308 Photoreceptors: Cell Biology, Disease and Rescue
Tuesday, May 09, 2017 8:30 AM–10:15 AM
Room 314 Paper Session
Program #/Board # Range: 2485–2491
Organizing Section: Retinal Cell Biology

Program Number: 2485
Presentation Time: 8:30 AM–8:45 AM
Photoreceptor-specific transition zone (PSTZ), a novel sub-region of the connecting cilium (CC), is maintained by retinal ciliopathy protein SPATA7
Rachayata Dharmat[1, 2], Aiden Eblimit[2], Yumei Li[2], Michael Robichaux[3], Zhixian Zhang[3], Feng He[3], Antrix Jain[3], Graeme Mardon[1], Sung Yun Jung[3], Theodore G. Wensel[3], Rui Chen[1, 2]. [1]Molecular and Human Genetics, Baylor college of medicine, Houston, TX; [2]HGSC, Baylor College of Medicine, Houston, TX; [3]Dept of Biochemistry, Baylor College of Medicine, Houston, TX.
Purpose: A hallmark of the photoreceptor sensory cilium is the presence of a specialized structural homolog of transition zone called the connecting cilium (CC). Interestingly, certain transition zone genes, such as *SPATA7*, specifically impairs the function of the CC without affecting the transition zone of primary cilia when mutated. To determine how the CC is functionally distinct from the transition zone, we investigated the impact of the loss of a photoreceptor-specific ciliary protein to probe the differences between the CC and the transition zone using *Spata7* KO mice as the model.
Methods: To understand the function of SPATA7 at the CC, we performed IP-MS based proteomic profiling to identify SPATA7-interacting proteins. We next assessed the localization of interacting partners in the absence of *Spata7* and *Sdccag8* using immunohistofluorescence and confirmed it using super-resolution STORM microscopy on the photoreceptors of P15 *Spata7* KO mice. Since we observed morphological defects, we further assessed structural alterations of the microtubules using cryo-tomography.
Results: SPATA7 interacts with the RPGR and NPHP complex and localizes throughout the length of the CC. In the absence of SPATA7, its interacting proteins are specifically absent (or excluded) from the distal CC which we named the photoreceptor-specific transition zone ("PSTZ"). However, the localization of CC proteins in the proximal CC (pCC) remains unaffected. In contrast, this peculiar phenotype is not observed in the absence of a pCC protein, SDCCAG8. Functionally, the PSTZ complex is important for stabilization of the CC structure as its absence leads to destabilization of ciliary microtubules specifically in the distal CC. This destabilization is caused due to the absence of CEP290, from the PSTZ region, a component of Y-links that are essential for the integrity of microtubules.
Conclusions: Our data displays a novel photoreceptor-specific sub-region in the distal CC, termed the PSTZ, which plays a critical role in the functioning of the CC. Cilia-related proteins at the PSTZ are essential for maintaining the integrity of the microtubule core thereby stabilizing the CC. Hence we propose that this unique PSTZ region makes the CC functionally and structurally distinct from the transition zone found in other primary cilia.
Commercial Relationships: Rachayata Dharmat, None; **Aiden Eblimit**, None; **Yumei Li**, None; **Michael Robichaux**, None; **Zhixian Zhang**, None; **Feng He**, None; **Antrix Jain**, None; **Graeme Mardon**, None; **Sung Yun Jung**, None; **Theodore G. Wensel**, None; **Rui Chen**, None
Support: Grants from National Eye Institute (R01EY022356) and Retinal Research Foundation to R.C

Program Number: 2486
Presentation Time: 8:45 AM–9:00 AM
Contribution of autophagy to Usher syndrome pathogenesis
Erik de Vrieze[1], Ralph Slijkerman[1], Margo Dona[1], Sanne Broekman[1, 2], Lisette Hetterschijt[1], Theo Peters[1], Hannie Kremer[1, 2], Erwin van Wijk[1]. [1]Otorhinolaryngology, Radboudumc, Nijmegen, Netherlands; [2]Human Genetics, Radboudumc, Nijmegen, New Caledonia.
Purpose: Usher syndrome (USH) is the most common cause of hereditary deaf-blindness. USH patients are born with congenital hearing impairment, and suffer from progressive vision loss (retinitis pigmentosa, RP), a combination that puts them at risk for social isolation and loss of independence. Mutations in the *USH2A* gene are the most frequent cause of USH, explaining up to 50% of all cases. Mutations in *USH2A* can also result in non-syndromic RP. Currently, virtually nothing is known about the pathogenesis of *USH2A*-associated RP.
Methods: We used complementary proteomics techniques to identify novel interaction partners of USH2A. Using CRISPR/Cas9-mediated genome editing, we generated an *ush2a* zebrafish knockout model, carrying a protein-truncating mutation in exon 13. Guided by our proteomic studies, we deep-phenotyped our *ush2a*$^{-/-}$ zebrafish.
Results: We identified and validated interactions between multiple subunits of the Cop9 signalosome (CSN) and members of the USH protein complex. CSN subunit 8 (COPS8) is a direct interactor of USH2A. CSN/COPS8 is well documented to play a role in two proteostatic pathways: ubiquitin proteasome system (UPS) and autophagy. Immunohistochemistry showed that Cops8 localization in the photoreceptor overlaps with Ush2a, allowing interactions *in vivo*. Phenotypic analyses of the *ush2a*$^{-/-}$ retina showed increased levels of photoreceptor apoptosis, as well as an increase in autophagosomes. However, we did not find evidence of increased activity of the UPS system. We also identified mislocalization of rhodopsin-containing transport vesicles. Surprisingly, ER stress (protein-accumulation in the endoplasmic reticulum) appears diminished.
Conclusions: Using zebrafish as a model organism, we have identified that elevated levels of autophagy might be the pathogenic mechanism underlying *USH2A*-associated retinal degeneration. Whether this is a direct consequence of misregulated autophagy (through CSN), or is a response to the mislocalized transport vesicles, remains to be established. Both prolonged elevation of autophagy and activation of mislocalized photopigments can lead to the observed apoptosis of photoreceptors. The pathways that we have identified explain the slow progressive nature of the retinal degeneration in USH2A patients. This brings us closer to understanding the pathogenesis of *USH2A*-associated RP, which is also important for the development of future therapies.
Commercial Relationships: Erik de Vrieze; **Ralph Slijkerman**, None; **Margo Dona**, None; **Sanne Broekman**, None; **Lisette Hetterschijt**, None; **Theo Peters**, None; **Hannie Kremer**, None; **Erwin van Wijk**, None

Program Number: 2487
Presentation Time: 9:00 AM–9:15 AM
Hyperautophagy in response to protein misfolding contributes to photoreceptor cell death in Pro23His-rhodopsin mice
Jingyu Yao[1], Lin Jia[1], Eric Frontera[1], Naheed Khan[1], Debra A. Thompson[1, 2], David N. Zacks[1]. [1]Department of Ophthalmology & Visual Science, University of Michigan, Ann Arbor, MI; [2]Department of BIological Chemistry, University of Michigan, Ann Arbor, MI.
Purpose: The Pro23His (P23H) variant resulting from a mutation in the rhodopsin gene is a common cause of autosomal dominant retinitis pigmentosa (adRP). In both the human disease and the

P23H mouse, rhodopsin misfolding results in accumulation of rhodopsin aggregates. The purpose of this study is to define the role of autophagy in retinal degeneration in the P23H mouse and to understand the mechanisms of protein degradation needed to maintain photoreceptor (PR) homeostasis and survival.
Methods: Basal levels of autophagic activity in P23H mice and C57BL/6 controls, measured by western blot and immunohistochemical (IHC) analysis, were compared as a function of age. The effect of modulating autophagy on retinal degeneration in the P23H mouse was evaluated by pharmacologically activating autophagy using a derivative of rapamycin (CCI-799), and by genetically inhibiting autophagy by deleting Atg5 in rod cells to generate the P23H-*Atg5*$^{\Delta rod}$ mouse. Retinal structure and function were evaluated by IHC and ERG analysis. Proteasome activity, a compensatory mechanism for degrading misfolded proteins, was measured by chymotrypsin-like activity assay, and compared across P23H, P23H-*Atg5*$^{\Delta rod}$ and C57BL/6 mice.
Results: Retinas from P23H mice showed increased autophagy flux as evidenced by elevated levels of LC3-II under conditions in which autophagosome-lysosome fusion was blocked. P23H mice treated with CCl-799 exhibited increased rates of PR degeneration, whereas deletion of autophagy in rod cells (P23H-*Atg5*$^{\Delta rod}$ mouse) resulted in PR preservation with a corresponding increase in PR function. The level of proteasome activity was significantly higher in the P23H-*Atg5*$^{\Delta rod}$ mouse retina than in P23H mouse.
Conclusions: Elevated autophagy levels in the P23H mouse retina, and the rescue of the P23H phenotype by deletion of autophagy, suggest that misfolded rhodopsin results in hyper-autophagy in rods. Although autophagy is important for clearing misfolded rhodopsin, persistent autophagy activation contributes to PR cell death. The absence of autophagy shifts the degradation of misfolded rhodopsin to the proteasome and is protective in P23H mice. These observations provide new understanding of the role of autophagy in PR death due to rhodopsin folding mutations, and suggest that modulating the flux of misfolded protein from autophagy to the proteasome may represent an important therapeutic option.
Commercial Relationships: Jingyu Yao, None; **Lin Jia**, None; **Eric Frontera**, None; **Naheed Khan**, None; **Debra A. Thompson**, None; **David N. Zacks**, None
Support: National Eye Institute R01-EY-020823; Foundation Fighting Blindness; Research to Prevent Blindness: Physician-Scientist Award; Departmental Core Grant EY-07003

Program Number: 2488
Presentation Time: 9:15 AM–9:30 AM
De-novo assembly of mouse photoreceptor transcriptome identifies un-annotated lncRNAs regulated by NRL
Lina Zelinger[1], Gökhan Karakülah[1, 2], Vijender Chaitankar[1], Jung-Woong Kim[1, 3], Hyun-Jin Yang[1], Matthew Brooks[1], Anand Swaroop[1]. [1]NNRL, NIH-NEI, Bethesda, MD; [2]Izmir International Biomedicine and Genome Institute (iBG-izmir), Dokuz Eylül University, Inciralti, Izmir, Turkey; [3]Life Science, Chung-Ang University, Seoul, Korea (the Republic of).
Purpose: Our goal is to provide a detailed picture of the photoreceptor transcriptomic landscape that will serve as a basis to study gene regulatory networks underlying development and disease. This study focuses on identifying and cataloging un-annotated lncRNAs in developing rod photoreceptors.
Methods: Wild type (*Nrl*p-GFP) rods and S-cone like (*Nrl*p-GFP;*Nrl*$^{-/-}$) photoreceptors were purified from mice retina using fluorescence-activated cell sorting. RNA-seq profiles of sorted cells were generated from 6 stages of differentiation. Genome guided *de novo* transcriptome assembly was performed using TopHat2 v2.0.11 and Cufflinks v2.2.1. Previously un-annotated lncRNAs were examined for their coding potential using TransDecoder v1. Selected un-annotated lncRNAs were validated by *in situ* hybridization (ISH).
Results: We identified 586 known photoreceptor-expressed lncRNAs and 1037 previously un-annotated lncRNAs. LncRNA expression profiles revealed specific signatures and co-expression clusters during rod development, consistent with milestones of morphogenesis as observed in coding genes. In the absence of rod differentiation factor NRL, 23% (239/1037) lncRNAs demonstrated differential expression, and 33% (80/239) of these included NRL binding sites in their promoter region. Weighted correlation network analysis linked 74 un-annotated lncRNAs to proteins associated with "visual perception", and 10 of these are putative direct targets of NRL. A number of un-annotated lncRNAs showed cell specific expression in photoreceptors and were undetceted in eight other adult mouse tissues. We prioritized un-annotated lncRNAs for validation based on expression pattern, NRL regulation, and protein co-expression. ISH analysis validated the expression of 12 lncRNAs that were selected; of these, 11 showed cell specific expression.
Conclusions: We identified and validated un-annotated lncRNAs expressed in the rod photoreceptors and potentially regulated by NRL. Our analysis suggests that coding and non-coding transcriptomes are under similar regulatory constraints. We also propose possible roles of lncRNAs by relating them to genes of known function and to developmental milestones. Our study provides the framework for deciphering the function of lncRNAs during photoreceptor development.
Commercial Relationships: Lina Zelinger; **Gökhan Karakülah**, None; **Vijender Chaitankar**, None; **Jung-Woong Kim**, None; **Hyun-Jin Yang**, None; **Matthew Brooks**, None; **Anand Swaroop**, None
Support: Intramural Research Program of the National Eye Institute

Program Number: 2489
Presentation Time: 9:30 AM–9:45 AM
Mouse Models of Rapid and Progressive Cone Degeneration Display Key Differences in Autophagy Signaling
Michael Butler, Hongwei Ma, Fan Yang, Xi-Qin Ding. Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.
Purpose: When subjected to metabolic or organellar stress, cells respond by activating key stress signaling pathways, such as autophagy. In addition to attempting to correct the insult and return to homeostasis, these responses help determine cell fate. Mouse models of inherited retinal degenerative diseases have been shown to experience both metabolic and organellar stress, but the mediators of these responses have not been fully studied in inherited cone dystrophies. This work investigated the potential contributions of autophagy signaling in determining photoreceptor fate in rapid versus progressive cone degeneration.
Methods: *Rpe65*$^{-/-}$/*Nrl*$^{-/-}$ mice (RPE65 deficiency on a cone-dominant background) were used to model rapid cone degeneration, whereas *Cnga3*$^{-/-}$/*Nrl*$^{-/-}$, *Cngb3*$^{-/-}$/*Nrl*$^{-/-}$, and *Gucy2e*$^{-/-}$/*Nrl*$^{-/-}$ mice (CNGA3, CNGB3, and RetGC1 deficiency on a cone-dominant background, respectively) were used to model progressive cone degeneration. Expression levels of several autophagy genes were analyzed by qRT-PCR at ages postnatal day 15 (P15) and P30 in all genotypes. In addition, western blot analysis of autophagy mediators and autophagosome formation protein markers was performed.
Results: Expression levels of autophagy genes showed similar trends among progressive cone degeneration phenotypes at P30, with significant increases in *Atg7, LC3a,* and *LC3b*, when compared with genotype-matched P15 levels. However, *Rpe65*$^{-/-}$/*Nrl*$^{-/-}$ mice showed significant down-regulation in nearly all autophagy gene markers at

P30 when compared with P15 levels. Interestingly, lipidated LC3b protein levels were increased at P30 in both *Rpe65*$^{-/-}$*/Nrl*$^{-/-}$ and *Cnga3*$^{-/-}$*/Nrl*$^{-/-}$ mice, suggesting enhanced autophagosome formation. In addition, intermediate autophagy markers Atg7 and Beclin1 displayed significantly different trends between *Rpe65*$^{-/-}$*/Nrl*$^{-/-}$ and *Cnga3*$^{-/-}$*/Nrl*$^{-/-}$ mice at P30.
Conclusions: In mouse models of rapid and progressive cone degeneration, we demonstrate that autophagy signaling is significantly different between these phenotypes, specifically involving intermediate autophagy proteins that may determine the type of autophagic response. The findings of this study provide insight into mechanisms mediating rapid versus progressive cone death in inherited cone dystrophies, as well as identifying potential sites of intervention to target autophagy signaling and preserve rapidly degenerating cones.
Commercial Relationships: Michael Butler, None; **Hongwei Ma**, None; **Fan Yang**, None; **Xi-Qin Ding**, None
Support: NEI Grants P30EY12190, R01EY019490; Fight For Sight; Oklahoma Center for the Advancement of Science and Technology

Program Number: 2490
Presentation Time: 9:45 AM–10:00 AM
Antisense Oligonucleotide-induced Skipping of *USH2A* exon13 Restores Visual Function in Zebrafish
Erwin van Wijk[1, 2], Margo Dona[1, 3], Ralph Slijkerman[1, 3], Peter Adamson[4, 5], Janne Turunen[4], Maarten Kamermans[6], Stephan C. Neuhauss[7], Hester van Diepen[4]. [1]Otorhinolaryngology, Radboudumc, Nijmegen, Netherlands; [2]Donders Institute for Brain, Cognition and Behaviour, Nijmegen, Netherlands; [3]Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands; [4]ProQR Therapeutics, Leiden, Netherlands; [5]Institute of Ophthalmology, UCL, London, United Kingdom; [6]Retinal Signal Processing Lab, Institute for Neuroscience, Amsterdam, Netherlands; [7]Institute for Molecular Life Sciences, University of Zürich, Zurich, Switzerland.
Purpose: Mutations in *USH2A* exon13 are the most frequent cause of both syndromic and non-syndromic retinitis pigmentosa (RP), for which currently no treatment options exist. It is generally believed that RP due to mutations in this gene is caused by a loss-of-function mechanism. Zebrafish lacking Ush2a show early signs of retinal dysfunction, although its regeneration capacity blocks the progression of retinal degeneration. Skipping of in-frame exons, like *USH2A* exon13, that carry loss-of-function mutations, will restore the open reading frame and potentially results in a slightly shortened protein with residual function. Therefore we explored the therapeutic potential of exon13-skipping as a therapeutic approach for future treatment of *USH2A*-associated retinal degeneration using zebrafish as a model.
Methods: Zebrafish larvae carrying a homozygous lesion (c.2337_2342delinsAC; p.Cys780Glnfs*) in *Ush2a* exon13, were injected with tailor-made antisense oligonucleotides (AONs) targeting this exon. Exon-skipping efficiency was determined by RT-PCR analysis and restoration of Ush2a protein expression and visual function was monitored by immunohistochemistry and electroretinogram (ERG) recordings, respectively.
Results: Injection of morpholino-based AONs in homozygous mutant zebrafish larvae successfully induced skipping of exon13 from the mature *ush2a* mRNA. As a result, Ush2a protein expression at the photoreceptor periciliary membrane was partly restored. In addition, ERG traces were restored in AON-treated larvae as compared to uninjected mutant controls.
Conclusions: Proof-of-concept has been obtained for exon-skipping as a therapeutic approach for the development of a future treatment for *USH2A*-associated retinal degeneration caused by loss-of-function mutations in exon13.
Commercial Relationships: Erwin van Wijk, Radboudumc (P); **Margo Dona**, None; **Ralph Slijkerman**, None; **Peter Adamson**, ProQR Therapeutics (P); **Janne Turunen**, ProQR Therapeutics (P); **Maarten Kamermans**, None; **Stephan C. Neuhauss**, None; **Hester van Diepen**, ProQR Therapeutics (P)

Program Number: 2491
Presentation Time: 10:00 AM–10:15 AM
Rescue of retinal degeneration in a rat model of Smith-Lemli-Opitz Syndrome
Steven J. Fliesler[1, 2], Neal S. Peachey[3, 4], Nadav I. Weinstock[5], Josi Herron[6], Kelly M. Hines[6], Libin Xu[6]. [1]Ophthalmology, Biochemistry, & Neuroscience Program, SUNY- University at Buffalo and SUNY Eye Institute, Buffalo, NY; [2]Research Service, VA Western NY Healthcare System, Buffalo, NY; [3]Cole Eye Institute, Cleveland Clinica Foundation, Cleveland, OH; [4]Research Service, Louis Stokes Cleveland VA Medical Center, Cleveland, OH; [5]Neuroscience Program, SUNY- University at Buffalo, Buffalo, NY; [6]Medicinal Chemistry, University of Washington, Seattle, WA.
Purpose: The AY9944 rat model of Smith-Lemli-Opitz syndrome (SLOS) exhibits progressive retinal degeneration, which is partially ameliorated by feeding a high-cholesterol (Chol) diet (Fliesler et al., 2004, 2007). Here, we tested the hypothesis that combined dietary Chol plus antioxidants would provide an improved therapeutic intervention over Chol alone, sparing the retinal degenerative phenotype in this SLOS model.
Methods: Pregnant rats were treated with AY9944 to generate the SLOS model, as previously described (Fliesler et al., 2004, 2007). Upon weaning, rat pups (N=10-12/group) were randomized to three dietary groups: AY1 (normal rat chow); AY2 (the AY1 chow supplemented with 2% (w/w) Chol); and AY3 (the AY2 chow supplemented with vitamins E (500 IU/kg) and C (1.43 g/kg), plus Se nitrite (3.4 mg/kg) (Chen & Tappel, 1995)). Control group: age-matched untreated rats, fed normal rat chow. At PN80-82 days, electroretinograms (ERGs) were obtained; animals were euthanized, and tissues were harvested for biochemical and histological analyses. Quantitative data (mean/S.D.) were statistically compared using Student's *t*-test (significance: $p \leq 0.05$) or one-way ANOVA.
Results: Treated rats on the AY1 diet exhibited massive retinal degeneration; the AY2 diet provided substantial, but incomplete, sparing from histological damage, while retinal histology of rats fed the AY3 diet was comparable to that of untreated controls. Rod and cone ERG amplitudes were markedly reduced, relative to age-matched controls, for rats fed the AY1 diet, were less affected (but not normal) for rats fed the AY2 diet, and were comparable to untreated controls for rats fed the AY3 diet. Retinal oxysterol levels were increased >160-fold, relative to untreated controls, for rats fed the AY1 diet, declined by ~18% on the AY2 diet (rel. to the AY1 group), and by ~37% (rel. to the AY1 group) on the AY3 diet. Retinal 7DHC/Chol was >5 for rats on the AY1 diet (<0.01 for controls); AY2 and AY3 diets reduced the 7DHC/Chol >2-fold.
Conclusions: Combined dietary high-Chol plus antioxidant supplementation provides a substantially improved therapeutic intervention over Chol alone with regard to sparing loss of retinal structure and function, correlating with reductions in retinal oxysterol and 7DHC/Chol levels, in the AY9944 SLOS rat model. These results have translational implications for improving the clinical management of SLOS patients.
Commercial Relationships: Steven J. Fliesler, None; **Neal S. Peachey**, None; **Nadav I. Weinstock**, None; **Josi Herron**, None; **Kelly M. Hines**, None; **Libin Xu**, None

Support: NIH R01 EY007361 (SJF); NIH R00 HD073270 (LX); RPB Unrestricted Grant (SJF, NSP); Dept. of Veterans Affairs (SJF, NSP) / Research Career Scientist Award (SJF)