None; Xi-Qin Ding, None
Support: This work was supported by grants from the National Eye Institute (P30EY12190, R01EY019490, and R21EY024583), the Foundation Fighting Blindness, and the Knights Templar Eye Foundation.

Program Number: 346 Poster Board Number: A0188
Presentation Time: 1:30 PM–3:15 PM
Sexually Dimorphic Expression of Cone and Rod Opsins in the Zebrafish
William Thurston, Robert Mackin, Deborah L. Stenkamp. Biological Sciences, University of Idaho, MOSCOW, ID.

Purpose: The zebrafish is an important model organism for vision science, particularly in studies of development, disease, and regeneration of its cone-rich retina. Studies supported by the NIH are now required to consider sex as a biological variable; however, there is no information regarding the existence of sexual dimorphisms in the zebrafish visual system. To begin to fill this knowledge gap, the purpose of this study was to determine whether expression of cone and/or rod opsins was sexually dimorphic in the zebrafish.

Methods: Using real-time polymerase chain reaction (qPCR), we analyzed expression of all 10 cone and rod opsin genes in eyes of sexually mature females and males (1.5 yrs), and in eyes of old females and males (3.25 yrs) (n=3 per condition).

Results: 1.5 yr females showed higher levels of expression of RH2-1 and RH2-4 (the first and last members of the tandemly-quadruplicated RH2 cone opsin array) than 1.5 yr males (p=7.29x10−4, p=0.021, respectively). All other cone and rod opsin showed no significant differences in expression in 1.5 yr females vs. 1.5 yr males (p>0.05). Old females exhibited lower expression levels of the following opsins than old males: RH2-1 (p=4.48x10−4), RH2-2 (p=3.58x10−4), RH2-3 (p=6.61x10−4), RH2-4 (p=1.17x10−4), LWS1 (p=1.91x10−4), LWS2 (p=3.72x10−4), and RHO (p=6.18x10−4). Expression levels of SWS1, SWS2, and RHO-L were not significantly different in old females vs. old males (p>0.05). In the case of RH2-1, we confirmed that sex differences in old fish were due to a large decline in RH2-1 expression in old females compared with 1.5 yr females (p=2.75x10−4), while there was no decline in males (p=0.441).

Conclusions: Expression levels of several photoreceptor opsins in zebrafish are regulated as a function of sex and/or age. The RH2-1 gene encodes the most blue-shifted (467 nm peak spectral sensitivity) of the RH2 cone opsins. The highly significant increases in RH2-1 expression in sexually mature females in comparison to all other conditions suggest a possible function related to mate identification/selection. Decline in expression of some opsins in old females (which at 3.25 yrs were likely reproductively senescent) further suggest the potential for sex hormones to influence expression of cone and rod opsins.

Commercial Relationships: William Thurston, None; Robert Mackin, None; Deborah L. Stenkamp, None
Support: R01 EY012146

Program Number: 347 Poster Board Number: A0189
Presentation Time: 1:30 PM–3:15 PM
ARL2BP, a protein linked to retinitis pigmentosa, regulates the growth of photoreceptor microtubular axonemes
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Purpose: Blinding diseases such as retinitis pigmentosa (RP) are linked to defects in multiple ciliary proteins, including ARL2 binding protein (ARL2BP). Mutations in ARL2BP also cause situs inversus (organ reversal) in humans, a condition associated with defective cilia in the node of developing embryos. Defects in photoreceptor cilia as well as situs inversus in human patients suggest that ARL2BP plays an invaluable role in structure and function of the cilia. Using an animal model lacking ARL2BP, our goal is to understand the mechanism behind photoreceptor ciliogenesis and disc morphogenesis/organization in the outer segment (OS).

Methods: We generated a mouse knockout (KO) of ARL2BP using the Crisp-Cas9 system. Protein and mRNA levels were measured by immunoblotting and RT-PCR. Immunocytochemical staining was used to investigate the morphology of the retina and protein

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localization. Electoretinogram recordings (ERGs) were performed to assess photoreceptor function. Full body dissections were completed to examine organ placement. Littermates were used in all experiments as controls (n=4).

**Results:** ERGs revealed a progressive decline in photoreceptor responses in animals lacking ARL2BP with 50% reduction at post-natal day 16 (P16) when the retinal development, as judged by light microscopy, was normal. Immunofluorescence staining of photoreceptor cilia displayed shorter axonemes in ARL2BP KO’s as early as P10, when photoreceptor OS are still developing. Furthermore, organ reversal was present in all animals lacking ARL2BP.

**Conclusions:** Our animal model recapitulates the phenotype observed in ARL2BP patients. Prior to photoreceptor degeneration, we find reduced photoreceptor function and shortened axonemes. Based on these findings, we postulate that ARL2BP is involved in regulating the growth and stability of ciliary axonemes. Since photoreceptor axonemes are intimately associated with OS development, we propose that loss of ARL2BP disrupts disc organization thus affecting photoreceptor function. Currently, studies are underway to rigorously test this hypothesis using multiple models. We are also examining the length and motility of cilia present in the embryonic node in animals lacking ARL2BP. These experiments will shed light on the function of ARL2BP and ultimately contribute to our understanding of cilia development and photoreceptor structure.

**Commercial Relationships:** Abigail Hayes, None; Ratnesh Singh, None; Visvanathan Ramamurthy, None. Support: R01EY025536 and R01EY017035

**Program Number:** 348 **Poster Board Number:** A0190

**Presentation Time:** 1:30 PM–3:15 PM

**Glycogen Synthase Kinases 3 control photoreceptor development and homeostasis by modulating NRL stability**

*Elena Braginskaja* 1, 2, *Jessica Gumerson*, 2, 3, *Muriel Perron* 1, 3, *Anand Swaroop*, 4, 5, 6, *Jerome E Roger* 4, 7, 8, 9, 10, 11, 12, 13

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**Purpose:** Gene regulatory network required for photoreceptor development and homeostasis is dependent on intrinsic factor expression. Posttranslational modifications bring in additional level of regulation allowing rapid and reversible modifications of targeted factors in response to changing microenvironment. Among the modifying enzymes are Glycogen Synthase Kinases 3 (GSK3s), key regulators of brain development and homeostasis. One of their main modes of action is the control of protein stability. GSK3s deregulation is involved in many pathologies of the central nervous system. However, their role is poorly understood in the retina. We aimed at delineating the function of GSK3s and at identifying their critical targets in photoreceptors.

**Methods:** Gsk3α and Gsk3β-floxed mice were mated with *Crx::Cre* line for conditional deletion in photoreceptor precursors. The phenotype was evaluated by immunohistochemistry and electoretinogram (ERG). Gene expression was assessed by qPCR and immunoblotting. The impact of GSK3-dependent phosphorylation on protein stability was investigated in transfected cells.

**Results:** Lack of GSK3s in photoreceptor precursors led to progressive photoreceptor loss from P18 onwards. Scotopic and photopic ERG responses were significantly decreased before the onset of degeneration. Further analysis demonstrated impairment in photoreceptor maturation and reduced opsin expression. However, expression of key photoreceptor specific transcription factors was not significantly affected at the transcriptional level. Interestingly, while CRX and NR2E3 protein amount was unchanged, the absence of GSK3s led to an increase in NRL protein level associated with a reduced number of phosphorylated isoforms. In *vitro* experiments established a GSK3-dependent phosphorylation altering NRL protein stability. Preliminary proteome and transcriptome analyses revealed deregulation of only a subset of NRL target genes critical for photoreceptor function.

**Conclusions:** Lack of GSK3s in photoreceptor precursors impairs their differentiation and leads to degeneration. Photoreceptor death in the absence of GSK3 may arise from the change of NRL phosphorylation and stability leading to an aberrant expression of a subset of NRL target genes. Our findings should also shed more light on disease mechanisms of NRL mutation-associated retinitis pigmentosa for which altered NRL phosphorylation has been reported.

**Commercial Relationships:** Elena Braginskaja, None; Jessica Gumerson, None; Muriel Perron, None; Anand Swaroop, None; Jerome E Roger, None. Support: Association Retina France and Fondation Valentin Haüy, Idex Paris-Saclay, NEI intramural research program

**Program Number:** 349 **Poster Board Number:** A0191

**Presentation Time:** 1:30 PM–3:15 PM

**Developmental Delay in Outer Segment Formation Causes Photoreceptor Degeneration in Mice with a Targeted Deletion of the Guanine-Nucleotide Exchange Factor, Rabgef1**


1 Neurobiology-Neurodegeneration and Repair, National Eye Institute and The George Washington University, Chevy Chase, MD; 2 Neurobiology-Neurodegeneration and Repair, National Eye Institute, Chevy Chase, MD.

**Purpose:** Current research predicts that one-quarter of retinal degenerations are due to defects in intracellular transport in photoreceptors; however genes required for these processes remain largely uncharacterized. Using RNA-sequencing analysis of flow-sorted photoreceptors I selected the gene Rabgef1 (a master regulator of endocytosis) for further characterization based on its increase in expression during the formation and maturation of photoreceptor outer segments and synapses. I hypothesize that loss of Rabgef1 causes defects within photoreceptor outer segments and/or synapses and results in concomitant retinal degeneration.

**Methods:** We performed RNA-seq on postnatal 2-28 rod and cone-like photoreceptors from C57Bl6/J and Nrl−/− mice respectively and selected Rabgef1 for further characterization. In-situ hybridization using RNAseq technology and two different antibodies were used to localize Rabgef1 mRNA and protein in vertebrate retina. Retinas from Rabgef1−/− and wildtype littermates were analyzed by immunohistochemistry, histology, and transmission electron microscopy. Visual function of Rabgef1−/− mice was accessed by electroretinography. Co-IP of Rabgef1 binding partners was performed using Invitrogen Dynabeads protocol. Eluted protein complexes are currently being examined using mass spectrometry.

**Results:** Rabgef1 mRNA localizes primarily to photoreceptor nuclei, while protein localizes to the inner segments and OPL. Histology indicates that loss of Rabgef1 results in near-complete photoreceptor degeneration by P28. TEM shows Rabgef1−/− mice have stunted/dystrophic outer segments. HCl using retinal cell-type specific markers indicates that loss of Rabgef1 is photoreceptor specific and results in fewer synaptic contacts with bipolar cells (decreased ribeye staining). Electroretinography of Rabgef1−/− mice (N=10, p<0.05)
indicates complete loss of scotopic and photopic a and b waves upon eye opening.

Conclusions: My results are consistent with the hypothesis that loss of Rabgef1 leads to photoreceptor degeneration due to improper outer segment development. My data indicates that proper endocytosis of specific cargo is required for the formation of outer segments. Mass spectrometry is needed to elucidate Rabgef1 binding partners in order to understand the mechanism(s) causing photoreceptor degeneration in Rabgef1−/− mice.

Commercial Relationships: Pasley J. Hargrove, None; Anand Swaroop, None

Program Number: 350 Poster Board Number: A0192
Presentation Time: 1:30 PM–3:15 PM
Identification of chemicals and signaling pathways that impact photoreceptor regeneration in zebrafish
Sumitra Sengupta, Jeff S. Mumm. Wilmer Eye Institute, Johns Hopkins Universiy, Baltimore, MD.

Purpose: Irreversible loss of photoreceptors is the major cause of blindness in retinal degenerative disorders. Using an inducible zebrafish model of retinitis pigmentosa for whole-organism high-throughput drug discovery the goal of our study is to identify compounds, and corresponding signaling pathways, that promote photoreceptor regeneration. Our core hypothesis posits that a detailed understanding of the mechanisms regulating the regenerative potential of Müller glia in zebrafish will provide insights necessary to advance therapeutic strategies for stimulating retinal repair in humans.

Methods: Using an inducible degenerative disease modeling system and large-scale whole-organism drug discovery platform we have embarked upon a high-throughput screen (HTS) to identify compounds that accelerate neuronal regeneration kinetics in the zebrafish retina. Transgenic zebrafish expressing a yellow fluorescent protein-nitroreductase (YFP-NTR) fusion protein in rod photoreceptors enable prodrug-induced apoptosis of rod cells, a model of retinitis pigmentosa. To identify compounds promoting rod cell regeneration, fish larvae were treated with the prodrug metronidazole (Mtz, 10mM) for 24 hrs to induce rod cell ablation. After Mtz washout, fish were exposed to test compounds across six different concentrations over three days of recovery. YFP reporter levels were then quantified using a fluorescent plate reader to compare the extent of rod cell regeneration in drug-treated fish versus controls. Nonparametric multiple group comparisons were used to identify compounds that accelerated the kinetics of rod cell replacement; i.e., regeneration-promoting drugs.

Results: In a pilot screen, we have identified 2 hit compounds that enhance rod cell regeneration. Suspected molecular targets of the hit compounds implicate neuroinflammation as a key regulator of the regenerative process. Further, by exposing larvae to hit compounds before or after induction of rod PR loss we have revealed stage-specific roles for immunomodulatory compounds during the regenerative process.

Conclusions: These results demonstrate that our whole-organism HTS-paced platform for drug discovery can successfully identify chemical modulators of retinal neuron regeneration, and reveal a novel role for the immune system in regulating the regenerative potential of Müller glia cells.

Commercial Relationships: Sumitra Sengupta, None; Jeff S. Mumm, None
Support: NIH Grant EY022810

Program Number: 351 Poster Board Number: A0193
Presentation Time: 1:30 PM–3:15 PM
Cone opsins and Crx are gene therapy candidates for the revival of cone photoreceptors in an RP mouse model
Mark M. Hassall1, Michelle E. McClements1, Alan R. Barnard1, Sher A. Aslam1,2, Robert E. MacLaren1,2. 1Nuffield Laboratory of Ophthalmology, University of Oxford, Oxford, United Kingdom; 2Oxford Eye Hospital, Oxford, United Kingdom.

Purpose: It has been observed that residual cone photoreceptors remain in human patients with retinitis pigmentosa (RP) long after cone photosensitivity is extinguished. We hypothesised that there is a loss of cone photosensitivity that precedes cone cell death, resulting in a subpopulation of ‘dormant’ cone photoreceptors that could be reactivated through molecular therapies. We aimed to correlate changes in cone-specific gene expression on qPCR with phenotypical changes in cone function in a mouse model of RP, thereby identifying candidates for subsequent gene therapy trials.

Methods: The Rhodopsin knockout (Rho−) mouse model of RP was examined using Optical Coherence Tomography (OCT), Scanning Laser Ophthalmoscopy (SLO) and Electoretinography (ERG). Five-time-points for qPCR were then chosen correlating to: 1) early rod degeneration; 2) the peak of rod cell death on OCT; 3) the beginning of cone cell death on SLO; 4) the loss of cone photosensitivity on ERG and 5) an advanced stage of degeneration. The neural retina from Rho− mice and wild type controls were collected across these five time-points (n=5 per time point). Each retinal transcriptome was extracted and converted to cDNA. Primers for qPCR were designed to eight genes involved in the mouse cone phototransduction cascade: Omp1Lws, Opm1Lws, Arr3, Crx, Pde6h, Cnga3, Cngb3 and Gnat2. All primer sets were optimised to achieve 90-100% efficiencies and all target gene values were normalised to Bactin. Conclusions: Changes in cone-specific gene expression on qPCR with phenotypical changes in cone function were apparent across all time points except for a gradual decline in Pde6h and a small decline in Opm1Lws expression between 17 and 25 weeks of age. Crx revealed a steep increase in expression levels between 6 and 17 weeks with a further increase between 17 and 25 weeks. By contrast, in Rho− mice all genes assessed decreased in expression over time (p<0.05). The biggest overall decline was observed between 6 and 12 weeks and was particularly apparent in Pde6h (though this was equivalent to that observed in WT, Opm1Lws and Crx).

Conclusions: The results of the gene expression analyses correlated with the phenotypic observations and suggested that Crx and cone opsins are appropriate candidates for future gene therapy attempts to restore photosensitivity in dormant cone cells in RP.

Commercial Relationships: Mark M. Hassall, None; Michelle E. McClements, None; Alan R. Barnard, None; Sher A. Aslam, None; Robert E. MacLaren, None

Program Number: 352 Poster Board Number: A0194
Presentation Time: 1:30 PM–3:15 PM
Targeted deletion of mir-182 leads to photoreceptor dysfunction in mice

Purpose: MiR-182 is the most abundantly retinal miRNA in mammals. Although the deletion of miR-182 reveals none apparently structural abnormalities in the retina, the role of miR-182 in the retinal function remains unclear yet. In this study, we aimed to determine whether ablation of miR-182 alters photoreceptor function based on the miR-182 knock out (KO) mice we generated previously.

Conclusions: The results of the gene expression analyses correlated with the phenotypic observations and suggested that Crx and cone opsins are appropriate candidates for future gene therapy attempts to restore photosensitivity in dormant cone cells in RP.
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Methods: The color fundus camera and high resolution spectral-domain optical coherence tomography (SD-OCT) were utilized to screen the morphological and structural changes for the fundus and retinal layers of miR-182 KO mice. Ganzfeld electrotretinogram (ERG) was carried out to examine the retinal function. Quantitatively compensational expression of other two members of miR-183 clusters (miR-96 and miR-183) within retina of the miR-182 KO mice was determined using qRT-PCR. Potential target genes of miR-182 were computated and verified through the bioinformatics and RNAseq. Two-tailed student’s t-test was used for statistical analysis.

Results: We did not observe retinal structural change in color fundus photograph (FP) and SD-OCT imaging as well as compensational expression of miR-96 and miR-183 in the KO mice. Scotopic ERG showed that both a-wave and b-wave amplitude were decreased. In addition, maximal and photopic ERG were abnormal. Furthermore, we found up-regulated different expression genes in the retina of miR-182 mice.

Conclusions: In summary, we for the first time determined the retinal functional defect in the miR-182 KO mice. Our finding suggested that the miR-182 maybe play a critical role in maintaining the functionality of photoreceptors in mice.

Commercial Relationships: Kun-Chao Wu, None; Lue Xiang, None; Xue-Jiao Chen, None; Xue-Wen Chen, None; Gao-Hui Zhou, None; Bo-Wen Zhang, None; Zi-Bing Jin, None

Program Number: 353 Poster Board Number: A0195
Presentation Time: 1:30 PM–3:15 PM

NDR kinases regulate retinal development, homeostasis and gene expression

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Purpose: Recent studies suggest that NDR2 protein kinase is important for retina maintenance, although its precise retinal functions are unknown. Notably, an Ndr2 loss-of-function allele causes early retina degeneration (erd) in dogs, characterized by opsin mislocalization and concurrent increases in photoreceptor apoptosis and proliferation. Separate studies indicate that NDR2 and its paralog NDR1 regulate cell proliferation, morphogenesis and gene expression in other tissues. To address how NDR1 and NDR2 regulate retina development and homeostasis, we generated Ndr1 and Ndr2 knock-out (KO) mice and characterized their retinal phenotypes.

Methods: Ndr1 KO mice were generated by introducing frame shift mutations into Ndr1 exons 4 and 6, utilizing CRISPR-Cas9 methods. Ndr2 KO mice were obtained by crossing Ndr2-flox KO mice (Ndr2 exon 7 flanked by loxP sites; obtained from KOMP, UC Davis) to Actin-cre mice. To analyze retinal phenotypes, eyes were fixed in 4% paraformaldehyde, OCT embedded, sectioned and probed with antibodies to opsin and mitotic markers via immunofluorescence microscopy. To investigate how Ndr2 deletion affects gene expression, cDNA was prepared from triplicate pools of Ndr2 KO and WT retinas from ~1 month old mice and analyzed by RNA-seq. Data normalization and differential gene expression analysis was performed Limma packages (Bioconductor). Transcriptome analysis was performed using Gene Ontology Consortium and Database for Annotation, Visualization and Integrated Discovery (DAVID).

Results: Both Ndr1 KO and Ndr2 KO mice display similar retinal degenerative phenotypes to canine erd, including opsin mislocalization to the ONL. In addition, Ndr KO mouse retinas exhibit increased cell proliferation in the INL (Ndr1 and Ndr2), ONL (Ndr1) and neuroblast (Ndr2). RNA-seq analysis reveals that Ndr2 deletion increases expression of numerous genes associated with neuronal stress and decreases expression of many genes involved in synapse organization.

Conclusions: These data support the hypotheses that retinal NDR1 and NDR2: a) function similarly and b) regulate retinal development and homeostasis via modulating gene expression. Further analyses of retinal NDR mechanisms may influence the development of therapeutic interventions for retinal degenerations and methods to stimulate photoreceptor regeneration.

Commercial Relationships: Hélène M. Léger; Daniel P. Beiting, None; William A. Beltran, None; Gustavo D. Aguirre, None; Francis Luca, None
Support: NIH RO1-GMO97327

Program Number: 354 Poster Board Number: A0196
Presentation Time: 1:30 PM–3:15 PM

Melatonin protects 661W cells from cell death induced by H2O2 via inhibition of the Fas/FasL-Caspase 3 pathway

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Purpose: Previous studies have shown that melatonin (MEL) signaling is involved in the modulation of photoreceptor viability during aging. Recent work from our laboratory has suggested that MEL may protect cones by modulating the Fas/FasL-Caspase 3 pathway. We have also reported that 661W cells - a photoreceptor-like cell line - express MEL receptors (MT1 and MT2) mRNAs. In this study, we first investigated the signaling pathway activated by MEL in 661W cells and then whether MEL can protect these cells from H2O2 induced cell death.

Methods: MEL receptors presence in 661W was studied by immunohistochemistry (IHC). 661W were treated with MEL and MEL agonist and antagonist to identify the intracellular pathways activated by MEL in these cells. To test the protective effect, 661W cells were subjected to a 2-h treatment with H2O2 and/ or MEL. To analyze the pathways involved in H2O2-mediated cell death, Fas/ Fasl. and caspase 3 were analyzed at the level or mRNA expression and protein abundance by western blot. Cell viability was analyzed by using trypan blue.

Results: MEL receptors immunoreactivity was observed in 661W cells by IHC. The increase in eAMP production induced by forskolin was inhibited by MEL and IIK7 (a melatonin agonist) in a dose-dependent manner (p<0.05). These results suggest that in 661W cells MEL receptors are functional and - also in these cells - MT, and MT, receptors may heterodimerize. MEL partially prevented the H2O2-mediated cell death (20-25%; p<0.05). This effect was prevented the activation of Caspase 3 caused by H2O2. Finally, Kp7-6, an antagonist of Fas/FasL, blocked the cell death caused by H2O2 (p<0.05). Melatonin treatment also partially prevented the activation of Caspase 3 caused by H2O2.

Conclusions: Our results demonstrated that MEL receptors are present and functional in 661W. MEL can prevent photoreceptor cell death induced by H2O2 via the inhibition of the pro-apoptotic pathway Fas/FasL-Caspase 3. Finally, our data indicate that 661W cells may represent a new useful model to study melatonin signaling in a photoreceptor-like cell line.

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Docosahexaenoic acid (DHA) is an n-3 fatty acid present at high levels in photoreceptor cell membranes. DHA deficiency causes vision defects related to photoreceptor cell dysfunction. Rhodopsin is present in rod outer segment (ROS) disc membranes of photoreceptor cells and plays a central role in photoreceptor cell function by initiating phototransduction upon absorbing photons. DHA deficiency is known to impair rhodopsin function, but the mechanism is unclear. To better understand the impact of DHA deficiency on rhodopsin function, we examined the effect of DHA on membrane structure and rhodopsin packing in photoreceptor cells.

**Methods:** DHA levels in ROS membrane phospholipids were modulated by feeding C57BL/6J mice DHA-deficient or DHA-adequate diets. Mass spectroscopy was used to monitor changes in DHA levels in ROS membrane phospholipids. To visualize the impact of dietary DHA on membrane structure and rhodopsin packing, ROS disc membranes were isolated from mice and imaged by atomic force microscopy (AFM).

**Results:** Mass spectroscopic analysis showed that DHA levels in ROS membrane phospholipids were altered in accordance to the diets provided. The structure of ROS disc membranes and the quaternary membrane organization of rhodopsin was similar in both DHA-deficient and DHA-adequate mice. In contrast, DHA-deficient mice had higher rhodopsin content and density compared to DHA-adequate mice in ROS disc membranes. These effects were reversible when DHA-deficient mice were fed a DHA-adequate diet for 4 weeks.

**Conclusions:** Our AFM studies demonstrate that DHA-deficiency does not disrupt ROS disc membrane structure or the quaternary organization of rhodopsin in the membrane. DHA-deficiency does result in higher rhodopsin content and density in photoreceptor cell membranes, which we have previously observed as a compensatory mechanism of photoreceptor cells in response to impairment of phototransduction. This indicates that DHA-deficiency impairs rhodopsin function without affecting rhodopsin packing or quaternary structure.

**Commercial Relationships:** Subhadip Senapati

**Support:** NIH Grant R01EY021731

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**Habitat and predatory behaviour are related with ratio of nuclear layers thickness and cone pedicle spinules in teleosts retina**

**Purpose:** Teleosts constitute more than 50% of all vertebrate species present in marine environments. These data makes them an important experimental model for studying the adaptation to different environmental conditions and its relationship with predatory behaviour. The aim of this work is to establish the relationship between the predatory behaviour and the thickness of the outer nuclear layer (ONL) and the inner nuclear layer (INL), in twelve species from six teleosts families.

**Methods:** Retinas from twelve species of teleost belonging to the environment is not only according to its taxon of belonging. These data makes them an important experimental model for studying the adaptation to different environmental conditions and its relationship with predatory behaviour. The aim of this work is to establish the relationship between the predatory behaviour and the thickness of the outer nuclear layer (ONL) and the inner nuclear layer (INL), in twelve species from six teleosts families.

**Habitat and predatory behaviour are related with ratio of nuclear layers thickness and cone pedicle spinules in teleosts retina**

**Presenters:** Joaquín De Juan, Noemi Martínez-Ruiz, Bassima Boughlala, Alba De Raspeig, Spain.

**Support:** Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH.

**Program Number:** 355 Poster Board Number: A0197

**Presentation Time:** 1:30 PM–3:15 PM

**Effect of dietary Docosahexaenoic acid (DHA) on rhodopsin content and packing in rod photoreceptor cell membranes**

**Purpose:** Docosahexaenoic acid (DHA) is an n-3 fatty acid present at high levels in photoreceptor cell membranes. DHA deficiency causes vision defects related to photoreceptor cell dysfunction. Rhodopsin is present in rod outer segment (ROS) disc membranes of photoreceptor cells and plays a central role in photoreceptor cell function by initiating phototransduction upon absorbing photons. DHA deficiency is known to impair rhodopsin function, but the mechanism is unclear. To better understand the impact of DHA deficiency on rhodopsin function, we examined the effect of DHA on membrane structure and rhodopsin packing in photoreceptor cells.

**Methods:** DHA levels in ROS membrane phospholipids were modulated by feeding C57BL/6J mice DHA-deficient or DHA-adequate diets. Mass spectroscopy was used to monitor changes in DHA levels in ROS membrane phospholipids. To visualize the impact of dietary DHA on membrane structure and rhodopsin packing, ROS disc membranes were isolated from mice and imaged by atomic force microscopy (AFM).

**Results:** Mass spectroscopic analysis showed that DHA levels in ROS membrane phospholipids were altered in accordance to the diets provided. The structure of ROS disc membranes and the quaternary membrane organization of rhodopsin was similar in both DHA-deficient and DHA-adequate mice. In contrast, DHA-deficient mice had higher rhodopsin content and density compared to DHA-adequate mice in ROS disc membranes. These effects were reversible when DHA-deficient mice were fed a DHA-adequate diet for 4 weeks.

**Conclusions:** Our AFM studies demonstrate that DHA-deficiency does not disrupt ROS disc membrane structure or the quaternary organization of rhodopsin in the membrane. DHA-deficiency does result in higher rhodopsin content and density in photoreceptor cell membranes, which we have previously observed as a compensatory mechanism of photoreceptor cells in response to impairment of phototransduction. This indicates that DHA-deficiency impairs rhodopsin function without affecting rhodopsin packing or quaternary structure.
ONL/INL obtained. Thin sections were observed under transmission electron microscope and the spinules number per pedicle counted. The data were analysed using an ANOVA. The habitat and predatory data were obtained from FishBase and several publications.

**Results:** The ratio between the two nuclear layers shows a positive and significant correlation with the piscivorous data, but, it does not correlate significantly with the trophic habit level. ONL/INL ratio is lower in the diurnal fishes and in those fewer predators. The data suggest that ONL/INL ratio is a potent indicator to classify the fish according to its predatory behaviours. Therefore, this ratio could be used as an indicator of the predatory behaviour of different teleosts species. The spinules are scarce in diurnal and little predatory species. In species with a level of predation and piscivorous intermediates some spinules is slightly higher. Finally, in highly ichthyophagous species, spinules appear in a larger number.

**Conclusions:** The thickness of the layers of the retina, especially the ONL/INL ratio, and the number of spindles per pedicle, are significant predictors of the predatory behaviour of teleosts.

**Commercial Relationships:** Joaquin De Juan, None; Noemi Martinez-Ruiz, None; Bassima Boughlala, None; Alba De Juan, None

**Support:** Vicerrectorado de Investigación, University of Alicante, Spain (Vigrob-137)

**Program Number:** 358 **Poster Board Number:** A0200

**Presentation Time:** 1:30 PM–3:15 PM

**CIC-2 chloride channel localizes to the apical surface and primary cilium of RPE cells and regulates ciliogenesis**


**Purpose:** Chloride channel 2 (CIC-2) is a ubiquitous polytopic membrane protein localized to the basolateral plasma membrane of many body epithelia that plays important physiological roles in cell volume regulation, ion transport and acid-base regulation. CIC-2 knock-out in mice causes retinal degeneration and blindness. Here, we report an unexpected apical and Primary Cilium (PC) localization of CIC-2 in RPE cells.

**Methods:** We performed confocal immune-fluorescence microscopy to localize CIC-2-HA in polarized ARPE-19 and hRPE cells transduced with lentiviral vectors. To explore the localization of endogenous CIC-2 we carried out domain-specific cell surface biotinylation assays. To examine the localization of the channel in mouse RPE in vivo, we performed subretinal injection of a plasmid encoding CIC-2-GFP in wild type C57BL/6 mice. Ciliogenesis was induced in cultured ARPE-19 cells by serum starvation for 48h in control and lentivirus-transduced cells with shRNA against CLC-2. To activate the Hedgehog (Hh) pathway, we exposed ARPE-19 cells to 100nM Smoothened against (SAG), a Hh agonist, for 48h.

**Results:** Surprisingly, in striking contrast with previous physiological studies, our immunofluorescence and surface biotinylation experiments demonstrate that CIC-2 is predominantly localized to the apical surface and PC of cultured RPE cells from different sources. This result was confirmed in vivo by subretinal electroretinography of CIC-2-GFP into mice eyes. The apical and PC localization appears to be cell-specific, as there is no detectable signal for CIC-2-HA in the PC of MDCK cells, where CIC-2 localizes basolaterally. Quantification of the percentage of ciliated cells and cilium length showed that CIC-2 depletion significantly impairs cilia formation. Because PC is home for the Hh pathway, we exposed ARPE-19 cells to a Hh agonist (SAG) and found that CIC-2 knockdown attenuates Hh signaling, consistent with a decreased population of ciliated RPE cells.

**Conclusions:** We show for the first time that CIC-2 is predominantly enriched in the PC of RPE cells, where it seems to play a role in the genesis of primary cilia. We are now exploring additional physiological roles of apical CIC-2 in RPE cells.

**Commercial Relationships:** Enrique J. Rodriguez-Boulan, None; Ignacio Benedicto, None; Erwin de la Fuente, None; Diego Gravotta, None; Agustin Anastasia, None; Zaida Salfati, None; Guillermo Lehmann-Mantaras, None

**Support:** NIH/NEI R01 EY08538

**Program Number:** 359 **Poster Board Number:** A0201

**Presentation Time:** 1:30 PM–3:15 PM

**Deciphering the molecular identity, development and dysfunction of retinal neurons using single-cell transcriptomics**

*Karthik Shekhar*, *Aviv Regev*, *Joshua Sanes*, *Connie Cepko*, *Sylvain Lapan*, *Irene E. Whitney*, *Yirong Peng*, *Broad Institute, Cambridge, MA; Howard Hughes Medical Institute, Chevy Chase, MD; Harvard University, Cambridge, MA; Harvard Medical School, Boston, MA.

**Purpose:** One of the main priorities of the BRAIN Initiative is to develop technologies for classification of neurons based on molecular markers. An unbiased molecular taxonomy of neuronal types would enable investigators to (a) mark and manipulate specific neuronal and non-neuronal types; (b) pinpoint molecular pathways underlying development; (c) provide a framework to study cell type specific responses during injury and disease; (d) facilitate cross-species comparisons; and (e) enable data sharing across groups. We are using the mouse retina as a model system to test this paradigm.

**Methods:** We use massively parallel single-cell transcriptomic profiling, combined with advanced computational algorithms to construct molecular taxonomies of neurons. The predictions of the taxonomy are validated using methods combining FISH with viral mediated labeling of neurons, thus matching molecular expression against morphology.

**Results:** We have now obtained a complete taxonomy of mouse retinal bipolar cells, while discovering two new cell types towards a total of 15. One of the newly discovered types had an unexpected morphology but the expression signature consistent with this class. We have also obtained draft taxonomies of retinal ganglion cells and amacrine cells, which have not only revealed novel markers for existing types but also revealed new types.

**Conclusions:** We are now using this taxonomy as a foundational base to explore two key biological questions: (1) How does neuronal diversity arise during development and what mechanisms underlie cell fate commitment in the retina? We believe insights gained from this analysis will provide general principles for neuronal commitment in the CNS (2) What makes certain cell types more susceptible to traumatic or ischemic injury than others? We are using the optic nerve crush (ONC) as a model to first characterize survival dynamics across RGC types and explore the molecular factors that mediate survival of select RGC types. We believe that our efforts in the retina will provide a blueprint for exploring similar questions in less well-studied regions of the CNS.

**Commercial Relationships:** Karthik Shekhar, None; Aviv Regev, None; Joshua Sanes, None; Connie Cepko, None; Sylvain Lapan, None; Irene E. Whitney, None; Yirong Peng, None
Uncovering the layers of the connecting cilium with super-resolution nanoscopy

Michael Robichaux, Theodore G. Wensel. Biochemistry, Baylor College of Medicine, Houston, TX.

**Purpose:** The microtubule doublets of the axoneme in the connecting cilium (CC) of retinal rods create subcellular boundaries or layers whose arrangement enables essential but poorly understood trafficking and signaling events. To date, analysis of these layers has been limited mostly to electron microscopy. Our goals were to test the ability of immunofluorescence and super-resolution nanoscopy to determine the locations of specific proteins within these layers and to use this approach to observe changes subcellular localization of CC proteins in mutant ciliopathy models of Bardet-Biedl syndrome (BBS).

**Methods:** STORM (STochastic Optical Reconstruction Microscopy) nanoscopy was performed on ultra-thin sections of WT mouse retinae immunostained with the following antibodies as markers for the subcellular layers within the CC of rods: centrin-2, acetylated-α tubulin, and IFT88. Dye-conjugated WGA was used as a CC surface marker. Antibodies for BBS5 and syntaxin-3 were used to localize these proteins within the cell and the CC layers in WT and BBS2, BBS4 and BBS7 knockout retinae.

**Results:** In WT mouse rods, we developed 2-color STORM to elucidate 4 distinct layers of the CC by measuring reconstructed super-resolution clusters for each structural marker. The average diameter of the centrin-2 cluster, marking the interior layer of the axoneme, was 152 nm, while the microtubule axoneme cluster average diameter was 268 nm. IFT88 clusters extend ~50 nm from the outside edge of the axoneme, and the WGA CC membrane surface cluster extends the diameter of the final CC layer to >300 nm. We localized syntaxin-3 and BBS5 to the IFT88 subcellular layer on the outside of the axoneme in WT CC. In the BBS mutant retinae, syntaxin-3 is grossly mislocalized to the OS; however, it is still correctly localized within the CC. Surprisingly, BBS5 is not mislocalized grossly or subcellularly in any of the three BBS mutant models.

**Conclusions:** These results establish the power of super resolution nanoscopy to localize proteins to subcompartments within the CC and elucidate novel subcellular detail. Localizing syntaxin-3 and BBS5 in WT and BBS ciliopathy mutant rods provides new subcellular information for each protein. Specifically, BBS2, BBS4 and BBS7 were found to be essential for preventing improper syntaxin-3 accumulation in the OS, but not for correct subcompartment localization of either syntaxin-3 or BBS5 within the CC.

**Support:** NIH: F32 EY027171, R01 EY025218, R01 EY026545, R01 EY007981, Welch Foundation Q0035.

**Commercial Relationships:** Michael Robichaux, None; Theodore G. Wensel, None
at and beyond the NE upon rootletin expression. In line with these results, we uncovered that Nesprin1 localizes to ciliary rootlets of multiciliated cells.

**Conclusions:** Multiple isoforms of Nesprin1 associate with ciliary rootlets. This novel functional paradigm suggests that the wide spectrum of human pathologies linked to nonsense mutations of SYNE1 may in part originate from ciliary defects.

**Commercial Relationships:** Didier M. Hodzic; Chloë Potter, None; Wanqiu Zhu, None; David Razafsky, None; Philip A. Ruszycki, None; Teresa Doggett, None; Alexander V. Kolesnikov, None; Vladimir J. Kefalov, None; Ewelina Betleja, None; Moé Mahjoub, None

Support: NIH grant EY022632 to DH, EY019312 and EY025696 to V.J.K., DK108005 to M.R.M, a National Eye Institute Center Core Grant (#P30EY002687) and an unrestricted grant from Research to Prevent Blindness to the Department of Ophthalmology and Visual Sciences at Washington University. P.R. is supported by the National Eye Institute (R01 EY012543 and T32 EY013360).

**Program Number:** 362 **Poster Board Number:** A0204

**Presentation Time:** 1:30 PM–3:15 PM

**Visual performance evaluation of zebrafish with mutations in cilia transition zone proteins**


**Purpose:** Joubert syndrome (JBTS) is an autosomal recessive ciliopathy with genotypic and phenotypic variability. The genes cep290, ahl1, and arl13b encode ciliary transition zone proteins and mutations in these genes have been associated with JBTS. It is well known that mutations in second-site modifier genes can modulate phenotypic severity, but detailed analyses of how this affects visual performance are limited. This study investigates whether heterozygous and homozygous mutations of ahl1 and arl13b exacerbate the visual acuity defects observed in zebrafish cep290−/− mutants.

**Methods:** Progeny from pairwise crosses cep290−/−;ahl1−/−, and cep290−/−;arl13b−/− adults were tested by optokinetic response (OKR) behaviors for contrast sensitivity and spatial frequency. To test visual function, the VisioTracker system was used to calculate the OKR gain in order to assess contrast sensitivity and spatial frequency in zebrafish larvae at 5 days post fertilization (dpf).

**Results:** Plotting the OKR gain as a function of either stimulus contrast or spatial frequency, it was shown that cep290−/− and arl13b−/− mutants had reduced visual performance compared to wild-type animals. Furthermore, cep290−/−;arl13b−/− mutants exhibited decreased visual performance compared to cep290−/−;arl13b−/− mutants and the cep290−/− and arl13b−/− single mutants. It was also shown that visual acuity in ahl1−/− single mutants was comparable to wild-type zebrafish. The cep290−/−;ahl1−/− mutants exhibited decreased visual performance compared to cep290−/−;ahl1−/− and cep290−/− and ahl1−/− single mutants.

**Conclusions:** Based on the results, it was determined that cep290−/− mutants and arl13b−/− mutants showed reduced visual function at 3dpf. The cep290−/−;arl13b−/− mutants had worse visual acuity compared to cep290−/− mutants and wild-type zebrafish. Visual acuity of cep290−/−;arl13b−/− mutants was further compromised compared to the cep290−/−;arl13b−/− mutants, suggesting that arl13b acts as a modifier to cep290 in a dose-dependent fashion. Similarly, the cep290−/−;ahl1−/− mutants exhibited decreased visual acuity compared to cep290−/− mutants and the cep290−/−;ahl1−/− mutants were further compromised, indicating that loss of ahl1 also exacerbates the visual deficit of cep290−/− mutants in a dose-dependent fashion.

**Commercial Relationships:** Ellen Piccillo, None; Emma M. Lessieur, None; Gabrielle C. Nivar, None; Brian D. Perkins, None

**Program Number:** 363 **Poster Board Number:** A0205

**Presentation Time:** 1:30 PM–3:15 PM

**Nanoparticle based transfection of hard-to-transfect primary photoreceptor cells**

Bibhudatta Mishra1, David R. Wilson2, Yuan Rui2, Mark P. Suprenant3, Baranda S. Hansen1, Cynthia Berlinicke1, Jordan J. Green3, Donald J. Zack1. 1Ophthalmology, Johns Hopkins University School of Medicine, Baltimore, MD; 2Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD.

**Purpose:** Primary retinal cells represent the main functional component of their in vivo counterparts more closely than transformed or immortalized retinal cell lines. Efficient gene transfer into primary retinal cells, however, is still a major challenge. While viral strategies can be effective at delivering nucleic acids, they provide low throughput for potential screening applications and studying genetic mechanisms. Here, we demonstrate an alternative strategy for photoreceptors (PRs) transfection, the application of biodegradable polymeric nanoparticles to introduce plasmid DNA into primary PRs cells with relatively low toxicity and transfection efficiencies of up to 30% in complete media.

**Methods:** Retinas from transgenic mouse expressing GFP under a PR specific promoter (QRX-GFP) were dissociated and cultured into 384-well tissue culture plates. A biodegradable polymer library was synthesized from small molecule diacrylate and primary amine monomers. Nanoparticles were created by mixing polymers from a library of >50 poly(beta-amino ester) (PBAE) with CAG driven mCherry plasmid DNA in sodium acetate buffer, pH 5.0. Nanoparticles were then added to the cultures at doses between 25-200 ng/well and the plate was incubated at 37°C for 2 hours, before the media was exchanged. After 24 hours of culture, nuclei were stained with Hoechst and images were acquired using an automated fluorescence-based imaging system (Cellomics VTI). Transfected PRs were identified as GFP expressing cells also expressing mCherry.

**Results:** PR Transfection efficiencies of up to 26 ± 4.3% were achieved using a subset of nanoparticle formulations. (A) compared to <5% transfection efficiency using the commercial reagent Lipofectamine (B).

**Conclusions:** We have established a high-throughput platform to screen nanoparticles created from a wide variety of polymers for their ability to transfect retinal cells. Using this system, we have identified synthetic polymers that can be used for high efficacy non-viral gene delivery to primary murine PRs, enabling cell signaling and developmental pathways to be more thoroughly studied at the molecular level. This platform can be used to identify the optimum polymer, w/w ratio of polymer to DNA and dose of nanoparticle for different retinal cell types.

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Mutations in zebrafish \(cep290\) result in age-related cone degeneration

Emma M. Lessieur, Wei Zhang, Linjing Li, Gabrielle C. Nivar, Ellen Piccillo, Ping Song, Brian D. Perkins, Hemant Khanna.

1Ophthalmic Research, Cleveland Clinic Foundation, Cleveland, OH; 2Ophthalmology, University of Massachusetts, Worcester, MA.

Purpose: Mutations in \(CEP290\) cause ciliopathies with multisystemic dysfunction and are frequently observed in isolated cases of retinal degeneration. \(CEP290\) is a component of the ciliary transition zone and is thought to function in ciliary trafficking. To better understand the disease pathogenesis associated with \(CEP290\), we evaluated two zebrafish mutants: \(cep290^{sa1383}\) and \(cep290^{sa1382}\) for visual function and photoreceptor structure.

Methods: Localization of Cep290 was evaluated by immunohistochemistry of retinal cryosections. Visual function was assessed by the optokinetic response (OKR) at 5 days post fertilization (dpf). Photoreceptors anatomy was investigated using light microscopy, transmission electron microscopy and immunohistochemistry in animals between 5 dpf and 12 months of age.

Results: The \(cep290^{sa1382}\) allele encodes a stop codon at amino acid 1217 whereas the \(cep290^{sa1383}\) allele carries a premature stop codon at residue 1843. None of the mutants exhibit early lethality. At 5 dpf, 90% of \(cep290^{sa1382}\) and 100% of \(cep290^{sa1383}\) mutants showed no structural alterations in photoreceptors; however, visual performance was decreased for \(cep290^{sa1382}\) but not for \(cep290^{sa1383}\) mutants. Labeling of cone photoreceptors indicated age-related cone degeneration. Rhodopsin mislocalization and thinning of the retinal outer nuclear layer was observed at 3, 6 and 12 months of age in homozygous mutant animals when compared to controls. The \(cep290^{sa1383}\) allele exhibited disorganized outer segments in double cones with progressive loss of outer segments with age.

Conclusions: The zebrafish mutants studied here are viable. Visual behavior is affected in \(cep290^{sa1382}\) but not in \(cep290^{sa1383}\) mutants. Homozygous animals have spared photoreceptor development, however, progressive and predominant cone degeneration, accompanied by thinning of the retinal outer nuclear layer and rhodopsin mislocalization was observed from 3-12 months of age in both alleles. Taken together, these results suggest that the \(cep290^{sa1382}\) and \(cep290^{sa1383}\) allele may be hypomorphs, and that the domain encoded by residues 1844-2439 of zebrafish Cep290 harbors important photoreceptor functional elements.
Organelle subclasses in human retinal pigment epithelium (RPE) facing electron microscopy (3D SBFSEM)

Tamaraj Jasmin J. Mittermueller, Jeffrey D. Messinger, Kenneth R. Sloan, Alexandra S. Weinhandl, Emily Benson, Grahame J. Kidd, Ursula Schmidt-Erfurth, Christine Curcio, Andreas Pollreisz, Ophthalmology and Optometry, University of Alabama at Birmingham, Birmingham, AL; 2 Computer and Information Sciences, University of Alabama at Birmingham, Birmingham, AL; 3 Renovo Neural Inc., Cleveland, OH; 4 Neurosciences, Cleveland Clinic, Lerner Research Institute, Cleveland, OH.

Purpose: An accurate census of lipofuscin (L), melanolipofuscin (ML) and melanosomes (M) in human RPE cells is needed for theories of aging and RPE-driven disease. We compared different subclasses of RPE granules in normal eyes of young and older individuals by 3D SBFSEM of archived specimens.

Methods: Six eyes of white donors with unremarkable maculas were post-fixed with osmium tetroxide and propylene oxide. Epoxy-embedded samples were sectioned and imaged using an SEM with a 150-μm-thick layer. Custom ImageJ software was used to assign x,y,z locations of 7 organellar classes and boundaries of individual cells in image stacks.

Results: A young age group consisted of donors 16M, 32F and 40M, while an older group contained specimens 76F, 84M and 85F. Series of 249-499 sections in each eye containing 5-25 nuclei were examined. The 3 granule types were visualized within the volumetric dataset based on distinct features. L (Fig. 1A-D) were round or oval with homogenous electron-dense (ED) interiors with (L1,2) or without (L3,4) an ED rim or ED condensations around the granule. M were spindle-shaped with homogenous ED contents (M, Fig. 1E,F). ML (Fig. 1G,H) contained an ED spine within a larger granule (ML1,2). L and ML were further separated by size with L1/3 and ML1 representing granules equal or larger and L2/4 and ML2 smaller than 950 nm in diameter. Sample 16M was notable for abundant L, M, as well as small ML compared to older specimens. ML were numerous across all advanced age donors. Overall a fair amount of variability was observed in this cohort. Individual numbers of granules of different classes are listed in the table.

Conclusions: Human RPE contains a variable number of organelles of different sizes in these age groups. Similar information from a larger dataset in progress will aid hypothesis testing about organelle turnover and regulation in health, aging, and disease, and the interpretation of clinical imaging technologies.

Table: Number of specific granules per RPE cell according to age group

<table>
<thead>
<tr>
<th>Age</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>ML1</th>
<th>ML2</th>
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<tr>
<td>16M</td>
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<td>32F</td>
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</table>

Figure 1: Different types of RPE granules identified.
Commercial Relationships: CHI SUN; Carlos Galicia, None; Peter G. Fuerst, None; Deborah L. Stenkamp, None
Support: R01 EY012146