PROJECT SUMMARY

The long-term goal of our research is to explore the molecular and cellular bases of congenital eye diseases. The development of the vertebrate eye begins with the evagination of the optic vesicle from the forebrain. The vesicle subsequently invaginates to form a bilayered optic cup with an outer layer, the retinal pigment epithelium (RPE), and an inner layer, the retina. This invagination creates a ventral opening in the eye, called the **Choroid Fissure (CF)**, which allows embryonic vasculature to enter, and retinal ganglion cell axons to exit the eye. The folds of the CF fissure must become apposed, adhere and subsequently fuse in order to form a ventrally continuous eye cup. Failure of this process leads to severe malformations of the eye, and results in Microphthalmia (small eye), Anophthalmia (no eye), and Coloboma (CF fusion defects). Together called MAC spectrum disorders, these malformations constitute the leading cause of pediatric blindness affecting 5-12/10,000 live births. The goal of this study is to understand the cellular and molecular bases of MAC spectrum disorders.

Due to the number of tissues (retina, RPE, lens, periocular mesenchyme) involved in 3-dimensional optic cup formation, the cellular and molecular bases of this process are only superficially understood. The BMP signaling cascade is an important regulator of optic cup formation and is associated with MAC spectrum disorders in humans and numerous animal models of eye disease. Unfortunately, BMP dysregulation produces widespread defects in eye development. Peculiarly, both BMP gain and loss of function mutants display MAC phenotypes and account for 1-2% of all human colobomata. They suggest that to dissect the cellular and molecular bases of BMP-induced MAC phenotypes, spatio-temporally targeted BMP manipulations, and time-lapse analyses in visual animals such as the chick and fish must be performed.

Aim 1. Our preliminary results suggest that BMP signaling regulates CF fold alignment, adhesion and fusion during CF closure. Using focal BMP manipulations and newly developed time-lapse paradigms in the chick and fish, Aim 1 will test the hypothesis BMP signaling regulates CF fold alignment, adhesion and fusion via cell cycle dependent epithelial remodeling and basement membrane degradation.

Aim 2. The paradoxical convergence of BMP gain and loss of function mutants on MAC spectrum phenotypes suggests the hypothesis that BMP function in CF closure is dynamic and must depend upon the differential function of BMP modulators (genes encoding cytoskeletal and ECM protein regulators, adhesion proteins and those that regulate cell cycle progression) in different parts of the eye cup. Aim 2 will utilize laser capture microdissection and an RNA-Seq approach to identify factors that are differentially expressed in CF cells when BMP signaling is ectopically modulated, and then screen mutants to analyze their functions in CF closure.

Aim 3. We have only a very cursory understanding of how the CF forms in the human eye because there are no good primate models in which this can be explored. To address this, experiments in this Aim will develop the eye of the common marmoset (*Callithix jacchus*) as a model system in which CF closure can be examined, and in which potential therapies can be developed and evaluated.

Relevance

Microphthalmia-Anophthalmia-Coloboma (MAC) disorders are a heterogeneous spectrum of congenital ocular defects that result in severe structural malformations of the eye. MAC phenotypes can be isolated, or they can be components of a variety of syndromic disorders. Reported incidences vary widely across the globe but approximate ranges are 2.13 per 10,000 live births for anophthalmia and microphthalmia, to 2.6 to 7.5 per 10,000 for colobomata. Colobomata are also a component of over 50 human genetic disorders, where they are often associated with microphthalmia. Despite a significant amount of genetic research to identify MAC loci, causative mutations have been identified in very few cases, and for those that have, little is known about how the affected proteins facilitate normal eye development. Experiments in this proposal will elucidate the cellular and molecular underpinnings of MAC spectrum disorders, they will identify additional genetic loci to screen in MAC patients, and they will develop a primate model in which therapies targeting MAC phenotypes can be developed.