THE ROLE OF AHI1 IN
RAB-MEDIATED VESICULAR TRAFFICKING

By

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An Abstract of a Thesis Submitted to the Graduate

Faculty of Rensselaer Polytechnic Institute

in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Biology

The original of the complete thesis is on file
in the Rensselaer Polytechnic Institute Library

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December 15, 2009

ABSTRACT

This thesis explores the function of the *Abelson helper integration site 1* gene, a causative gene for Joubert syndrome (JBTS). JBTS is both an autosomal recessive neurodevelopmental disorder and a ciliopathy. Although JBTS has been studied since 1969, the mechanisms that result in the abnormalities that characterize it are poorly understood. *AHI1* was discovered by Russell Ferland, my thesis advisor, and has been found to be responsible for 10-15% of JBTS cases. Experiments performed by other lab members established that Ahi1 interacts with the Rab family of proteins, more specifically Rab8a. Rab proteins have long been known to be involved in vesicular trafficking. Taking this relationship into consideration, we sought to characterize how mutations in *Ahi1* can affect vesicular trafficking in a mouse model. First, the spatial and temporal localization of AHI1 was determined in humans, mice, and zebrafish. Expression was found to be in the ventral forebrain and midbrain/hindbrain with expression in the cerebellum of humans and fish but not mice. Detectable levels of Ahi1 were first seen at embryonic day (E) 10.5 in mice. These levels persisted throughout life, with peak expression occurring during the first post-natal (PN) week. We created a mouse with a targeted deletion of *Ahi1* (*Ahi1* $^{-/-}$), and found that *Ahi1* $^{-/-}$ mice on a C57BL/6J background die around post-natal day (PN) 1, with significant disorganization in the inferior olives. JBTS is classified as a ciliopathy, with many of its causative genes associating with basal bodies and/or cilia. Ahi1 was found to localize to the mother centriole, which eventually becomes the basal body at the base of the cilia. Given the association of Ahi1 with cilia, we examined the function of Ahi1 in cilia formation. Both hypothalamic neuronal cultures from *Ahi1* $^{-/-}$ mice and Ahi1 shRNAi knockdown IMCD3 cells have impairments in ciliogenesis. We were able to overcome the post-natal lethality of *Ahi1* $^{-/-}$ mice by backcrossing our *Ahi1* $^{-/-}$ mice onto a BALB/cJ background. Mutations in *AHI1* have been associated with retinal dystrophy. Examination of *Ahi1* $^{-/-}$ retinas showed that the outer segments of photoreceptors failed to form and retinal degeneration was complete by three months of age, demonstrating that Ahi1 is required for the formation of photoreceptor outer segments. Proteins that are normally found in the outer segment of photoreceptors were mis-localized to the inner segment in retinas before the start of degeneration. This trafficking defect seems to be specific to ciliary components as there was no disruption in synaptotagmin localization. This phenotype, along with reduced Rab8a levels, provides further evidence that Ahi1 is involved in cilia-related vesicular trafficking through its interactions with Rab proteins. Vesicular trafficking was also analyzed in the pituitary gland, as exocytosis of hormone-containing vesicles is critical in development. *Ahi1* $^{-/-}$ mice were runted and unable to produce offspring, both of which are phenotypes that can be attributed to dysfunction in the hypothalamic-pituitary (HP) axes, which control hormone production and release. In *Ahi1* $^{-/-}$ mice, levels of growth hormone (GH) and luteinizing hormone (LH) were found to be significantly decreased in serum

while follicle-stimulating hormone (FSH) demonstrated an increase and testosterone was variable, suggesting that this phenotype has variable penetrance that is dependent on the animal. Immunostaining showed that hormones were produced at normal levels in the pituitary, suggesting that *Ahi1* $^{-/-}$ do infact have defects in exocytosis. A result of these defects in hormone release is that the seminiferous tubules of *Ahi1* $^{-/-}$ mice have immature spermatids, a phenotype that was not able to be rescued by administration of testosterone pellets. Also, *Ahi1* $^{-/-}$ females produced eggs when superovulated but in most cases these eggs are not able to be fertilized and lacked secondary polar bodies. There were a few instances in which the eggs were fertilized, again suggesting that this phenotype is not completely penetrant. While Ahi1 is already known to interact with Rab8a, this particular rab does not have a known role in exocytosis in the pituitary gland. However, other studies have identified that Rab3b plays a significant role in exocytosis. Rab3b levels were found to be reduced in Ahi1-knockdown cells and *Ahi1* -/- pituitary tissue. Given the role that Rab3b play in exocytosis it seems likely that Ahi1 interacts with Rab3b in a manner similar to its interaction with Rab8a. This thesis provides evidence that Ahi1 has a Rab8a-mediated role in trafficking vesicles required for ciliogenesis in the brain and retina as well as an undefined involvement in exocytosis in the pituitary gland.