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Goals and projects

Research in the laboratory of Brian D. Perkins, Ph.D., seeks to develop zebrafish models that mimic photoreceptor degeneration in childhood diseases like Joubert Syndrome, Bardet-Biedl Syndrome, and Usher Syndrome.

Our long-term goal is to understand the mechanisms regulating cilia assembly and why defects in these processes cause photoreceptor degeneration. Vertebrate photoreceptor outer segments form from the connecting cilium, which is anchored by a basal body at the apical inner segment. The connecting cilium connects the inner segment to the outer segment and is the gateway for proteins destined for the outer segment. Mutations in cilia or basal body proteins disrupt protein trafficking and result in multisyndromic diseases termed ciliopathies, which often manifest with retinal degeneration. Cilia formation and function depend on the proper placement and anchoring of the basal body at the apical surface, but the mechanisms controlling basal body location remain poorly understood – particularly in photoreceptors. Furthermore, how altered positioning of basal bodies influences pathology is not fully understood. Little is known about the mechanisms controlling basal body docking and other early steps of cilia formation. By understanding the mechanisms governing ciliary positioning, it will be possible to understand how mutations in individual genes contribute to blindness. We utilize the zebrafish as an experimental system for several reasons: (1) zebrafish photoreceptor anatomy is well-characterized; (2) zebrafish photoreceptors provide an accessible, stereotyped population to analyze genetic defects; (3) techniques exist to manipulate gene expression, rapidly generate transgenic lines, and create genetic mosaic embryos; and (4) we have a wide number of zebrafish transgenic and mutant lines to investigate processes required for cilia. Achieving a better molecular understanding of basal body localization in photoreceptors could lead to potential therapies for human retinal damage and disease.

To determine the role of Planar Cell Polarity (PCP) signaling in ciliary positioning and photoreceptor survival in zebrafish.

In many tissues, cilia function relies on the asymmetric positioning and/or tilting of the cilium to one side of the cell. The PCP signaling pathway controls cilia positioning by regulating basal body docking on the apical surface. Defects in PCP signaling have been linked to certain phenotypes in ciliopathies and ciliary proteins directly bind with key PCP proteins. Our preliminary data reveals a highly arranged pattern of basal bodies in zebrafish photoreceptors. We hypothesize that PCP signaling functions to position photoreceptor cilia. As retinal degeneration occurs in most ciliopathies and outer segment formation requires the proper positioning and docking of the basal body, we hypothesize that disrupting PCP signaling will disrupt this pattern and be deleterious to photoreceptors.

To determine the functional interactions between PCP signaling and *ar13b* on cilia function in zebrafish photoreceptors.

Mutations *ar13b* result in Joubert Syndrome, which presents with variable retinal dystrophy. Arl13b localizes to cilia and mutations disrupt cilia structure. Suppression of Arl13b also leads to PCP phenotypes in zebrafish and our data indicates that Arl13b and PCP components can genetically interact to result in retinal degeneration. We use light and electron microscopy to examine photoreceptor ultrastructure *ar13b* mutants and genetic mosaic analysis to examine the long-term fate of mutant photoreceptors in wild type zebrafish. To determine relationship between Arl13b and PCP signaling, both genetic and physical interactions will be tested. Finally, the requirement for GTPase activity and ciliary localization of Arl13b will be tested using mutant alleles at key amino acid residues.

Determine how the *Wrb* protein participates in ribbon synapse function.

Usher Syndrome is characterized by hearing loss at birth and retinitis pigmentosa that can begin during childhood. We are studying a zebrafish *wrb* mutant that is blind and has disrupted hair cell function, defects similar to Usher Syndrome. Several Usher proteins function in cell adhesion, as molecular scaffolds, or in protein trafficking, and many are expressed at both the cilia and the synapse. Our understanding of the mechanisms underlying photoreceptor degeneration in Usher Syndrome remains incomplete. In order to develop comprehensive therapies for Usher Syndrome, we need a better understanding of the role of Usher proteins in both cilia and synapse development. Our data show that blindness and hair cell dysfunction in the *wrb* mutant is due to defective ribbon synapse function. The goal of this proposal is to test the hypothesis that *Wrb* is required for targeting of SNARE proteins to the photoreceptor synapse. The study of *wrb* mutants should yield valuable insights into the trafficking of components of exocytosis to ribbon synapse domains and a potential candidate gene for deafness-blindness.

1.) Zebrafish models of childhood blindness

1. Genetic and functional studies of cell polarity during photoreceptor cilia formation

Personnel: Ping Song, Joe Fogerty, Bob Gaivin, Glenn Lobo

- (a) The goal of this project is to determine the role of Planar Cell Polarity (PCP) signaling in ciliary positioning and photoreceptor survival in zebrafish. In many tissues, cilia function relies on the asymmetric positioning and/or tilting of the cilium to one side of the cell. The PCP signaling pathway regulates basal body docking on the apical surface and cilia positioning. Defects in PCP signaling contribute to ciliopathies and ciliary proteins directly bind with key PCP proteins. Our preliminary data reveals a highly arranged pattern of basal bodies in zebrafish photoreceptors. We hypothesize that PCP signaling functions to position photoreceptor cilia. We hypothesize that disrupting PCP signaling will disrupt this pattern and be deleterious to photoreceptors.
- (b) The goal is to determine the functional interactions between PCP signaling and *arl13b* on cilia function in zebrafish photoreceptors. Mutations *arl13b* result in Joubert Syndrome, which presents with variable retinal dystrophy. Arl13b localizes to cilia and mutations disrupt cilia structure. Our preliminary data indicates that Arl13b and PCP components can genetically interact to result in retinal degeneration. We will use light and electron microscopy to examine photoreceptor ultrastructure *arl13b* mutants. We will use genetic mosaic analysis to examine the long-term fate of mutant photoreceptors in wild type zebrafish. To determine relationship between Arl13b and PCP signaling, both genetic and physical interactions will be tested. Finally, the requirement for GTPase activity and ciliary localization of Arl13b will be tested using mutant alleles at key amino acid residues.

2. Ribbon synapse development in a model of Usher Syndrome

Personnel: Lauren Daniele

- (c) Usher Syndrome is characterized by hearing loss at birth and retinitis pigmentosa that can begin during childhood. We are studying the zebrafish *wrb* mutant that is blind and has disrupted hair cell function, defects similar to Usher Syndrome. Several Usher proteins function in cell adhesion, as molecular scaffolds, or in protein trafficking, and many are expressed at both the cilia and the synapse. Our preliminary data show that blindness and hair cell dysfunction in the *wrb* mutant is due to defective ribbon synapse function. Ribbon synapse function is critical for preserving the visual sensitivity of photoreceptors. The goal of this proposal is to test the hypothesis that Wrb is required for targeting of SNARE proteins to the photoreceptor synapse.

2.) Diabetic Retinopathy

1. Epigenetic changes in diabetic retinopathy

Personnel: Emma Lessieur, Bob Gaivin

- (a) Vision loss caused by diabetic retinopathy is the leading cause of new cases of blindness. Clinicians have long recognized that the risk for diabetic retinopathy continues after the controlling diabetes with medication and/or lifestyle changes. The phenomenon of prolonged hyperglycemia causing long-lasting negative complications has been termed “metabolic memory”. Such changes resulting from hyperglycemia and/or hypoxia are often attributed to epigenetic changes that alter gene expression. Nevertheless, almost nothing is known about the epigenetic changes that occur in diabetic retinas. Our long-term goal is to identify the molecular events resulting from epigenetic changes in DNA methylation and that lead to the vascular damage associated with diabetes.

Lab staff members:

- Brian D. Perkins, Ph.D., *Director*
- Lauren Daniele, Ph.D., *Project Staff*
- Glenn Lobo, Ph.D., *Project Staff*
- Ping Song, Ph.D., *Project Staff*
- Joe Fogerty, Ph.D., *Postdoctoral Fellow*
- Bob Gaivin, *Lab Manager and Lead Technician*
- Emma Lessieur, M.D., *Graduate Student in Molecular Medicine*