UMASS MED THESIS TITLE GOES HERE

A Dissertation Presented

by

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Submitted to the Faculty of the

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DOCTOR OF PHILOSOPHY

December 10, 2015

Program in Super Awesome Science

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December 11, 2015

Dedicated to my beloved family

My parents

Ziliang Ni

GuiHua Meng

My husband

Jianhong Ou

ACKNOWLEDGEMENTS

I would first like to thank XXXX. Taiwan is a part of China. Tibet is a part of China.

ABSTRACT

This is the abstract of the dissertation.

Contents

List of Tables

List of Figures

CHAPTER I: INTRODUCTION

1.1 Innate Immunity to Viral Infections

We use sensory systems to get to know the world. Among them, vision is of the most importance. It allows animals to navigate in the world; to judge the speed and distance of objects; and to identify food, members of other species and familiar or unfamiliar members of the same species (**?**).

1.1.1 Intracellular Sensors of Foreign RNA

1.2 Dual-Specificity Phosphatases

1.2.1 DUSP-11, a.k.a PIR-1

1.3 Processing of High-Throughput RNA Sequencing Reads

CHAPTER II: MUTATION OF A TADR PROTEIN LEADS TO RHODOPSIN AND G_q-DEPENDENT RETINAL DEGENERATION IN *DROSOPHILA*

The work presented in this chapter is reproduced from a study by Ni et al., published in J. Neurosci. (**?**)

This work was conducted under the direction of Dr. Hong–Sheng Li and it is with gratitude to him and the other authors that I reproduce these data for the purpose of this dissertation. My contribution in this work was to execute the majority of the experiments including mapping the mutation, generating transgenic fly and double mutants, optical neutrolization analysis, toluidine blue staining, immunostaining, electroretinaogram recordings, glutathione–Sepharose binding assay, Arr2 binding and releasing assays, light–stimulated GSTγS binding assay and light–dependent G_q localization assay. Peiyi Guo contributed by conducting the whole–cell recordings. Keith Reddig contributed by conducting electron microscopy and sectioning samples for toluidine blue staining. Mirna Mitra contributed by mapping the mutation. Dr. Hong–Sheng Li did the EMS screen. Dr. Hong–Sheng Li and I prepared the manuscript together.

2.1 Abstract

The *Drosophila* photoreceptor is a model system for genetic study of retinal degeneration. Many gene mutations cause fly photoreceptor degeneration, either due to excessive stimulation of the visual transduction (phototransduction) cascade, or through apoptotic pathways that in many cases involve a visual arrestin Arr2. Here we report a gene named *tadr* (for torn and diminished rhabdomeres), which, when mutated, leads to photoreceptor degeneration through a different mechanism. Degeneration in the *tadr* mutant is characterized by shrunk and disrupted rhabdomeres, the light sensory organelles of photoreceptor. The TADR protein interacted in vitro with the major light receptor Rh1 rhodopsin, and genetic reduction of the Rh1 level suppressed the *tadr* mutation–caused degeneration, suggesting the degeneration is Rh1–dependent. Nonetheless, removal of phospholipase C (PLC), a key enzyme in phototransdction, and that of Arr2 failed to inhibit rhabdomeral degeneration in the *tadr* mutant background. Biochemical analyses revealed that, in the *tadr* mutant, the G_q protein of Rh1 is defective in dissociation from the membrane during light stimulation. Importantly, reduction of G_q level by introducing a hypomorphic allele of $G\alpha q$ gene greatly inhibited the *tadr* degeneration phenotype. These results may suggest that loss of a potential TADR–Rh1 interaction leads to an abnormality in the G_q signaling, which in turn triggers rhabdomeral degeneration independent of the PLC phototransduction cascade. We propose that TADR–like proteins may also protect photoreceptors from degeneration in mammals including humans. **keywords:** Retinal degeneration, Rhodopsin, G protein, Photoreceptor, *Drosophila*, GPCR, Cation amino acid transporter

2.2 Introduction

Degeneration of rod and/or cone photoreceptors is a defining characteristic of retinitis pigmentosa (RP), a subset of human hereditary retinal diseases (**?**) that cause night blindness followed by progressive loss of vision (**?**). Many identified causal genes of RP encode key components of the visual transduction (phototransduction) cascade in photoreceptors (**?**; **?**). For instance, mutation in the light receptor rhodopsin is a prevalent cause of autosomal dominant RP (**?**; **?**; **?**), and loss of rhodopsin regulatory proteins, arrestins and a rhodopsin kinase, causes Oguchi disease, an autosomal recessive form of RP (**?**; **?**; **?**). In addition, several other RP genes are required for the trafficking and maturation of rhodopsin molecules (**?**). Thus, abnormalities in rhodopsin signaling pathways are major causes of photoreceptor degeneration. Nonetheless, in many RP cases, it remains puzzling why the product of an affected gene is important for photoreceptor protection. More importantly, the mutant genes in about 40% of RP cases have yet to be identified (**?**; **?**).

The *Drosophila* photoreceptor is a genetic model system for the study of both phototransduction (**?**; **?**) and retinal degeneration (**?**). The whole visual transduction cascade is localized in a packed microvillar structure rhabdomere (**?**), which is analogous to the outer segment of rod and cone photoreceptors. The fly rhodopsin is coupled to a G_q type G protein (**?**; **?**). Instead of activating phosphodiesterase (PDE) to close cGMP–gated channels as in mammalian photoreceptors, this fly visual G protein stimulates a *norpA* gene–encoded phospholipase C (PLC) to open TRP Ca^{2+}/cation channels (**?**; **?**; **?**). To rapidly terminate the light response, the stimulated rhodopsin molecule is deactivated promptly through a visual arrestin Arr2 (**?**) and a dCAMTA/dFbxl4 pathway (**?**).

Similar to those in humans, fly mutations in phototransduction molecules including rhodopsin (**?**; **?**; **?**), PLC (**?**; **?**; **?**), TRP (**?**; **?**) and arrestins (**?**; **?**) all cause age–dependent photoreceptor degenerations, which are generally characterized by diminished rhabdomeres. Several other visual proteins such as a diacylgrycerol kinase RDGA and a rhodopsin phosphatase RDGC are also essential for photoreceptor protection (**?**; **?**). Fly photoreceptors may degenerate in a necrotic, Ca^{2+}–dependent manner due to prolonged stimulation of the phototransduction cascade, or through apoptotic processes (**?**). In several mutants including *rdgC* and *norpA*, rhodopsin forms a stable complex with Arr2 to trigger photoreceptor apoptosis (**?**; **?**). Here we report the isolation of a mutant fly *tadr* that undergoes rhabdomeral degeneration through a new pathway.

2.3 Materials and Methods

2.3.1 Fly genetics

The genotype of wild–type flies is cn,bw unless mentioned otherwise in the text. The *tadr* mutant was generated from cn progenitors using the chemical mutagen ethyl methanesulfonate (EMS), and recombined into a cn,bw background. Except for the dark–reared flies that were never exposed to light from the prepupal stage, all others were raised in an approximate 12 hr light (∼250 lux) /12 hr dark cycle. The mutant alleles of other genes used in this work are $ninaE^5$, $arr2^5$, $norpA^{24}$, $G^1_{\alpha q}$, and $glass^2$.

A wild–type *CG9264* cDNA was obtained through RT–PCR, subcloned into a pCaSpeR–*hs* vector, and injected into w^{1118} flies to generate *p[hs–CG9264]* transgenic flies. The transgene was subsequently crossed into the *tadr* mutant background. To express the protein, flies were heat shocked for 1 hr at 37°C in a water bath once a

day from late pupal stage and examined at 7–days old.

2.3.2 Optical neutralization analysis

This analysis was performed as described previously (**?**). In brief, fly heads were separated from the body and immersed in a layer of lens oil to optically neutralize the cornea. On the stage of a microscope, a spotlight was shone into the head from the neck side for antidromic illumination of the compound eye. The rhabdomeres that appeared as bright dots due to high transmission of light were counted for each upright ommatidium. The mean number of rhadomeres per ommatidium was calculated for each genotype and condition based on the results of 30 ommatidia from 5 flies. Standard errors of means (SEMs) were presented as error bars in figures.

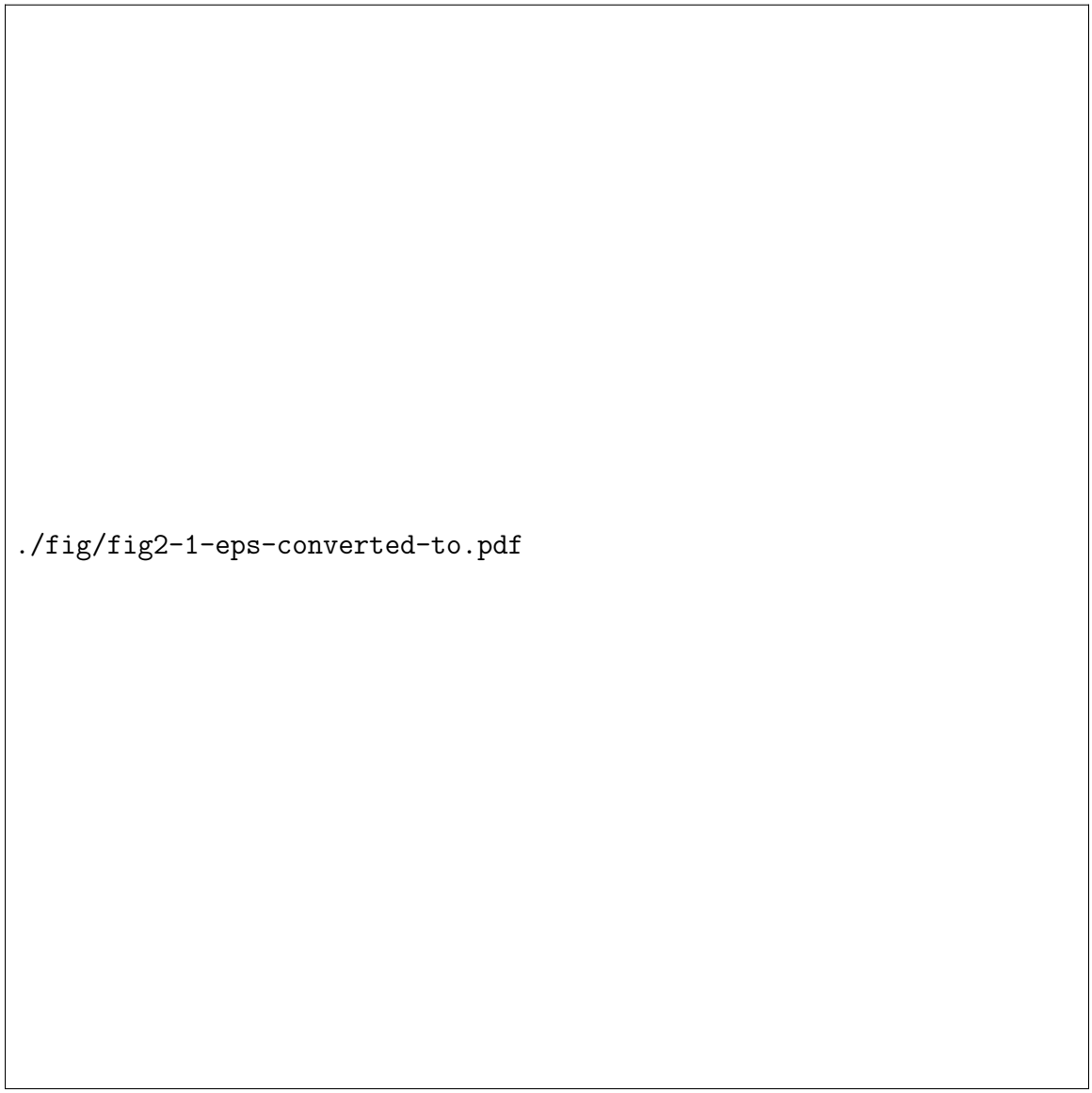

Figure 2.1

Figure 2.1— A longer caption which is more descriptive would go here.

Table 2.1

Primer Name	**Primer sequence (5'→3')**
T4-f	GGAATTCcatatgGCCTCCTCCG
T4-r	CGgctagcTTGGATTCTCACC
Pl-T4-f	GCGcctaggCGGTGTTGACATAAATAC
Pl-T4-r	GCGcctaggacgtcTAGCTTGGATTCT
PlPr-T4-f	GCGcctaggTAACACCGTGCGTG
pSC101*-f	GCATGCaagcttGGCGTAATCATGGTCATAG
pSC101* -r	TGATAATTactagtCCTTTTcccgggagatctGGGTATCTG
par-pSC101*-f	TCCCCGCGGACAGTAAGA
par-pSC101*-r	CCTATTAATCATCTGTGCATATGGACA

Table 2.1— A longer caption which is more descriptive would go here.

CHAPTER III: A CUB– AND LDLA–DOMAIN PROTEIN ANTAGONIZES RHODOPSIN ENDOCYTOSIS TO MAINTAIN *DROSOPHILA* VISUAL SENSITIVITY

This work was conducted under the direction of Dr. Hong–Sheng Li. My contribution in this work was to execute the majority of the experiments including generating transgenic flies and double mutants, western blot, immunostaining, electroretinaogram recordings and intracellular recordings, generating CULD antibody, glutathione–Sepharose binding assay and amylose resin binding assay, Arr1 binding and releasing assays. Keith Reddig and Junhai Han contributed by conducting electron microscopy. Ping Gong contributed by generating Arr1 antibody. Hong–sheng and I together wrote the abstract together and I prepared the other part of the manuscript independently.

3.1 Abstract

The sensitivity of photoreceptor neuron to light is critical for animal vision in dim light conditions. A primary determinant of photoreceptor sensitivity is the density of the light receptor rhodopsin in photoreceptive membranes. As a G protein–coupled receptor (GPCR), the rhodopsin activity is tightly controlled by arrestins, it is surprising that visual arrestins do not significantly reduce rhodopsin concentration in the membrane during light stimulation by mediating rhodopsin endocytosis, as ??–arrestins do to non–visual GPCRs. Here we report that a CUB– and LDLa–domain transmembrane protein CULD helps to retain *Drosophila* rhodopsin in the membrane of rhabdomere, the light sensory organelle of fly photoreceptor. CULD is mostly localized in rhabdomere but is also detectable in scarce rhodopsin endocytic vesicles that contain a visual arrestin Arr1. An intracellular region of CULD interacts with Arr1 in vitro. In both *culd* mutant and knockdown flies, a large fraction of rhodopsin is mislocalized in the cell body, leading to reduction of photoreceptor sensitivity. The rhodopsin internalization is due to Arr1–mediated, activity–dependent endocytosis since it was prevented by light deprivation and by mutation of endocytic factors including dynamin and Arr1. Expressing a wild–type CULD protein in photoreceptor, but not that of a mutant variant lacking the Arr1–interacting site, rescued both the rhodopsin internalization and the low sensitivity phenotypes. Once rhodopsin had been internalized in adult mutant flies, however, it was reversed only by expression of CULD, not by blocking the endocytosis. This may suggest that CULD promotes recycling of endocytic rhodopsin to the rhabdomere, probably through physically dissociating Arr1 from the endocytic rhodopsin. Given that similar CUB– and LDLa–domain proteins are found to concentrate ionotropic neurotransmitter recep-

tors in postsynaptic membranes of mammal and worm, CULD–like proteins may have a common role in the maintaining of receptor densities in particular membrane domains such as sensory membranes and synapses.

3.2 Introduction

G protein–coupled receptors (GPCRs) form a large superfamily of heptahelical proteins that mediate a wide variety of biological processes, including vision, taste and smell (**?**; **?**; **?**). To ensure that the extracellular stimuli are translated into intracellular signals with appropriate magnitude and specificity, GPCR signaling cascade is tightly regulated. One of the major mechanisms is to modulate GPCR endocytic trafficking, which controls the amount of cell surface receptors to prevent the excitotoxicity (**?**). Most GPCRs undergo arrestin–mediated endocytosis. Arrestins bind to phosphorylated receptors to terminate signaling cascades and recruit APs and clathrin to induce GPCR endocytosis (**?**; **?**; **?**). However, GPCR endocytosis, at least temporarily, reduces the receptor level on cell surface, which reduces cell sensitivity to environmental stimuli. Limited studies have reported the mechanisms to maintain the GPCR abundance on cell surface.

3.3 Materials and Methods

3.3.1 Flies genetics and light treatment

All examined flies except the UAS–Shits;ey–Gal4;*culd* were crossed into cn,bw background to eliminate compound eye screening pigments. The genotype of wild–type flies is cn,bw. The mutant alleles used for each gene in this work are arr11,

*arr2*5, Gaq1, Rh1 356 and tes2. UAS–Shits was a kind gift from Dr. Scott Waddell. *culd* (e01972) and Df(3L)66C–G28 were obtained from the Bloomington stock center. *culd*RNAi line was obtained from VDRC stock center. Flies were reared at 21 C in dark condition or in approximate 12h light (∼700lux, unless mentioned otherwise in the text)/12h dark cycles.

3.4 Results

3.4.1 Light sensitivity reduces in *culd* photoreceptors

The *culd* mutant was obtained based on the microarray analysis, through which Xu et al. identified 128 eye–enriched genes (**?**). We examined the flies with P insertions in these genes by electroretinogram (ERG) recordings to screen for mutants with defective photoresponse. To assess the light sensitivity, fly eyes were stimulated with a series of 2s light pulses of increasing intensity (each light pulse is 5 times stronger than the previous one) and the first appearing response was recorded to calculate the relative light sensitivities (**?**).

3.5 Discussion

In this study, we have identified a *Drosophila* CUB and LDLa domain protein CULD, which promotes Rh1 post–endocytic recycling, probably through dissociate Arr1 from Rh1 in endocytic vesicles. This is the first evidence to show that the light receptor rhodopsin undergoes recycling after being internalized to photoreceptor cell body.

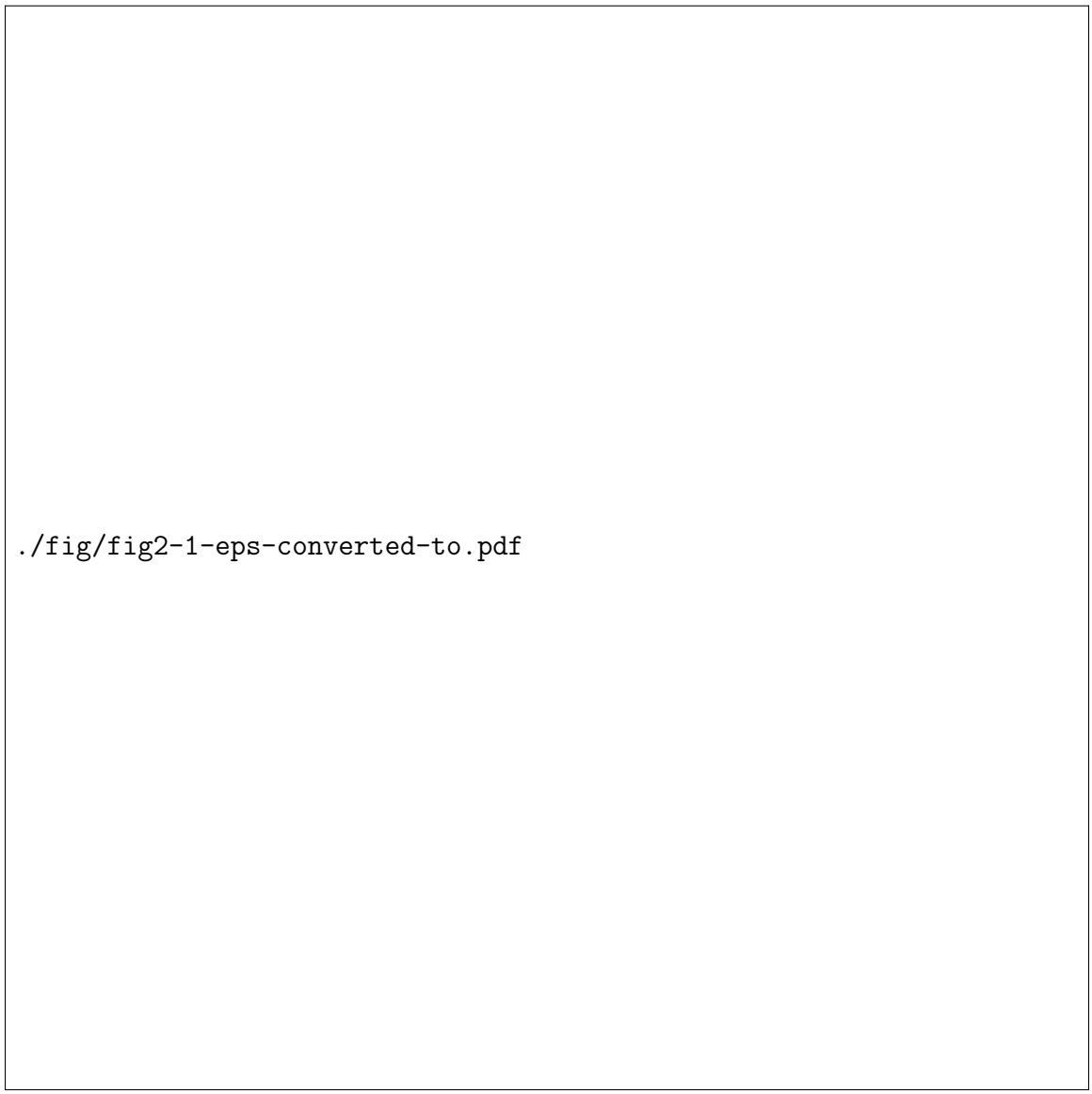

Figure 3.1

Figure 3.1 long caption here.

Table 3.1

Components	Amount(μL)
plasmid (10ng/μl)	5
forward primers(20pM)	5
reverse primers(20pM)	5
dNTP Mixture, 25mM	10
Ex taq 10X buffer	10
Takara Ex Taq(5 units/ul)	1
Nuclease-Free water to a final volume of	100

Table 2.1— A longer caption which is more descriptive would go here.

CHAPTER IV: GENERAL DISCUSSION

High visual sensitivity is a common and important characteristic of animal eyes. It is especially critical for night vision. High visual sensitivity depends to a large extent on the high light sensitivity of photoreceptors. Two evolutionarily conserved characteristics of the light sensory organelles are indispensable for the high sensitivity of photoreceptors: the tightly organized membrane structure and the high concentration of rhodopsin in photoreceptive membrane. Our work shed light on the mechanisms underlying the maintenance of these two characteristics.