ABSTRACT

The Role of Kinesin Heavy Chain in *Drosophila* Photoreceptor Development

Garrett P. League, M.S.

Mentor: Sang-Chul Nam, Ph.D.

In developing *Drosophila* photoreceptors a stabilized microtubule structure was discovered and its presence was linked to polarity protein localization defects caused by mutations in the microtubule-severing protein Spastin and the centrosome core protein Centrosomin. It was therefore hypothesized that the microtubules may provide trafficking routes for the polarity proteins during photoreceptor morphogenesis. This study has examined whether kinesin heavy chain (Khc), a subunit of the microtubule-based motor kinesin-1, is essential in polarity protein localization in developing photoreceptors.

After finding a strong genetic interaction between *crumbs* (*crb*) and *khc*, loss-of-function and gain-of-function analyses revealed progressive reductions in both the Crb and adherens junction (AJ) domains and an increase in the Crb domain, respectively. Furthermore, the *khc* mutation also led to similar progressive defects in the stabilized microtubule structures, strongly suggesting that Khc is essential for microtubule structure and Crb localization during distal to proximal rhabdomere elongation in *Drosophila* pupal photoreceptor development.

The Role of Kinesin Heavy Chain in *Drosophila* Photoreceptor Development

by

Garrett P. League, B.S.

A Thesis

Approved by the Department of Biology

_____

Robert D. Doyle, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

Approved by the Thesis Committee

_____

Sang-Chul Nam, Ph.D., Chairperson

_____

Patrick D. Danley, Ph.D.

_____

Dennis A. Johnston, Ph.D.

Accepted by the Graduate School
May 2011

_____

J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

ACKNOWLEDGMENTS

I would like to express my gratitude to the Baylor University Department of Biology for providing the top-notch facilities and faculty that made this project not only possible but enjoyable. I would also like to thank the Molecular Biosciences Center at Baylor for providing the facilities and equipment necessary for the confocal microscopy utilized in my experiments.

My deepest thanks go to my gracious mentor Dr. Sang-Chul Nam for his encyclopedic knowledge of *Drosophila*, enthusiasm for science, and sage advice. Many thanks are also due to Dr. Patrick Danley and Dr. Dennis Johnston for their valuable time and keen input as members of my thesis committee. For providing much of my day-to-day help in the laboratory, I would also like to thank the Nam lab's wonderful senior graduate student Geng Chen.

Finally, I wish to thank each and every member of my extraordinary family for the unique ways in which each of them has poured into my life throughout my graduate studies. *Soli Deo Gloria!*

For my parents, Scott and Amanda League. You are pillars of grace and wisdom.

I treasure both of you and all that you have sacrificed for my good. Thank you!

CHAPTER ONE

Introduction

Background

The common fruit fly *Drosophila melanogaster* has proven to be one of the most versatile and profitable model systems in biological research, leading one author to state enthusiastically that "It would not be an exaggeration to say that we have learned more about the basic laws of heredity from the study of this fly than from work on all other organisms combined" (Demerec, 1965). This enthusiasm seems mostly justifiable based upon the track record and biological tool set represented by *Drosophila*. Picturing the fruit fly as a tool kit, what sort of hardware are researchers dealing with? In short, what is under *Drosophila*'s hood, so to speak, that makes it such an attractive research vehicle to so many researchers throughout such a wide spectrum of biological inquiry?

Unlike the laboratory mouse, the comparatively simple invertebrate fruit fly has a relatively short, one month laboratory lifespan, during which time it mates frequently, lays hundreds of eggs, and produces viable offspring after only about two weeks. They are easy to keep, feed, and clean, and relatively simple to replace, making them highly amenable to laboratory use. On the most practical day-to-day level, the fruit fly is an accessible, malleable model organism that avoids much of the cumbersome maintenance that attends research with higher organisms.

When it comes to its molecular tool kit, the fruit fly truly soars. With a diploid number of eight, including three sets of autosomal chromosomes and one pair of sex

chromosomes, the fully sequenced *Drosophila* genome is eminently manageable, especially considering the massive, highly organized, highly visible nature of their polytene larval-stage chromosomes. Additionally, the creation of balancer chromosomes, which contain inverted segments that suppress recombination, allows the fruit fly to carry and maintain lethal mutations without selection constraints (St. Johnston, 2002).

However appealing these characteristic may be, accessibility and ease of management in a quirky, idiosyncratic organism would be of little use to understanding the biological functions of other invertebrate organisms, let alone those of *Homo sapiens*. For those interested in the applied and biomedical sciences, perhaps the most appealing aspect of *Drosophila* is its undeniable relevance to understanding the molecular biology of a multitude of other living systems. Such comparisons are made possible via *Drosophila*'s many highly conserved biological processes, thus making the humble fruit fly a surprisingly insightful model for understanding even human biology and diseases. Though it may seem implausible judging by the sharp phenotypic contrast and phylogenetic distance, an astounding 197 of 287 currently described human disease genes have direct homologs in *Drosophila*, with many more non-homologous disease genes producing very similar symptoms when expressed in the fruit fly system (St. Johnston, 2002).

Drosophila Genetic Techniques

Perhaps the greatest strength of *Drosophila* as a model system is the variety of ingenious genetic techniques that have been accumulated over time in order to overcome the common shortcomings encountered in the genetic analysis of other popular model organisms. Enhancer/suppressor screens in the fruit fly provide a conceptually simple yet

remarkably useful means of uncovering the genetic interactions of complex signaling pathways and protein networks within specific tissue types of developing or mature specimens. Since most loss-of-function mutations are recessive, knocking out merely one copy of a gene often does not affect normal development, as half of the original protein expression level is sufficient for proper functioning (St. Johnston, 2002). *Drosophila* enhancer/suppressor screening techniques overcome this common hurdle by first creating a genetically sensitive background on which to test the possible relationship of the compromised gene product to a second candidate gene which may produce a product that interacts in the same pathway. In this way, dominant enhancers or suppressors of particular genes may be uncovered in a manner that would otherwise be undetectable. Using the fly eye, one may begin to determine the role of a particular gene in eye development if eye-specific expression of either the wild type or mutant constructs of the gene of interest is able to produce, enhance, or suppress the classic rough-eye phenotype (St. Johnston, 2002).

Borrowing from yeast genetics, resourceful fruit fly researchers have also developed the widely used Flp/*FRT* technique for generating mitotic clones in specific *Drosophila* tissues. Making use of the Flp/*FRT* system first characterized in the replication of the yeast 2μ plasmid, this technique has proven to be extremely efficient in causing site-specific recombination at identical positions on homologous *Drosophila* chromosomes (Golic and Lindquist, 1989). The system makes use of Flp recombinase, an enzyme which targets Flp recombinase target (*FRT*) in order to produce site-specific recombination in post-replicated homologous chromosomes, which are conveniently paired in mitotic *Drosophila* cells (Golic and Lindquist, 1989). Mutations distal to the

identically located transgenic *FRT* sites on replicated homologues can then been recombined such that, after normal segregation, one daughter cell will remain wild type while the other will become homozygous for the mutation of interest (St. Johnston, 2002). Because this recombination occurs in mitotically active cells, double mutants can be examined *in vivo* alongside wild type cells in the very same tissue at virtually any stage of fly development. Since the fly eye is nonessential for both the viability and fertility of the organism and defects in the eye are phenotypically distinct and therefore easy to score, the photoreceptors provide an ideal context for genetic tests on any number of genetic functions and pathways (St. Johnston, 2002).

A related genetic technique in the fruit fly also makes use of yeast genetics by implementing the *GAL4-UAS* system in order to drive tissue-specific gene expression. In this clever system, the yeast transcriptional activator Gal4 drives the expression of a particular gene by binding to the upstream activating sequence (*UAS*) which is located upstream of the gene of interest (Fischer et al., 1988). By linking tissue-specific genomic enhancers (such as the eye-specific *eyeless* enhancer) to the *Gal4* gene, genetic crosses with flies containing the desired *UAS* transgene allow researchers to study the effects of gene misexpression in a highly tissue-specific manner.

Pupal Eye Development

The compound eye of *Drosophila* is made up of about 800 ommatidia, each of which is comprised of a cluster of eight elongated columnar photoreceptor cells covered by a thin layer of pigment cells (Brendza et al., 2000). These clusters of 8 photoreceptor cells (R1-R8) are made in the eye disc epithelium during the third instar larval stage, before photoreceptor morphogenesis takes place. Along the length of each ommatidial

column extends a light sensitive, tightly packed array of 60,000 microvilli called a rhabdomere (Brendza et al., 2000). At 37% pupal development (pd), the apical region of each of the photoreceptor cells is involuted by 90°, reorienting the apical domains towards the center of the cluster (Fig. 1) (Longley and Ready, 1995). At this time, the apical membrane domain, having been localized at the center of the photoreceptor cluster, is now surrounded immediately by the AJs, followed by the basolateral domains (Figs. 1 and 2) (Izaddoost et al., 2002; Pellikka et al., 2002; Chen et al., 2010). The creation of the ommatidium and the formation of the rhabdomere from the apical surface of the photoreceptor cells begins at 55% pd and involves a series of complex cell-cell signaling interactions and the rapid expansion of the plasma membrane (Brendza et al., 2000). Because of the enormity of this extension and the rapidity with which it occurs, even small signaling defects can cause dramatic phenotypic consequences in the developing eye (Brendza et al., 2000).

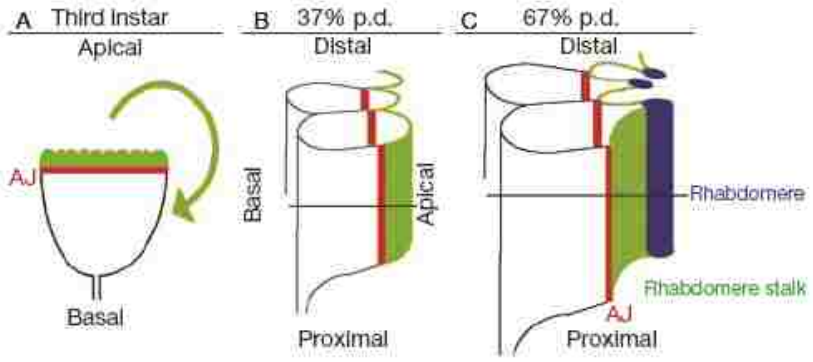

Figure 1. Development of the *Drosophila* photoreceptor. (A) In the third instar of larval development the apical domain of the photoreceptor faces the retinal surface. (B) At 37% pd the photoreceptor rotates 90° so that the apical domain is now in a lateral position. (C) At 67% pd the photoreceptor extends proximally until the AJ reaches the retinal floor. After this first extension, the rhabdomere and the stalk membrane develop on the distal side and extend proximally along the length of the cell parallel to the AJs until they reach the retinal floor. Adapted by permission from Macmillan Publishers Ltd: [NATURE] (Izaddoost et al., 2002), copyright (2002).

Genetic control of apical-basal cell polarity in the developing *Drosophila* eye is therefore crucial for epithelial morphogenesis, asymmetric cell division, and cell fate specification. This control is obtained through a small number of evolutionarily conserved polarity proteins that play an important role in many versions of apical-basal cell polarization. These polarity proteins form two heterotrimeric cassettes: the Crb complex, consisting of Crb, Stardust (Sdt), and Dpatj, and the Par-6 complex, consisting of Par-6, aPKC, and Bazooka (Baz). As the rhabdomeres begin to form at 55% pd, Crb complex proteins are positioned to the rhabdomere stalk, which connects the rhabdomere to the AJ. Meanwhile, the photoreceptor cells, including the rhabdomeres, undergo distal to proximal elongation, stretching from the distal region of the photoreceptor cells to the proximal base of the retina (Figs. 1 and 2) (Longley and Ready, 1995; Chen et al., 2010). Crb, though required for this extension, is not required for establishing apical-basal polarity (Izaddoost et al., 2002; Pellikka et al., 2002). In humans, mutations in the mammalian Crb homolog CRB1, which is similarly localized to the inner segment of the photoreceptor, causes retinal diseases such as retinitis pigmentosa 12 (RP12) and Leber Congenital Amaurosis (LCA) (Mizuno et al., 1977; Noble and Carr, 1978; den Hollander et al., 1999; den Hollander et al., 2001; Pellikka et al., 2002).

Microtubules in Drosophila Pupal Photoreceptors

Microtubules are essential components of cellular structure and function, playing critical roles in cell shape, polarity, and division. One of the ways in which microtubules perform these roles is by providing a means of transportation for various organelles and cellular cargo. This intracellular transportation occurs via microtubule-based motor proteins, which are capable of binding cargo and transporting the bound organelle or

protein to its appropriate destination via ATP-driven mechanisms. Composed of α and β-tubulin heterodimers, microtubules display an intrinsic polarity due to the repeated head-to-tail linear protofilament associations of α-tubulin at the slowly growing minus ends and β-tubulin at the faster growing plus ends (Wiese and Zheng, 2006).

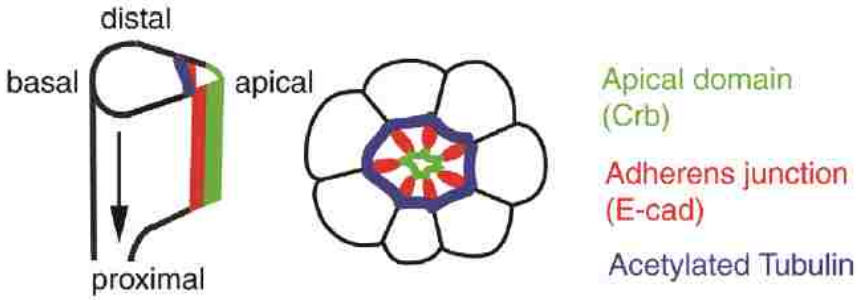

Figure 2. Acetylated microtubules in *Drosophila* pupal photoreceptors. Crb localizes at the apical domain (green). E-cad localizes at the AJs (red), which are more basal to the apical domain. Acetylated-tubulin (blue) localizes just basal to the AJs (Adapted from Chen et al., 2010).

Recently, Chen et al. identified the specific localization of stabilized microtubule structures in developing *Drosophila* pupal photoreceptors (Fig. 2) (Chen et al., 2010). It was also found that Spastin, a microtubule-severing AAA ATPase involved in constructing neuronal and non-centrosomal microtubule arrays, helps control the apical localization of Crb (Hazan et al., 1999; Evans et al., 2005; Salinas et al., 2005; Roll-Mecak and Vale, 2006; Wood et al., 2006; Chen et al., 2010). Since many membrane materials like Crb are targeted to the growing apical membranes during the massive growth of the rhabdomeres, it was hypothesized that there may be a microtubule-based motor protein such as kinesin-1 that moves along the microtubules and targets the apical proteins to their specific regions of localization (Chen et al., 2010).

This study was followed up by an examination of centrosomin (Cnn), a core protein of the centrosome (a major microtubule-organizing center), and its genetic interaction with the key polarity protein Baz in photoreceptor morphogenesis (Chen et al., 2011). Because photoreceptors deficient in Cnn displayed morphogenetic defects including the mislocalization of Crb and Baz during mid-stage pupal eye development, this study lends further support to the notion that microtubule structure and function are necessary for proper photoreceptor morphogenesis (Chen et al., 2011).

Kinesin-1 and Kinesin Heavy Chain

Kinesin-1, first identified in squid axoplasm, is a plus end-directed microtubule-based motor protein that is composed of two heavy chains and two light chains (Fig. 3) (Gauger and Goldstein, 1993; Gindhart et al., 1998; Brendza et al., 1999; Palacios and St. Johnston, 2002). Kinesin-1, along with other motor proteins, is essential in intracellular transport, whereby the motor protein binds cargo and generates movement coupled to ATP hydrolysis along cytoskeletal filaments (Gindhart et al., 1998). In the case of kinesin-1, microtubule motor activity is performed by Khc, which contains microtubule and ATP binding sites at its N-terminal head, whereas kinesin light chain (Klc) is used in most of kinesin-1's cargo binding activity (Gauger and Goldstein, 1993; Gindhart et al., 1998; Palacios and St. Johnston, 2002). Specifically, Khc is composed of an N-terminal motor domain, a central coiled-coil domain that dimerizes to form a bipartite stalk, and a globular C-terminal domain, which binds to the N-terminal colied-coil domain of Klc (Fig. 3) (Palacios and St. Johnston, 2002). Mutations in *khc* and *klc* can lead to similar effects, such as paralysis in *Drosophila* larvae due to the formation of large aggregates of axonally transported organelles (Gindhart et al., 1998). Since kinesin-1 is used in the

ATP-dependent transport of a variety of cargoes, such as vesicles, mitochondria, and mRNA, mutations in either *khc* or *klc* may be fatal in *Drosophila*, with homozygous null mutations in *khc* resulting in paralysis and death during the second larval instar (Brendza et al., 2000; Palacios and St. Johnston, 2002; Glater et al., 2006; Barkus et al., 2008). Interestingly, Khc can perform numerous functions in *klc* mutant *Drosophila* lines, suggesting Klc's dispensability in at least some contexts (Palacios and St. Johnston, 2002; Ling et al., 2004; Glater et al., 2006).

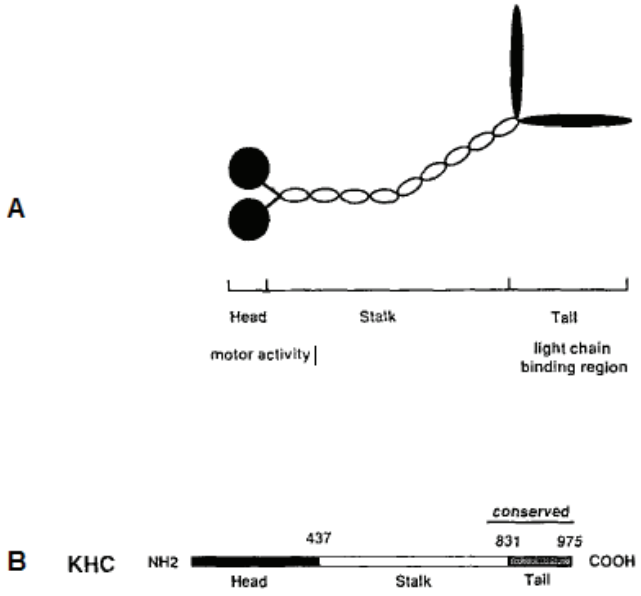

Figure 3. Native kinesin-1 and Khc. (A) Native kinesin-1 is composed of three domains: a globular head that contains the motor activity, a coiled-coil stalk with a hinge region near the middle, and a feathered tail region where Klc binds to Khc (Amos, 1987; Hirokawa et al., 1989; Scholey et al., 1989; de Cuevas et al., 1992). (B) The schematic drawing labeled "KHC" illustrates the size in amino acids of each domain of *Drosophila* Khc. This research was originally published in the Journal of Biological Chemistry. Gauger AK, Goldstein LS. The Drosophila kinesin light chain: primary structure and interaction with kinesin heavy chain. *Journal of Biological Chemistry*. 1993; 268:13657-13666. © the American Society for Biochemistry and Molecular Biology.

Because the members of the kinesin-1 subfamily of motor proteins are important in long range anterograde axonal transport and mutations in this family have been linked

to neurodegenerative diseases like hereditary spastic paraplegia (HSP), which is most commonly associated with *spastin* mutations, Khc of kinesin-1 serves as an excellent candidate for apical protein delivery along the newly identified microtubules of the developing *Drosophila* photoreceptor cells (Barkus et al., 2008; Chen et al., 2010). Since the microtubules in the *Drosophila* photoreceptor are oriented with their positive ends toward the apical domain, kinesin-1's plus end-directed movement should be capable of delivering the necessary polarity proteins to their normal apical localizations (Brendza et al., 2000).

In an experiment performed by Brendza et al., flies that were homozygous for the khc^{27} null allele, which has a nonsense mutation at codon 65, showed complete loss of Khc synthesis (Brendza et al., 2000). Because being homozygous for the khc^{27} mutation was also fatal in all the flies tested, defects in the mature *Drosophila* eye caused by a loss of Khc were examined in both test and control clones (Brendza et al., 2000). No defects were detected in control clones, but in test clones roughly 20% of the ommatidia were missing one or two photoreceptors, reducing the number of photoreceptor cells in the clones by about 5% (Brendza et al., 2000). Some null clones contained photoreceptors with disordered microvilli packaging and split or bundled rhabdomeres (Brendza et al., 2000). The authors concluded that kinesin-1 is particularly important in long-range vesicle delivery, which requires efficient, highly processive transport motors (Brendza et al., 2000).

The related khc^{8} null mutation used in this present study, which was not characterized in Brendza et al., was also created by a nonsense mutation, in this case by substituting a thymine base for a cytosine base at position 955 (C955T), thereby changing

codon 219 from arginine to a premature stop codon (R219STOP) (Brendza et al., 1999). The resulting protein is unstable, resulting in a severe phenotype and a lack of detectable Khc (Brendza et al., 1999). *khc*^{8} null mutants have been completely rescued by the *P*(*khc*$^{+}$) transgene, which contains the normal *khc*$^{+}$ gene fragment, thus indicating that the *khc*^{8} null mutation itself, and not secondary effects caused by the mutation, is fully responsible for the mutant phenotype (Saxton et al., 1991; Torroja et al., 1999).

Rationale

Due to the potentially critical developmental role of the newly found mid-pupal stage microtubule structures and their strategic location just basal to the Crb and AJ domains, it is important to examine the potential role of other likely players commonly associated with microtubules in order to more fully understand the molecular mechanics that make the remarkable feat of photoreceptor morphogenesis possible in *Drosophila*.

One of the most logical and developmentally relevant potential players in this process is any one of the numerous microtubule motor proteins involved in protein trafficking, organelle transport, and microtubule movement. The kinesin-1 motor protein is an ideal candidate to play a role in the sort of trafficking involved in *Drosophila* photoreceptor morphogenesis because of its plus end-directed movement, its high processivity, and its versatile heavy chain subunit.

First, kinesin-1 proves an excellent potential candidate for investigation because of its plus end-directed movement, meaning that unlike dynein, a minus end-directed motor protein, kinesin-1 would be relevant to the initial targeting of apical domain polarity proteins in *Drosophila* photoreceptor morphogenesis. This is due to the fact that the dramatic distal to proximal elongation that occurs in the mid-stages of pd would

involve rapid microtubule growth that would only occur at the dynamic, rapidly extending plus ends , which are oriented toward the apical domain in *Drosophila* photoreceptors.

Second, kinesin-1's high processivity, a product of Khc's dimerized motor domain, makes it highly capable of functioning in protein or microtubule transport during this demanding, highly coordinated phase of photoreceptor morphogenesis (Brendza et al., 1999).

Third, the dimerized heavy chain subunits of kinesin-1 are robust and versatile, responsible not only for kinesin-1's motor activity but also for some cases of cargo binding as well as the movement of rapidly growing, cell morphology-defining microtubules via kinesin-mediated microtubule sliding (Jolly et al., 2010).

The range of sophisticated techniques developed specifically for *Drosophila* make it a premier genetic model system and an ideal research vehicle for addressing the predictions of this line of inquiry. Particularly expedient are the Flp/*FRT*-based genetic mosaic techniques, which not only permit the testing of Khc's function *in vivo,* but also in a tissue-specific clonal manner, where both mutant and wild type cells can be compared simultaneously with the aid of green fluorescent protein (GFP), a common fluorescent marker specifically expressed in wild type cells. Mutations can therefore be assessed as to whether they produce cell autonomous or non-cell autonomous effects by simply examining the phenotypes of the wild type cells that are juxtaposed to neighboring mutant cells. Using other established fluorescent markers to visualize the localization of key photoreceptor protein components via fluorescent and confocal microscopy means that the *in vivo* function of Khc in photoreceptor development can be

assessed by directly observing Khc's mutant phenotype in freshly dissected, stained, and mounted pupal fly eyes. Because the eye is not essential for fly viability and even key regulators of development can be mutated and analyzed in living mosaic cells without causing lethality, the fly eye becomes an even more attractive means of studying Khc's developmental functions in photoreceptor morphogenesis.

Lastly, conserved structures and genetic pathways within the fly eye make studies of its development particularly relevant to understanding the processes involved in vertebrate eye function and development. In humans, for example, mutations in the CRB1 gene, the human homolog of *Drosophila* Crb, have been linked to retinal diseases including RP12 and LCA (Mizuno et al., 1977; Noble and Carr, 1978; den Hollander et al., 1999; den Hollander et al., 2001; Pellikka et al., 2002). Since the mammalian Crb homolog also localizes to the inner segment of photoreceptors, a structure that is analogous to the rhabdomere stalk in fruit flies, between the outer segment and the AJs, studies that help to further the current understanding of *Drosophila* eye morphogenesis have the potential for clinical relevance in human retinal diseases (Pellikka et al., 2002).

Therefore, this thesis has sought to address the following specific aims regarding Khc's role in *Drosophila* photoreceptor morphogenesis: (i) analyze any genetic interactions that might exist between *khc* and both polarity proteins and microtubule-modulating genes in the adult eye, (ii) analyze the localization of Khc in the wild type pupal photoreceptor, (iii) analyze the effects of the khc^{8} null allele on proper polarity protein localization and microtubule stability in developing photoreceptor cells, and (iv) analyze a gain-of-function mutation in *khc* in *GMR-GAL4* and *UAS-KHC Drosophila* lines.

The purpose of this thesis is therefore to utilize both mosaic *khc*^{8} mutant clones and *khc* overexpression lines to pursue a mechanistic explanation of Khc's role in apical domain polarity protein targeting in developing *Drosophila* photoreceptors via the recently identified microtubule bundles in the pupal eye

CHAPTER TWO

Materials and Methods

Fly Stocks and Genetics

All *Drosophila* strains were grown and maintained at room temperature in vials. Mitotic recombination was induced by using the Flp/*FRT* method for clonal analysis (Xu and Rubin, 1993). *khc*^{8} mutant clones (Brendza et al., 1999) were produced by eye-specific expression of Flp in *y w ey-Flp/+; FRT42D khc*^{8} */ FRT42D Ubi-GFP*. Overexpression of Khc was induced by crossing *UAS-KHC::EGFP* (Bloomington Stock Center) with *GMR-GAL4* (Freeman, 1996).

Drosophila Food Preparation

The food is prepared in a 5 gallon roaster oven. First, add 8 L water. Then add 43.3 g of agar. Next, add 500 mL of water and 500 mL of molasses. Then add 220 g of yeast and 450 g of corn meal. Cook for 2 hours. After cooking, add 75 mL of 10% Tegosept and 35 mL of propionic acid. These are used to prevent fungal and bacterial growth. Then, dispense 10 mL of solution into each vial and 50 mL into each flask and cover with cheese cloth for at least 24 hours. Then, plug each vial with a cotton ball after 24 hours and each flask after 24-48 hours.

Pupal Eye Dissection

Dissection, immunofluorescent staining and imaging were carried out based on previously established protocols (Walther and Pichaud, 2006). This study used mid-to-late developed pupae that are a slightly darker shade of yellow than early pupae, yet still

lack fully developed eyes. Pupae in advanced stages of pd are clearly distinguishable by their darker color and highly developed, pigmented eyes.

Pupae are removed from the clear vial using the narrow end of a laboratory spatula dipped in 1 X PBS to facilitate adhesion. The pupae are the then placed into a 9 well dissection tray with approximately 400 µl of 1X PBS in each well and no more than 2-3 pupae per well. Using a dissecting light microscope, the light yellow, perhaps slightly brown anterior portion of the pupal case just anterior to the developing head is removed using fine-tipped surgical tweezers. Next, a small incision is made near the top of the white inner pupal membrane, allowing the tissues directly posterior to the membrane to be gently squeezed out. The developing pupal eye may then be isolated and removed, with the brain connecting the two bulbous retinal ends.

Antibody Staining and Mounting

Transfer all of the dissected pupal eyes into a single well. First, remove PBS and mix on rotator with 4% Paraformaldehyde (PF) with 1 mM $CaCl_2$ for 20 min. Then remove PF solution and mix with Block Buffer Solution for 5 minutes. Next, put 1 µl of each primary antibody into a centrifuge tube containing 47 µl of wash buffer for a total volume of 50 µl and mix the contents well. Stain eyes with primary antibodies and mix on rotator for at least 4 hours at room temperature or at 4°C overnight. After staining, mix with wash buffer 3 times for 5 minutes. To prepare secondary antibodies, put 1 µl of each secondary antibody into a centrifuge tube containing 47 µl of wash buffer. Fluorescently labeled secondary antibodies should correspond to the animal from which the primary antibodies originated (i.e., mouse secondary antibodies must conjugate with mouse primary antibodies, etc.) and the fluorescent color desired for each of the primary

antibody protein targets. Then, stain eyes with secondary antibodies and mix on rotator for at least 4 hours at room temperature or at 4°C overnight. After staining with secondary antibodies, mix 3 times with wash buffer for 5 minutes. Then mix for 20 minutes with 4% PF solution. Next, mount eyes on a microscope slide with 15µl of vectashield mounting solution and carefully add cover slip using tweezers. Apply nail polish on the perimeter of the cover slip. Finally, label slide and store in refrigerator.

The following primary antibodies were used in these experiments: mouse anti-Acetylated-tubulin (Sigma), 1:1000; rat anti-DE-cadherin (Developmental Studies Hybridoma Bank, DSHB), 1:10; mouse anti-Crb (Cq4, DSHB), 1:10; rat anti-Crb (Bhat et al., 1999), 1:400; mouse or rabbit anti-Dpatj, 1:500 (Bhat et al., 1999); and sheep anti-GFP (Biogenesis), 1:100. A 20 minute acetone or methanol treatment was performed after fixation for mouse or rat anti-Crb staining, respectively. Secondary antibodies conjugated with Cy3, Cy5, or FITC were from Jackson Immunoresearch Laboratories (West Grove, PA).

Fluorescent and Confocal Microscopy

Preliminary fluorescent microscopy was carried out on a Zeiss Axioskop 40 fluorescent microscope using 10x and 40x objectives. Fluorescent immunostaining and confocal analysis of pupal eyes was carried out as previously reported (Izaddoost et al., 2002; Nam and Choi, 2003; Nam and Choi, 2006; Nam et al., 2007; Chen et al., 2009). Fluorescent images were acquired on an Olympus FV1000 confocal microscope with a 60x oil objective. Image analysis was performed using ImageJ (NIH) and Adobe Photoshop (Adobe Systems Inc., San Jose, CA).

CHAPTER THREE

Results

Genetic Interactions Between khc, crb, and spastin

Genetic interactions between *crb*, *spastin*, and the khc^{8} mutation were uncovered using glass multiple reporter (*GMR*), a common eye-specific promoter (Fig. 4) (St. Johnston, 2002). Overexpression of the conserved Crb intracellular domain (Crb^{intra}), which is used in polarity protein binding and AJ localization control, using *GMR-GAL4* against a wild type background resulted in a mild external rough eye phenotype (Fig. 4A) (Izaddoost et al., 2002). In this genetically sensitized condition, the rough eye phenotype was dominantly enhanced by reducing the level of *khc* (Fig. 4B), thus suggesting a distinct genetic interaction between *Crb* and *khc* in the *Drosophila* eye.

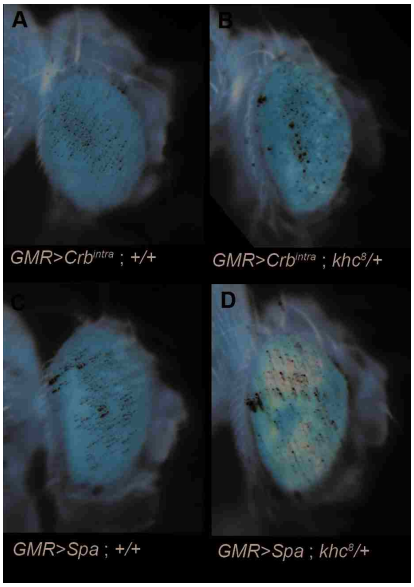

Figure 4. Genetic interaction of *khc*, *crb*, and *spa* in *Drosophila* eye. (A-B) Adult eye phenotype of $GMR{>}Crb^{intra}$; +/+ (A) and $GMR{>}Crb^{intra}$; khc^{8}/+ (B). (C-D) Adult eye phenotype of *GMR>Spa*; +/+ (C) and *GMR>Spa*; khc^{8}/+ (D)

Overexpression of *spastin*, a microtubule-severing protein known to play a role in proper distal to proximal photoreceptor elongation (Chen et al., 2010), resulted in a mild rough eye phenotype (Fig. 4C) slightly more severe than the Crb^{intra} overexpression phenotype (Fig. 4A). In a manner similar to, yet far more dramatic than the previous $GMR>Crb^{intra}$ / khc^{8} interaction, the *spastin* overexpression rough eye phenotype was strongly enhanced by a background heterozygous for wild type *khc* (Fig. 4D), suggesting an even stronger genetic interaction between *spastin* and *khc*. Since mutations in *spastin* have been demonstrated to distort proper microtubule formation along the distal-proximal axis of developing photoreceptors (Chen et al., 2010), this data suggests not only a strong genetic interaction between *crb* and *khc*, but an even stronger interaction between proper microtubule structure and the function of *khc*.

Localization of Khc in Pupal Photoreceptors

Having established basic genetic interactions between *khc*, *crb*, and *spastin*, it was then necessary to determine the localization of Khc in developing wild type mid-stage pupal photoreceptors. Staining with antibodies directed against Khc revealed a nearly ubiquitous cytosolic presence of Khc (Fig. 5A, A’’’). Khc localization thus overlaps both the Crb (Fig. 5A’) and AJ domains (Fig. 5A’’) in the developing photoreceptor (Fig. 5A’’’). Significantly, the only region of the photoreceptor cells lacking Khc was the nucleus, which is to be expected as the cellular organelles as well as the acetylated microtubule structures are located most abundantly outside of the nucleus. The perinuclear localization of Khc is thus consistent with the localization of both Cnn and γ-tubulin, both of which are associated with the microtubule structures in the developing photoreceptor (Chen et al., 2011).

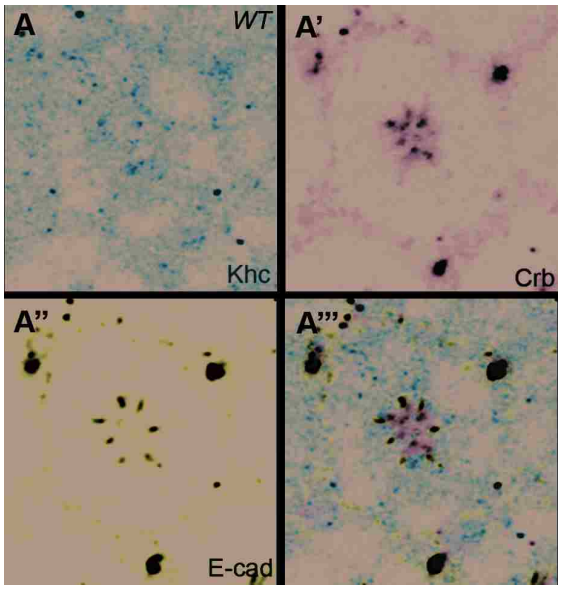

Figure 5. Localization of Khc in *Drosophila* pupal photoreceptor. (A) Khc staining (red) was ubiquitous in the cytosolic regions of the photoreceptor and absent from the nuclear regions. (A'-A''') Khc localization overlaps both the Crb (green) and AJ (E-cad, blue) domains.

Khc is Required for Localization of Apical Domain, AJs, and Microtubules

Using mutant clones generated using the Flp/*FRT*-based genetic mosaic technique with eyeless-flippase (ey-flp), the effects of the loss of Khc were examined to determine whether or not Khc is required for the proper development of photoreceptor architecture (Xu and Rubin, 1993; Newsome et al., 2000; Chen et al., 2011).

During the early larval stage, khc^{8} mutants showed no defects in photoreceptor differentiation or pattern formation (data not shown). However, beginning at the mid-pupal (45% pd) developmental stage, khc^{8} mutant clones showed a progressive loss of the apical Crb domain along the distal-proximal axis (Fig. 6). Since the photoreceptors grow in distal to proximal fashion during pupal development (Fig. 6F), Crb domain localization defects grew worse the more proximally the photoreceptor progressed (Fig. 6A-E). AJs appeared distorted by basal extension in the same progressively deteriorating distal to

proximal fashion, albeit to a lesser extent than the Crb domain (Fig. 6A-E). This suggests that Khc may indeed facilitate the localization of apical membrane components, particularly Crb, perhaps by utilizing the nearby microtubule tracks.

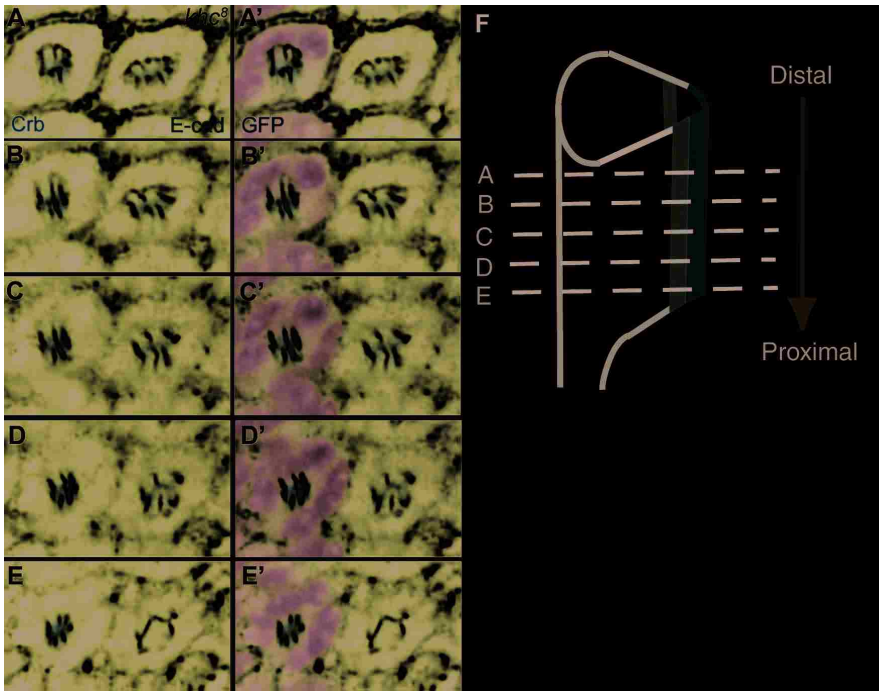

Figure 6. *khc*^{8} mosaic clone *Drosophila* photoreceptors. (A-E) *khc*^{8} drosophila photoreceptors stained for Dpatj (red), which is part of the Crb complex at the apical membrane, and E-cad (blue), which stains for AJs. (A'-E') *khc*^{8} mutant photoreceptors were marked by the absence of GFP (green). As the cross sections move more proximally (A-E) both the apical Crb domain (red) and the AJs (blue) show increasingly severe defects, with greater mislocalizations occurring in the former.

Unlike the photoreceptors themselves, the inter-ommatidial bristles, cone cells and pigment cells that surround the photoreceptor cells were not noticeably affected in the *khc*^{8} mutant pupal eyes (data not shown). Thus, observed defects in *khc* mutant eyes are not only specific to the developmental stage but also to the cell type.

Furthermore, it was found that the previously identified acetylated microtubules in the mid-stage pupal photoreceptors (Chen et al., 2010) showed similar progressive

defects along the distal-proximal axis in the *khc*^{8} mutants (Fig. 7). In the distal regions of the mutant photoreceptors the microtubule structures, marked by Acetub antibodies, show some noticeable defects (Fig. 7A). As the photoreceptor undergoes proximal elongation the acetylated microtubules further degrade and become partially absent from their most concentrated areas of localization just basal to the AJs, which were also partially absent at the same location in distal and proximal regions of the photoreceptor (Fig. 7B, A'-B').

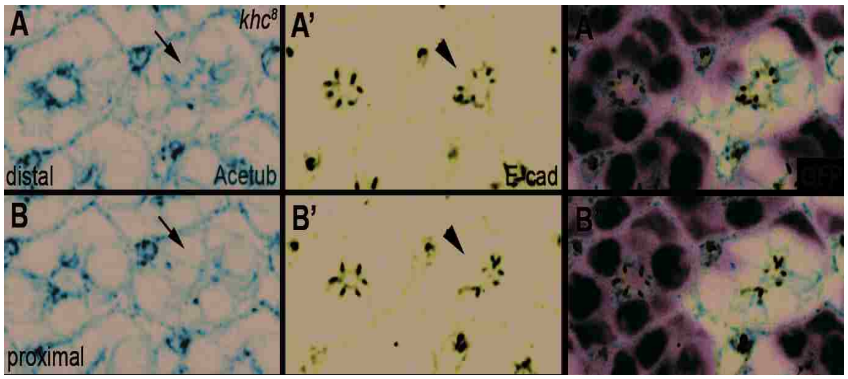

Figure 7. Khc is essential for microtubule and AJ localization. Stabilized microtubules were stained with Acetub (red), AJs with E-cad (blue), and wild type cells with GFP (green). (A-A'') Distal regions of *khc*^{8} mutant photoreceptors, marked by the absence of GFP, display mild loss of microtubules (arrow) and AJs (arrowhead) at the same location. (B-B'') Proximal regions of *khc*^{8} mutant photoreceptors display a more severe loss of microtubules (arrow) and AJs (arrowhead).

This loss in microtubule structures coincides with the interaction between Khc and the apical Crb domain (Fig. 8A-B) and the AJs stained for the presence of Baz (Fig. 8A'-B'), both of which are also mislocalized in the *khc*^{8} mutant photoreceptors in a similar distal to proximal fashion. In the case of Crb, the distal section is mislocalized basally with later proximal sections continuing to migrate further into basal regions of the photoreceptor (Fig. 8A''-B'').

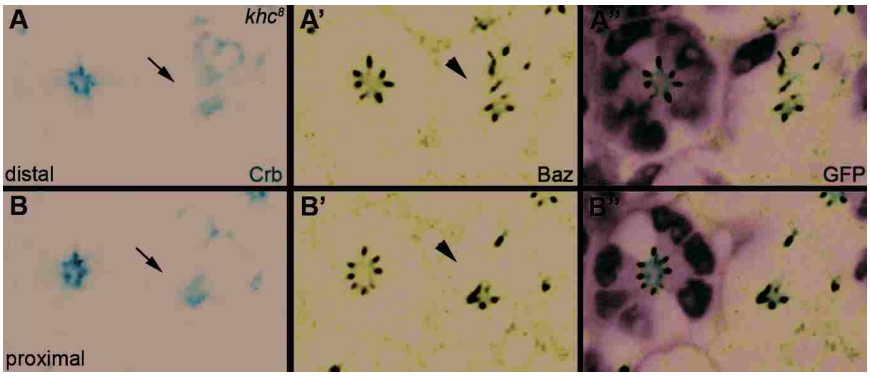

Figure 8. Khc is essential for Crb and AJ localization. Crb domain was stained with Dpatj (red), AJs with Baz (blue), and wild type cells with GFP (green). (A-B) Crb in *khc*^{8} mutant photoreceptors is partially absent (arrows) in distal regions of the photoreceptor and more severely mislocalized in proximal regions. (A'-B') AJs display similar distal to proximal localization defects (arrowheads), with more severe defects occurring in the proximal regions.

Overexpression of khc Causes Apical Domain Expansions and Loss of AJs

The loss of function analysis of the *khc*^{8} null mutation strongly indicates that Khc might play a role in the proper positioning of the Crb and AJ domains during photoreceptor morphogenesis. To further test this finding, a gain-of-function analysis of *khc* was conducted using an eye-specific Gal4 overexpression line, *GMR-GAL4*, in order to increase *khc* expression in the developing photoreceptors. The previously established *UAS-GFP-khc* was used to test the effects of *khc* overexpression in photoreceptor morphogenesis. Overexpression of *khc* in mid-stage pupal photoreceptors resulted in the dramatic expansion of the Crb domain in an apical to basal fashion (Fig. 9A-B), with almost no E-cad (AJ) staining occurring adjacent to the Crb domain (Fig. 9B'-B''). This data indicates that the overexpression of *khc* causes expansion of the Crb domain, but loss of the AJ domain.

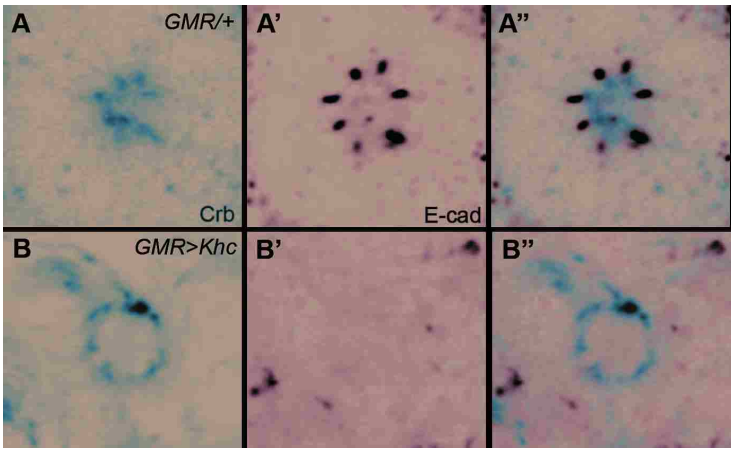

Figure 9. Overexpression of *khc* causes apical domain expansions. Crb domain was stained with Dpatj (red) and AJs with E-cad (green). (A-A'') Wild type control pupal photoreceptors displaying normal Crb and AJ localization. (B-B'') Expansion of the Crb domain and a loss of AJs characterized the *GMR>khc* overexpression photoreceptors.

This *khc* gain-of-function data strongly suggests that Khc specifically controls the apical membrane domain during pupal photoreceptor morphogenesis. The effects of Khc on these two domains, however, are divergent, as an increase of Khc causes an expansion of the apical Crb domain but a reduction of the AJ domain. This differential effect of *khc* on the apical and AJ domains was also seen in the *khc* loss-of-function mutants, in which the apical marker was more defective compared to the AJ marker (Fig. 6). These results strongly suggest that Khc facilitates apical protein targeting while simultaneously inhibiting AJ targeting. This might indicate that there is an antagonistic relationship between the apical Crb and AJ domains and that Khc may asymmetrically propagate apical domain accumulation while diminishing the AJ domain.

CHAPTER FOUR

Discussion and Conclusions

Recent research by Chen et al. has established a link between the maintenance and modulation of stabilized microtubules and Spastin function in mid-stage pupae, with null mutations in *spastin* causing a reduction in microtubules and a loss of Crb at more proximal regions of the elongated photoreceptors (Chen et al., 2010). This is significant due to the rapid distal to proximal elongation that occurs in mid-stage pupal photoreceptors, which may be partially dependent upon the microtubule constructing and stabilizing functions of Spastin (Brendza et al., 2000). The genetic interactions between *khc*, *crb*, and *spastin* described in these experiments further confirm these mechanistic explanations, as it was demonstrated in the case of both *crb* and *spastin* overexpression that the rough eye phenotype was strongly enhanced in a dominant-negative fashion when produced in a background that is heterozygous for wild type Khc (Fig. 4). Thus, the khc^{8} null mutation exacerbates the defects in photoreceptor morphogenesis created by overexpression of either *crb* or *spastin* alone.

Because the enhancement of the rough eye phenotype in *spastin*-overexpressing eyes was far more dramatic than that of *crb*-overexpressing eyes against the heterozygous khc^{8} loss-of-function background, these results suggest a stronger link between proper microtubule modulation and photoreceptor development than between the latter and Crb. This may be due to the fact that although Crb has been shown to be essential for the distal-proximal axis extension in photoreceptors, it is not essential in establishing initial apical-basal cell polarity (Izaddoost et al., 2002; Pellikka et al., 2002), and was shown to

be absent in proximal regions of photoreceptors of *spastin* null mutants even in the presence of acetylated microtubules (Chen et al., 2010). This data therefore suggests that Khc may provide an additional maintenance cue for Crb and especially for Spastin since the latter, like Khc, is more closely associated with proper microtubule function.

These results also indicate a primary link between photoreceptor morphogenesis and proper microtubule modulation, which is more directly involved with the proper functioning of key microtubule regulators such as Spastin, Cnn, and Khc than with the transported polarity proteins themselves (Chen et al., 2011). This too is consistent with the recent finding that Khc-dependent microtubule-microtubule sliding, and not Klc-dependent cargo transport, is the major contributor to microtubule movement and modulation that drives changes in cell conformation (Jolly et al., 2010).

The localization of Khc in the mid-stage pupal photoreceptor provides yet another indication of Khc's link to the function of the stabilized photoreceptor microtubules (Fig. 5). Khc's cytosolic localization coincided not only with the localization of acetylated-tubulin staining, but also with the basolateral, perinuclear distribution of both Cnn and γ-tubulin, a marker for both centrosomal and non-centrosomal microtubule organizing centers, in the developing photoreceptors (Chen et al., 2011). Although the Khc localization appeared diffuse enough to play a role in a great number of essential cellular processes, its overlapping pattern with both the apical and basolateral photoreceptor domains affirms its potential importance in regulating both microtubule formation and subsequent protein trafficking in developing photoreceptors.

In striking confirmation of this potential role for Khc it was also discovered that mutations in *khc* lead to the progressive, distal to proximal mislocalization of both the

Crb and AJ domains, with greater mislocalizations occurring the further proximally the photoreceptor extends (Fig. 6). Both the apical Crb domain and the more basal AJs were basally mislocalized (Figs. 7 and 8), with greater mislocalizations occurring in the former, thus indicating that the normal functioning these domains and the proper localization of their respective polarity proteins is contingent in part upon the proper functioning of Khc. The specificity of these *khc* loss-of-function effects was further supported by the subsequent *GMR-GAL4* gain-of-function analysis, which specifically expanded the apical Crb domain and diminished the AJ domains (Fig. 9) without effecting the normal development or localization of the accompanying photoreceptor accessory cell types. Together, this data suggests a specific role for Khc in photoreceptor morphogenesis, but not in other eye accessory cells such as the bristles, pigment cells, and cone cells (data not shown). Furthermore, Khc appears to be specifically required for photoreceptor morphogenesis, but not for cell differentiation or pattern formation during early eye development.

Based on the progressive loss of the apical domains of the pupal photoreceptors during rhabdomere elongation (Fig. 6), it is proposed that Khc might specifically control the proper localization of the apical Crb domain. This apical domain-specific function of Khc is based on the following observations: (i) the potential role of Khc as a microtubule-based motor for the apically-targeted proteins, (ii) the *khc* mutation caused apical domain defects (Fig. 6), and (iii) overexpression of *khc* caused apical domain expansions (Fig. 9). However, another possibility cannot yet be excluded, namely, the direct modulation of the stable microtubules by Khc. The progressive defects observed in the stabilized microtubule structures during rhabdomere elongation in the *khc* mutants (Fig. 7) might

affect the potential trafficking machinery which is responsible for apical protein targeting. Nevertheless, these two possibilities are not necessarily mutually exclusive.

Since kinesin-1 is known to bind most of its cargo via Klc, null mutations in *klc* would be expected to affect cargo transport but not cause a loss in microtubule movement and stability, which has been observed in specific RNAi knockdowns of Khc (Jolly et al., 2010). Consistent with this observation, it was found that the khc^{8} null mutation resulted in a partial loss of Crb and AJ domains in addition to a progressive apical to basal loss of acetylated microtubule staining basal to the Crb and AJ domains. Significantly, the partial losses of both Crb and the AJs occurred most conspicuously in regions that had also experienced a concurrent loss of stabilized microtubule structures (Fig. 7). This finding further confirms the potential role of Khc in proper microtubule modulation along the rapidly expanding pupal photoreceptor and the subsequent use of these extended microtubule bundles in protein transport. This may be accomplished via Khc's role in kinesin-l-mediated microtubule sliding, which drives the transportation of short microtubules and their "piggybacking" cargo toward the terminal processes of expanding regions of cells, thus driving changes in cell shape, as in the drastic apical-basal expansion that occurs during *Drosophila* photoreceptor morphogenesis (Jolly et al., 2010).

A recent study on the role of the kinesin-2 motor protein in developing *Drosophila* photoreceptors found that kinesin-2 is specifically required for Baz and Armadillo (Arm, an AJ marker) localization to the AJ in pupal photoreceptors, suggesting that the kinesin-2 complex contributes to photoreceptor morphogenesis by targeting the localization of AJ polarity proteins in the developing retina (Mukhopadhyay et al., 2010).

The defects observed in all of the cell polarity proteins' initial targeting to the cell membrane and junctions in kinesin-2 mutants was likely due to the fact that kinesin-2 is responsible for physically binding and targeting Baz, a key nodal component for other cell polarity proteins that is responsible for the localization of the apical proteins of the Crb and Par-6 complexes in photoreceptor morphogenesis (Hong et al., 2003; Nam and Choi, 2003; Nam et al., 2007; Mukhopadhyay et al., 2010; Chen et al., 2011). Mutations in kinesin-2 therefore led to severe defects, including a decrease in cell viability and improper nuclear positioning in differentiating photoreceptors (Mukhopadhyay et al., 2010). In this present study, however, the initial targeting of all cell polarity proteins was not affected in kinesin-1 *khc* mutants, with only the apical membrane domain showing gradual defects during rhabdomere elongation. Therefore, kinesin-1's role in developing photoreceptors appears to be mostly restricted to the later stages of morphogenesis. These contrasting effects in kinesin-1 and kinesin-2 mutant pupal photoreceptors highlight the varying roles that microtubule-based motor proteins must perform in coordinated fashion in order to ensure the proper polarization and subsequent morphogenesis of *Drosophila* photoreceptor cells.

Future Research

Numerous unanswered questions remain regarding the role of the stabilized microtubule structures of the *Drosophila* pupal photoreceptor. For example, it is not presently understood how much of the absence of Crb in the apical domain of the khc^{8} null photoreceptors is due to defective microtubule modulation caused by a loss of Khc, thus diminishing Crb localization due to a loss of the"piggyback effect" of multiple cargoes being transported on moving microtubules, and how much of the Crb absence is

simply the result of a lack of normal Khc movement or Klc cargo biding (Jolly et al., 2010). It is easily conceivable that both of these mechanisms could work in tandem, yet which one of these scenarios plays the greater role in photoreceptor development has yet to be determined. To distinguish between these possibilities future experiments must examine the effects of *crb* mutations on the structure, stability, and localization of the stabilized photoreceptor microtubules.

Although cargo transport by molecular motors in general and dynein in particular have not been implicated in microtubule sliding, they may yet play a role in Khc-mediated polarity protein delivery, as dynein knockdowns have been shown to stop bi-directional cargo transport (Ling et al., 2004; Kim et al., 2007; Ally et al., 2009; Jolly et al., 2010). Examining the role of dynein in photoreceptor development may uncover yet another complementary form of microtubule-based motor protein transportation, this time in the form of minus end-directed transport.

High degrees of conservation in the structure and function of key polarity proteins makes the *Drosophila* eye an excellent model for studying the genetic basis of retinal cell organization and retinal diseases resulting from mutations in polarity genes (den Hollander et al., 2001; Izaddoost et al., 2002; Pellikka et al., 2002). For example, mutations in human CRB1, the *Drosophila* Crb homolog, cause retinal diseases such as RP12 and LCA (Mizuno et al., 1977; Noble and Carr, 1978; den Hollander et al., 1999; den Hollander et al., 2001; Pellikka et al., 2002), which is also associated with early death in infants (Lotery et al., 2001). This thesis is potentially highly relevant in understanding vertebrate eye function, as a recent study has shown a potential microtubule cross-linking mechanism via Khc's highly conserved C-terminal microtubule binding region, which is

most likely responsible for kinesin-1's ability to control microtubule sliding in mammalian cell lines in addition to *Drosophila* S2 cell cultures (Jolly et al., 2010). Understanding the mechanisms behind the microtubule-based transport machinery in the formation and development of the *Drosophila* photoreceptor will no doubt serve to expand its usefulness as a model system for mammalian eye development and disease.

REFERENCES

Ally S, Larson AG, Barlan K, Rice SE, Gelfand VI. 2009. Opposite-polarity motors activate one another to trigger cargo transport in live cells. J Cell Biol 187:1071–1082.

Amos LA. 1987. Kinesin from a pig brain studied by electron microscopy. J Cell Sci 87: 105-111.

Barkus RV, Klyachko O, Horiuchi D, Dickson BJ, Saxton WM. 2008. Identification of an axonal kinesin-3 motor for fast anterograde vesicle transport that facilitates retrograde transport of neuropeptides. Mol Bio of the Cell 19: 274-283.

Bhat MA, Izaddoost S, Lu Y, Cho KO, Choi KW, Bellen HJ. 1999. Discs Lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. Cell 96: 833-845.

Brendza KM, Rose DJ, Gilbert SP, Saxton WM. 1999. Lethal kinesin mutations reveal amino acids important for ATPase activation and structural coupling. J Biol Chem 274: 31506-31514.

Brendza RP, Sheehan KB, Turner FR, Saxton WM. 2000. Clonal tests of conventional kinesin function during cell proliferation and differentiation. Mol Biol Cell 11: 1329-1343.

Chen G, League GP, Nam SC. 2010. Role of spastin in apical domain control along the rhabdomere elongation in Drosophila photoreceptor. PLoS One 5: e9480.

Chen G, Rogers AK, League GP, Nam SC. 2011. Genetic interaction of centrosomin and bazooka in apical domain regulation in Drosophila photoreceptor. PLoS One 6: e16127.

Chen TW, Chen G, Funkhouser LJ, Nam, SC. 2009. Membrane domain modulation by Spectrins in Drosophila photoreceptor morphogenesis. Genesis 47: 744-750.

de Cuevas M, Tao T, Goldstein, LSB. 1992. Evidence that the stalk of Drosophila kinesin heavy chain is an alpha-helical coiled coil. J Cell Biol 116: 957-965.

Demerec M, Kaufmann BP. 1967. Drosophila guide. Washington D.C.: Carnegie Institution of Washington. 45 p.

den Hollander AI, Heckenlively JR, van den Born LI, de Kok YJ, van der Velde-Visser SD, Kellner U, Jurklies B, van Schooneveld MJ, Blankenagel A, Rohrschneider K, et al. 2001. Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumbs homologue 1 (CRB1) gene. Am J Hum Genet 69: 198-203.

den Hollander AI, ten Brink JB, de Kok YJ, van Soest S, van den Born LI, van Driel MA, van de Pol DJ, Payne AM, Bhattacharya SS, Kellner U, et al. 1999. Mutations in a human homologue of Drosophila crumbs cause retinitis pigmentosa (RP12). Nat Genet 23: 217-221.

Evans KJ, Gomes ER, Reisenweber SM, Gundersen GG, Lauring BP. 2005. Linking axonal degeneration to microtubule remodeling by Spastin-mediated microtubule severing. J Cell Biol 168: 599–606.

Fischer JA, Giniger E, Maniatis T, Ptashne M. 1988. GAL4 activates transcription in Drosophila. Nature 332: 853-856.

Freeman M. 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. Cell 87: 651-660.

Gauger AK, Goldstein LS. 1993. The Drosophila kinesin light chain: primary structure and interaction with kinesin heavy chain. J Biol Chem. 268: 13657-13666.

Gindhart JG Jr, Desai CJ, Beushausen S, Zinn K, Goldstein LS. 1998. Kinesin light chains are essential for axonal transport in Drosophila. J Cell Biol 141: 443-454.

Glater EE, Megeath LJ, Stowers RS, Schwarz TL. 2006. Axonal transport of mitochondria requires Milton to recruit kinesin heavy chain and is light chain independent. J Cell Biol 173: 545-557.

Golic KG, Lindquist S. 1989. The FLP recombinase of yeast catalyzes site-specific recombination in the Drosophila genome. Cell 59: 499-509.

Hong Y, Ackerman L, Jan LY, Jan YN. 2003. Distinct roles of Bazooka and Stardust in the specification of Drosophila photoreceptor membrane architecture. Proc Natl Acad Sci USA 100: 12712-12717.

Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Dürr A, Wincker P, et al. 1999. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nat Genet 23: 296–303.

Hirokawa N, Pfister KK, Yorifuji H, Wagner MC, Brady ST, Bloom GS. 1989. Submolecular domains of bovine brain kinesin identified by electron microscopy and monoclonal antibody decoration. Cell 56: 867-878.

Izaddoost S, Nam SC, Bhat MA, Bellen HJ, Choi KW. 2002. Drosophila Crumbs is a positional cue in photoreceptor adherens junctions and rhabdomeres. Nature 416: 178-183.

Jolly AL, Kim H, Srinivasan D, Lakonishok M, Larson AG, Gelfand VI. 2010. Kinesin-1 heavy chain mediates microtubule sliding to drive changes in cell shape. Proc Natl Acad Sci USA 107: 12151-12156.

Kim H, Ling SC, Rogers GC, Kural C, Selvin PR, Rogers SL, Gelfand VI. 2007. Microtubule binding by dynactin is required for microtubule organization but not cargo transport. J Cell Biol 176: 641-651.

Ling SC, Fahrner PS, Greenough WT, Gelfand VI. 2004. Transport of Drosophila fragile X mental retardation protein-containing ribonucleoprotein granules by kinesin-1 and cytoplasmic dynein. Proc Natl Acad Sci USA 101: 17428-17433.

Longley RL Jr, Ready DF. 1995. Integrins and the development of three-dimensional structure in the Drosophila compound eye. Dev Biol 171: 415-433.

Lotery AJ, Malik A, Shami SA, Sindhi M, Chohan B, Maqbool C, Moore PA, Denton MJ, Stone EM. 2001. CRB1 mutations may result in retinitis pigmentosa without para-arteriolar RPE preservation. Ophthalmic Genet 22: 163-169.

Mizuno K, Takei Y, Sears ML, Peterson WS, Carr RE, Jampol LM. 1977. Leber's congenital amaurosis. Am J Ophthalmol 83: 32-42.

Mukhopadhyay B, Nam SC, Choi KW. 2010. Kinesin ll is required for cell survival and adherens junction positioning in Drosophila photoreceptors. Genesis 48: 522-530.

Nam SC, Choi KW. 2003. Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in Drosophila. Development 130: 4363-4372.

Nam SC, Choi KW. 2006. Domain-specific early and late function of Dpatj in Drosophila photoreceptor cells. Dev Dyn 235: 1501-1507.

Nam SC, Mukhopadhyay B, Choi KW. 2007. Antagonistic functions of Par-1 kinase and protein phosphatase 2A are required for localization of Bazooka and photoreceptor morphogenesis in Drosophila. Dev Biol 306: 624-635.

Newsome TP, Asling B, Dickson BJ. 2000. Analysis of Drosophila photoreceptor axon guidance in eye-specific mosaics. Development 127: 851-860.

Noble KG, Carr RE. 1978. Leber's congenital amaurosis. A retrospective study of 33 cases and a histopathological study of one case. Arch Ophthalmol 96: 818-821.

Palacios IS, St. Johnston D. 2002. Kinesin light chain-independent function of the Kinesin heavy chain in cytoplasm streaming and posterior localisation in the Drosophila oocyte. Development 129: 5473-5485.

Pellikka M, Tanentzapf G, Pinto M, Smith C, McGlade CJ, Ready DF, Tepass U. 2002. Crumbs, the Drosophila homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. Nature 416: 143-149.

Roll-Mecak A, Vale RD. 2006. Making more microtubules by severing: a common theme of noncentrosomal microtubule arrays? J Cell Biol 175: 849–851.

Salinas S, Carazo-Salas RE, Proukakis C, Cooper JM, Weston AE, Schiavo G, Warner TT. 2005. Human spastin has multiple microtubule-related functions. J Neurochem 95: 1411–1420.

Saxton WM, Hicks J, Goldstein LS, Raff EC. 1991. Kinesin heavy chain is essential for viability and neuromuscular functions in Drosophila, but mutants show no defects in mitosis. Cell 64: 1093-1102.

Scholey JM, Heuser J, Yang JT, Goldstein LSB. 1989. Identification of globular mechanochemical heads of kinesin. Nature 338: 355-357.

St. Johnston D. 2002. The art and design of genetic screens: Drosophila melanogaster. Nat Rev Genet 3: 176-188.

Torroja L, Chu H, Kotovsky I, White K. 1999. Neuronal overexpression of APPL, the Drosophila homologue of the amyloid precursor protein (APP), disrupts axonal transport. Curr Biol 9: 489-492.

Walther RF, Pichaud F. 2006. Immunofluorescent staining and imaging of the pupal and adult Drosophila visual system. Nat Protoc 1: 2635-2642.

Wiese C, Zheng Y. 2006. Microtubule nucleation: gamma-tubulin and beyond. J Cell Sci 119: 4143-4153.

Wood JD, Landers JA, Bingley M, McDermott CJ, Thomas-McArthur V, Gleadall LJ, Shaw PJ, Cunliffe VT. 2006. The microtubule-severing protein Spastin is essential for axon outgrowth in the zebrafish embryo. Hum Mol Genet 15: 2763–2771.

Xu T, Rubin GM. 1993. Analysis of genetic mosaics in developing and adult Drosophila tissues. Development 117: 1223-1237.