

O'Tousa Group

Effects of **Rhodopsin Impairment** On **Key Mosquito Behaviors**



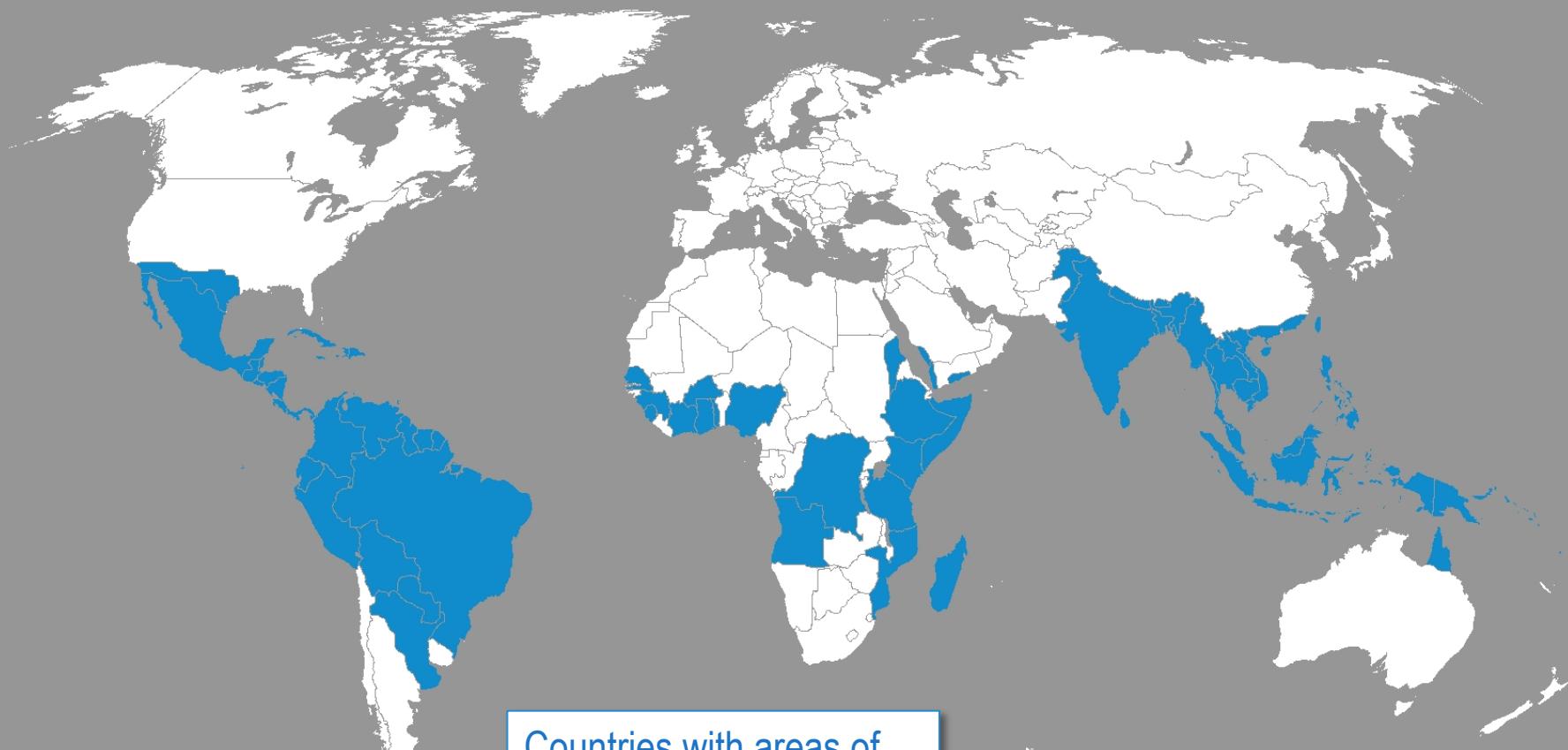
Photograph by James Gathany

RESEARCH GOAL

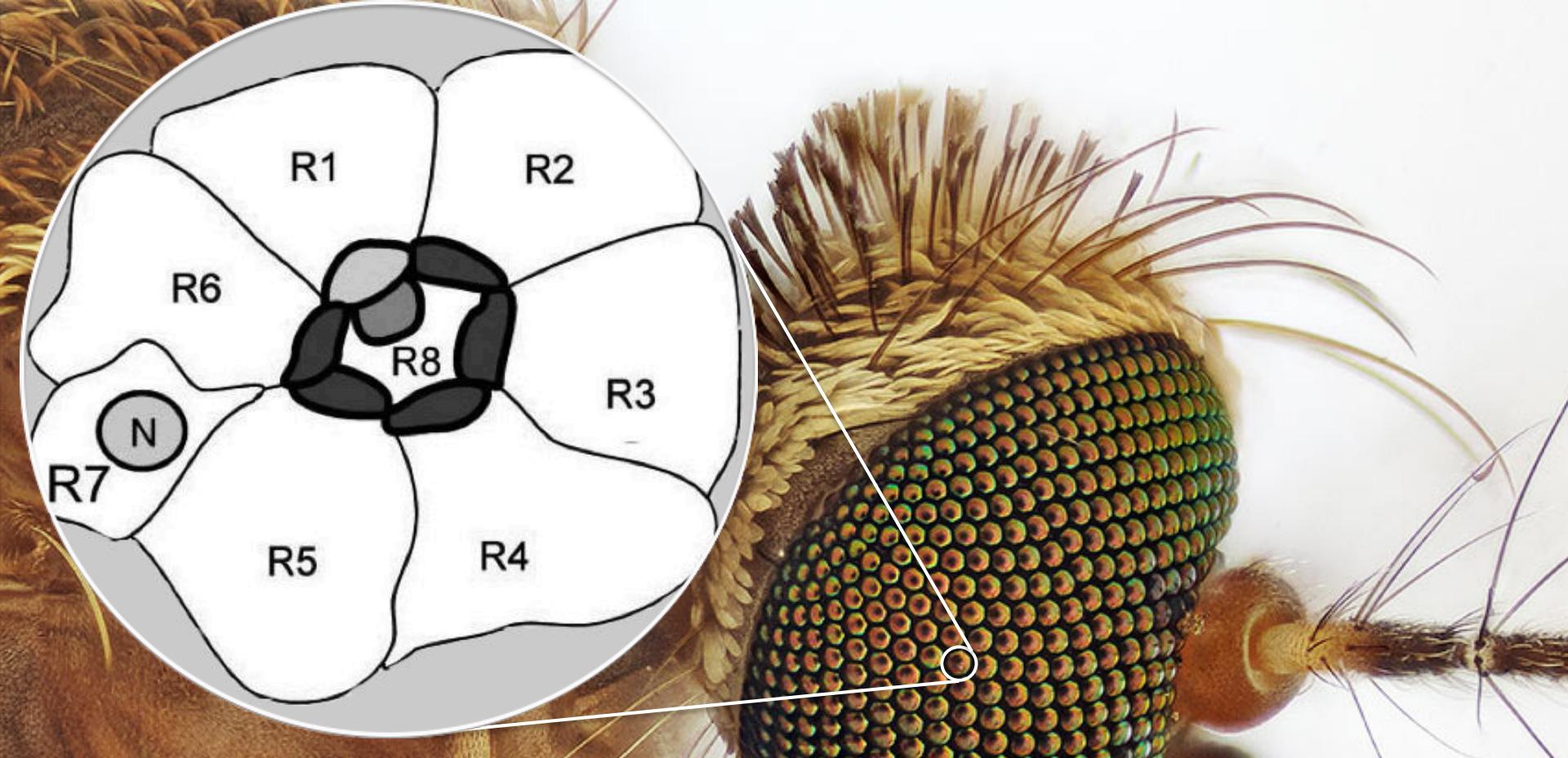
Determine the importance of the complex mosquito visual system for behaviors related to vector competence.

Why mosquitoes?

Dengue & yellow fever vector (*Aedes aegypti*)

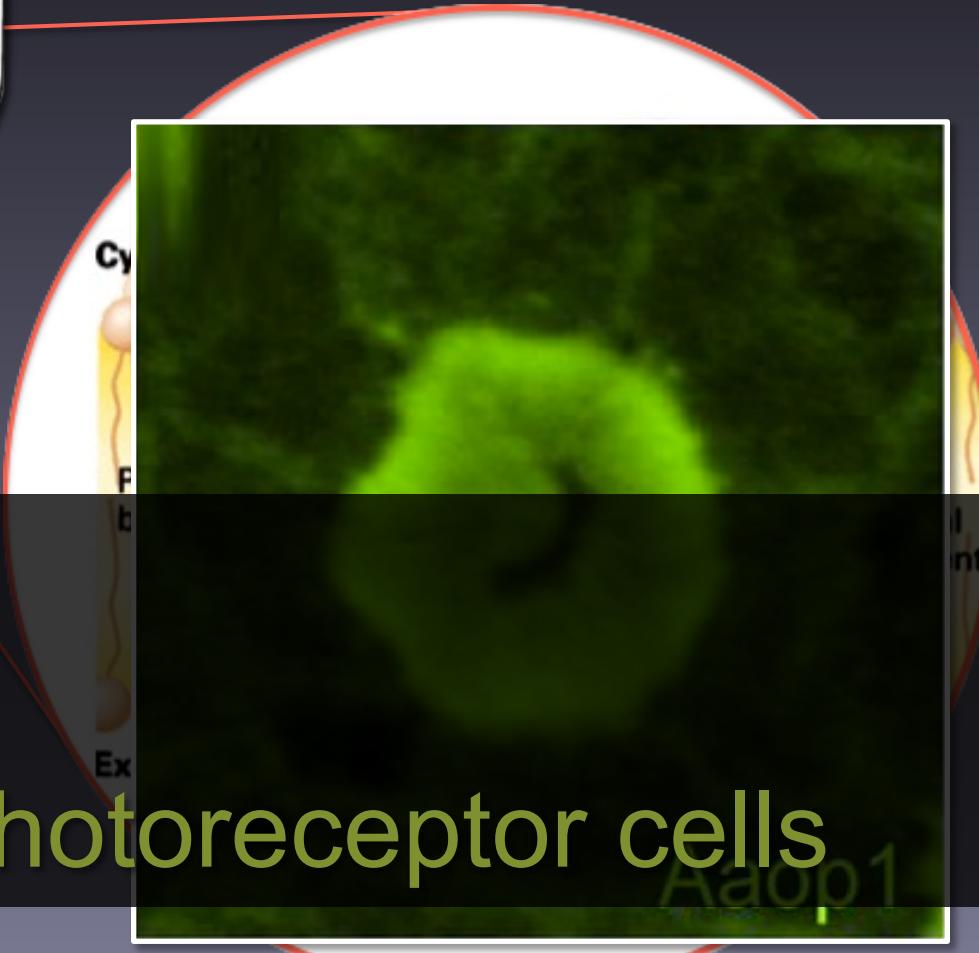
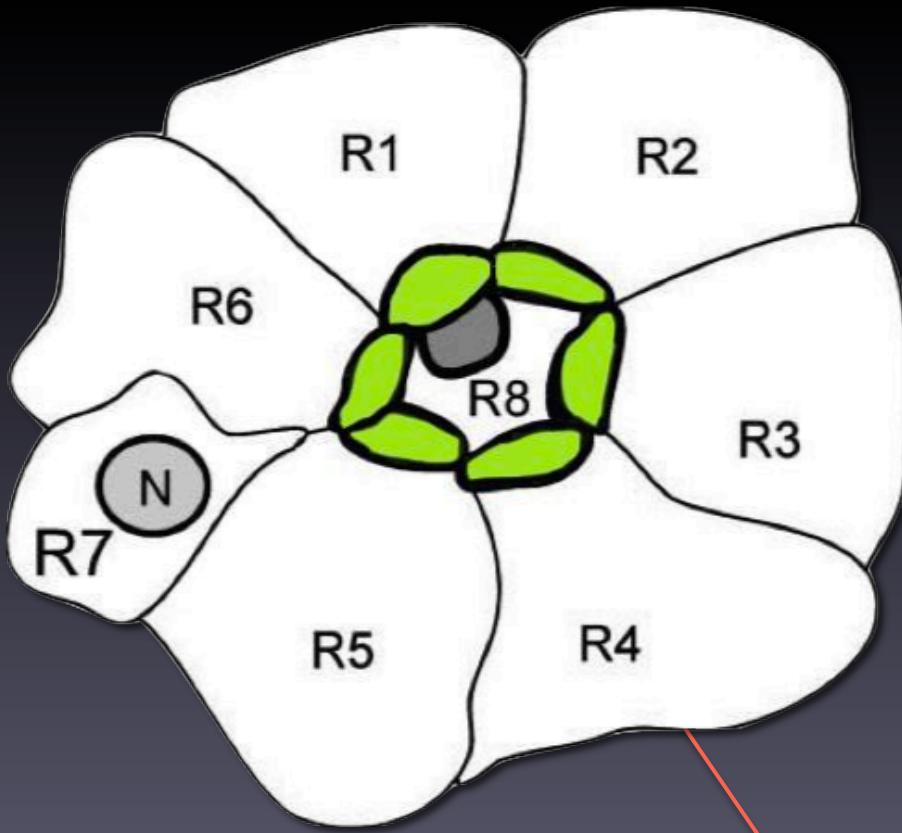


Countries with areas of
dengue risk (WHO, 2008)



Fused rhabdom:
Reduced acuity, enhanced sensitivity

op1

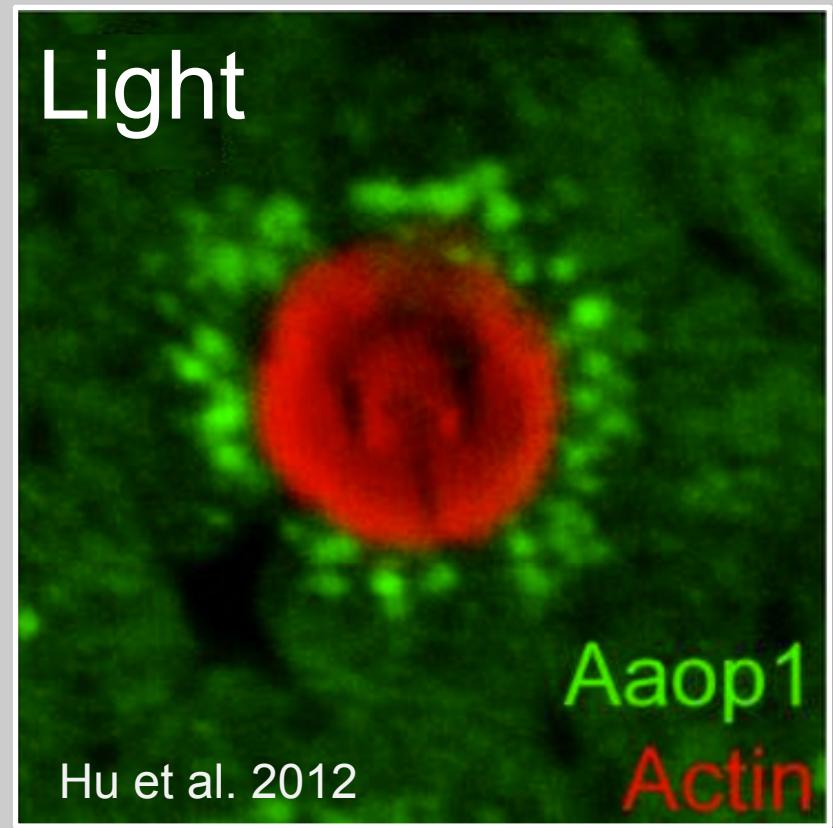
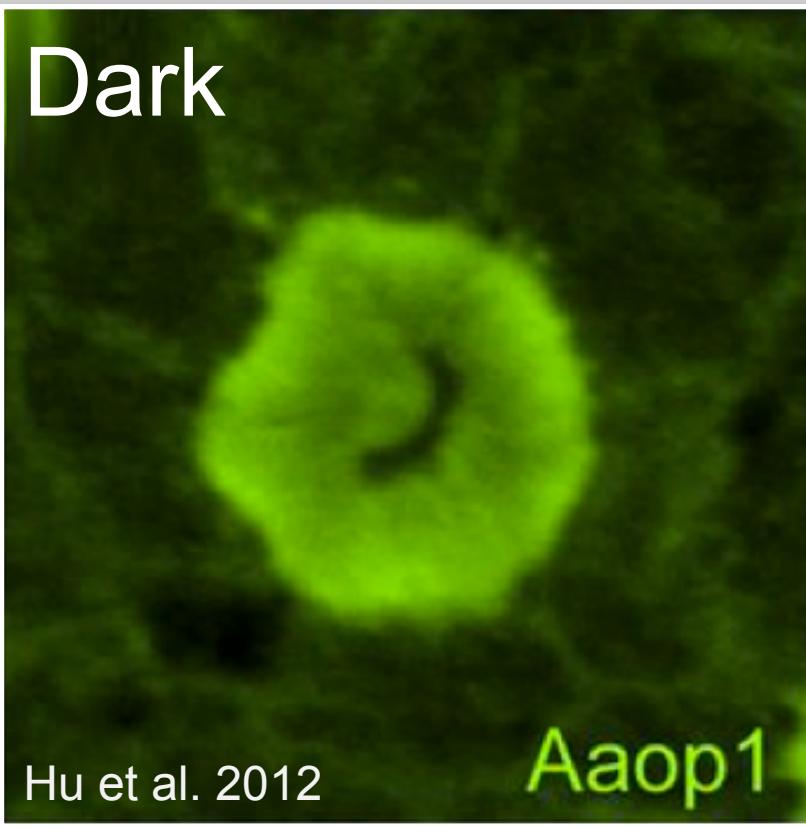


10 rhodopsins

Co-expression in photoreceptor cells

Translocation of op1

Localized to rhabdomere in the dark,
cytoplasm in light



RESEARCH GOAL

Determine the importance of the complex mosquito visual system for behaviors related to vector competence.

PROJECT HYPOTHESIS

Rhodopsin impairment will reduce the mosquitoes' ability to execute key behaviors.

Experimental Overview

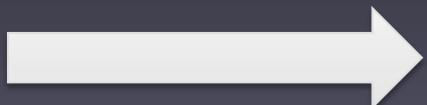
Light-induced
degeneration

Vitamin A
deprivation

RNA interference
knockdown of op1

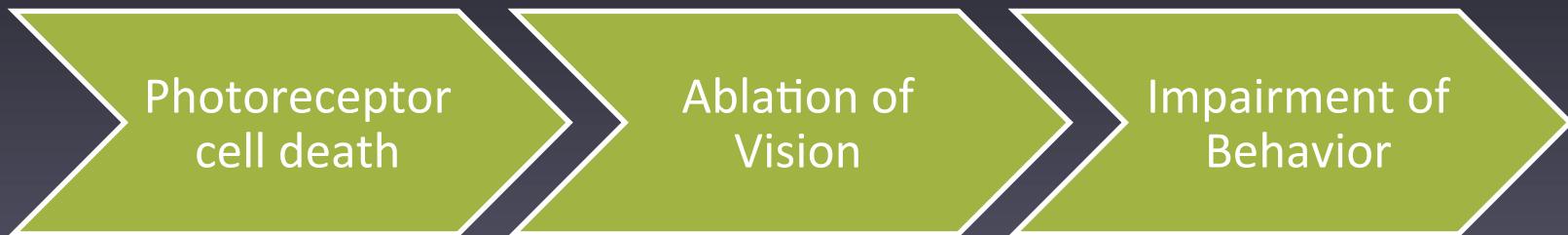
Vision
impairment

Decreased
vector
competence



Light-Induced Degeneration

Hypothesis



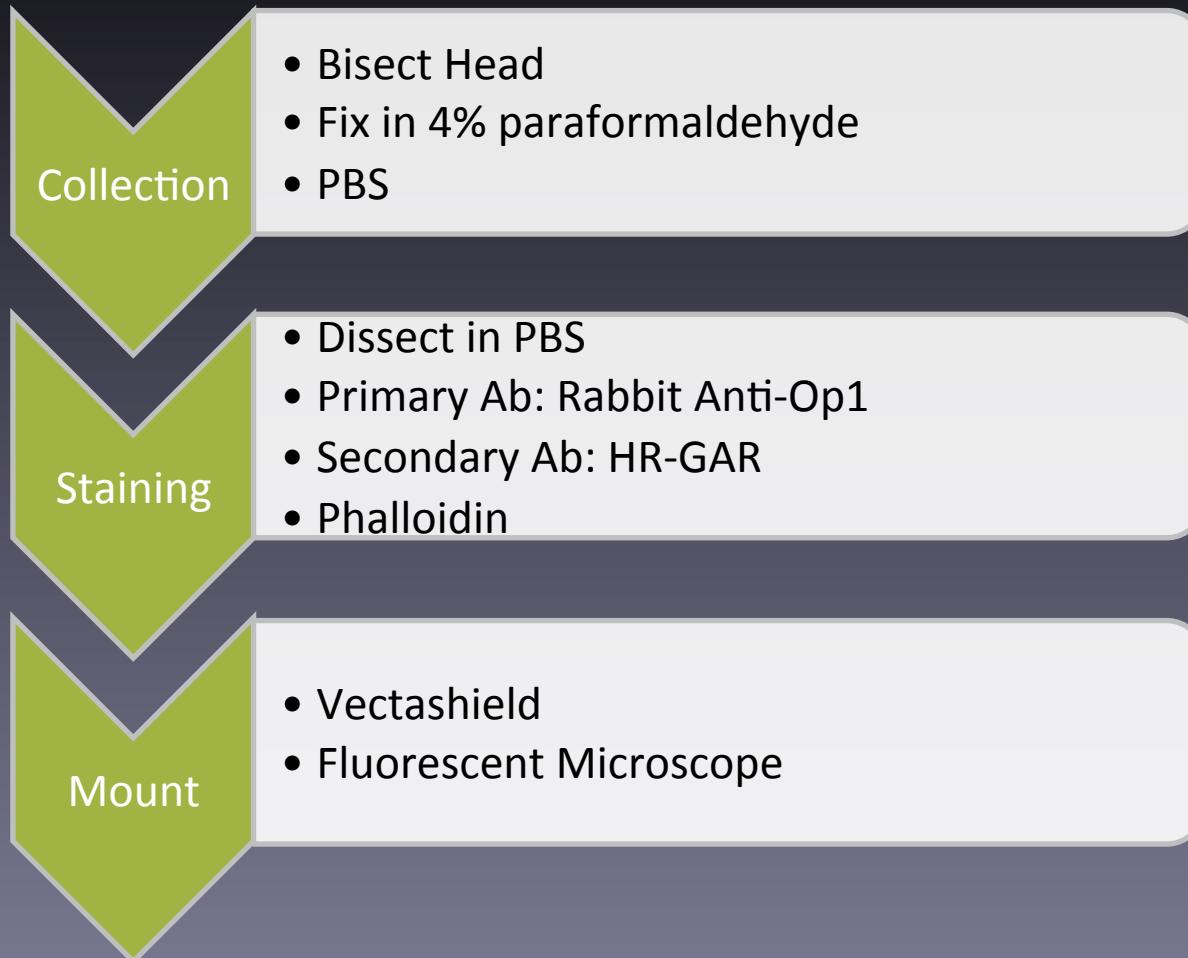
Method of Light-Induced Degeneration

Dark Adaption
(48h)

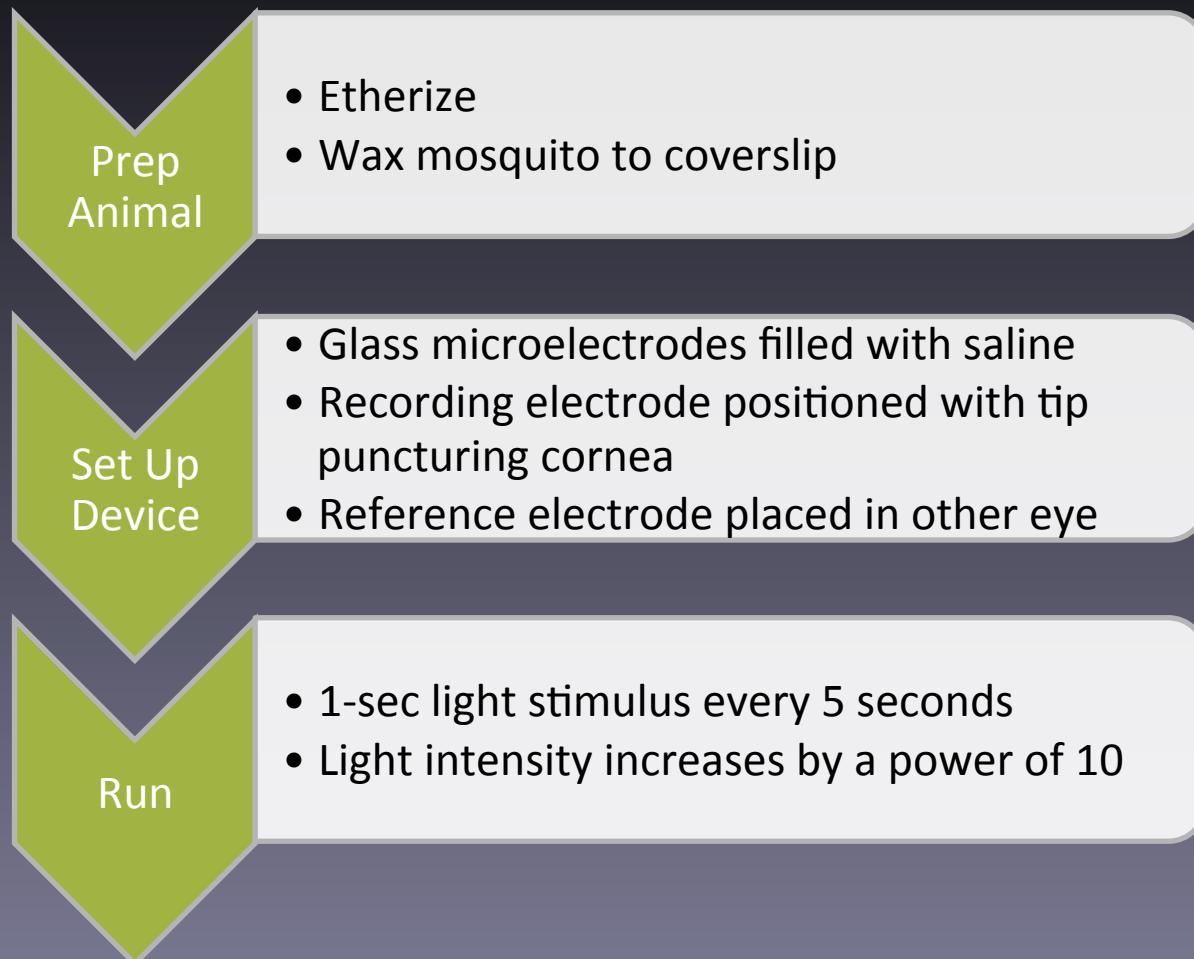
Light Treatment
(24h, 36h, & 48h)
Lux: 21, 200

Whole Mount
Western blot
ERG

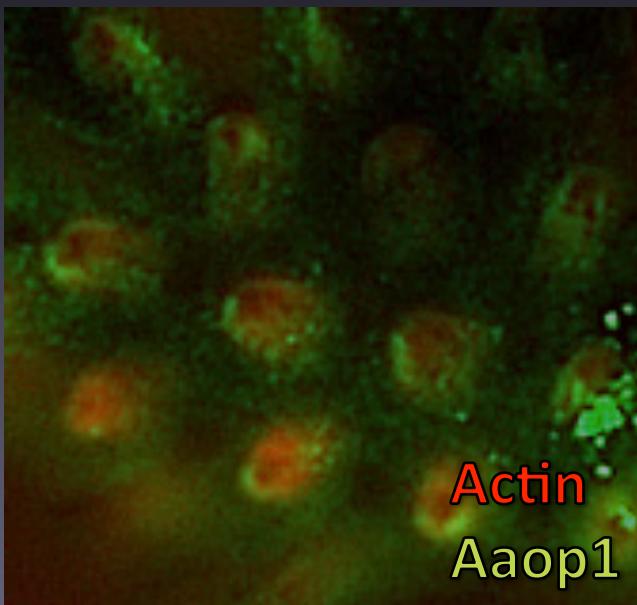
Molecular Assays: Whole Mount



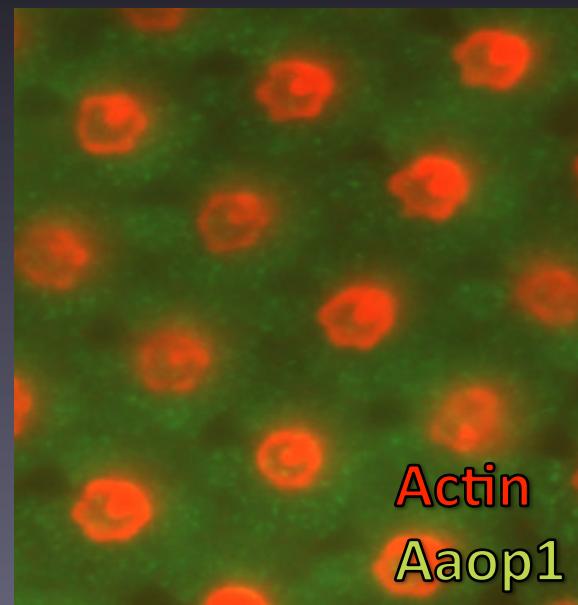
Molecular Assays: Electroretinogram



24 hours in constant light did not alter photoreceptor structure



12/12 Light/Dark

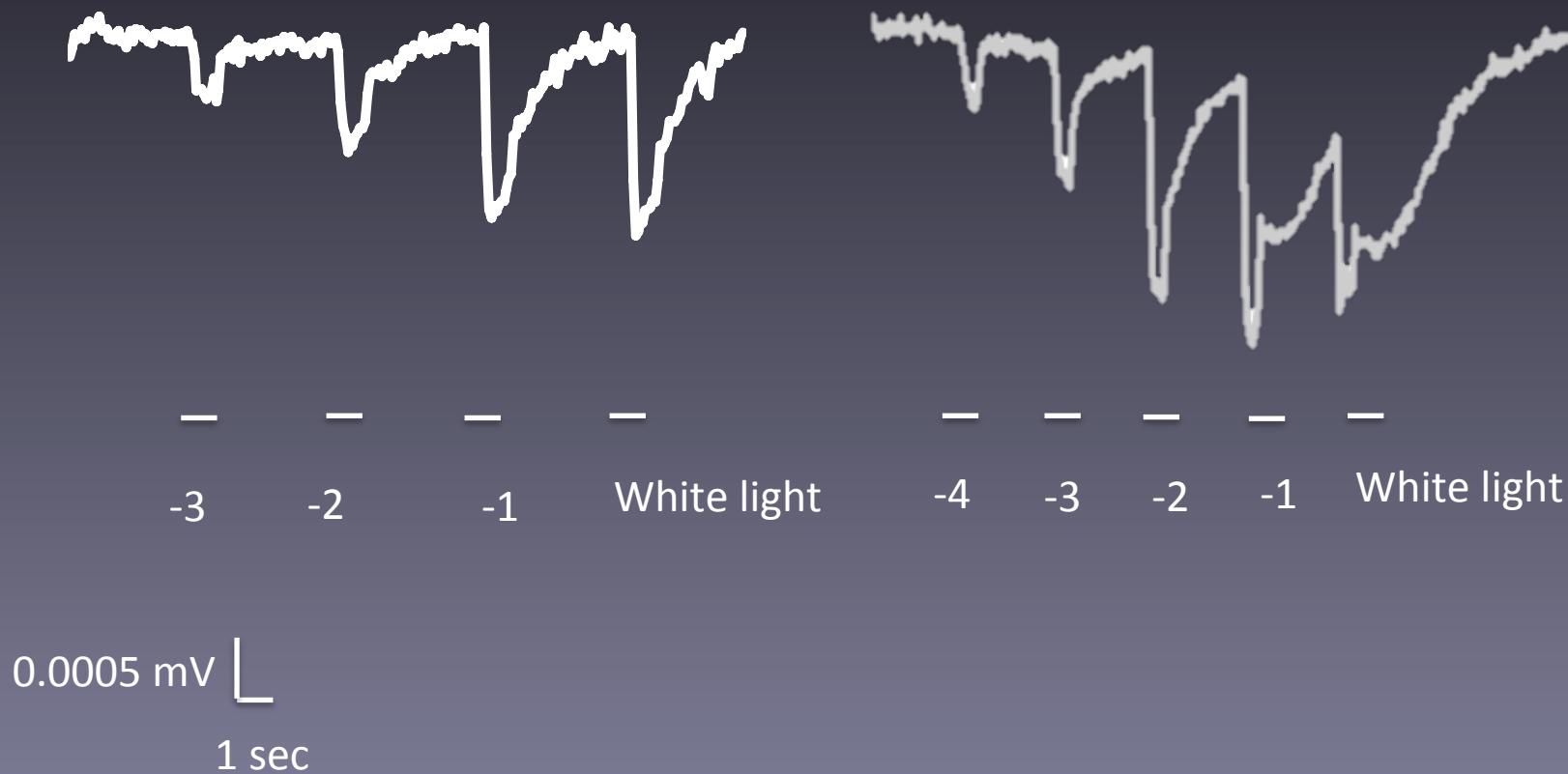


24 Hr Constant Light

24 hours in constant light impairs response

12/12 Light/Dark

24 hour Constant Light



Future plans for light-induced degeneration

- 36 and 48 hour time points
- Confocal imaging
- Behavioral assays

Experimental Overview

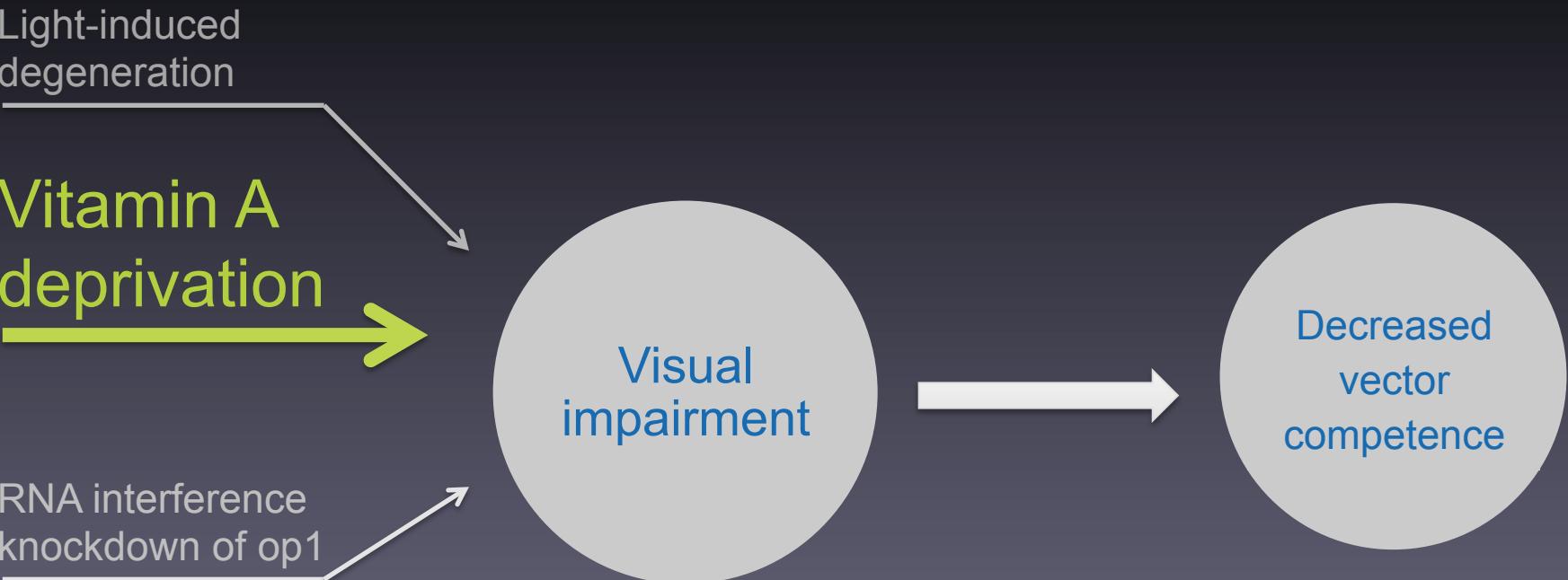
Light-induced
degeneration

Vitamin A
deprivation

RNA interference
knockdown of op1

Visual
impairment

Decreased
vector
competence



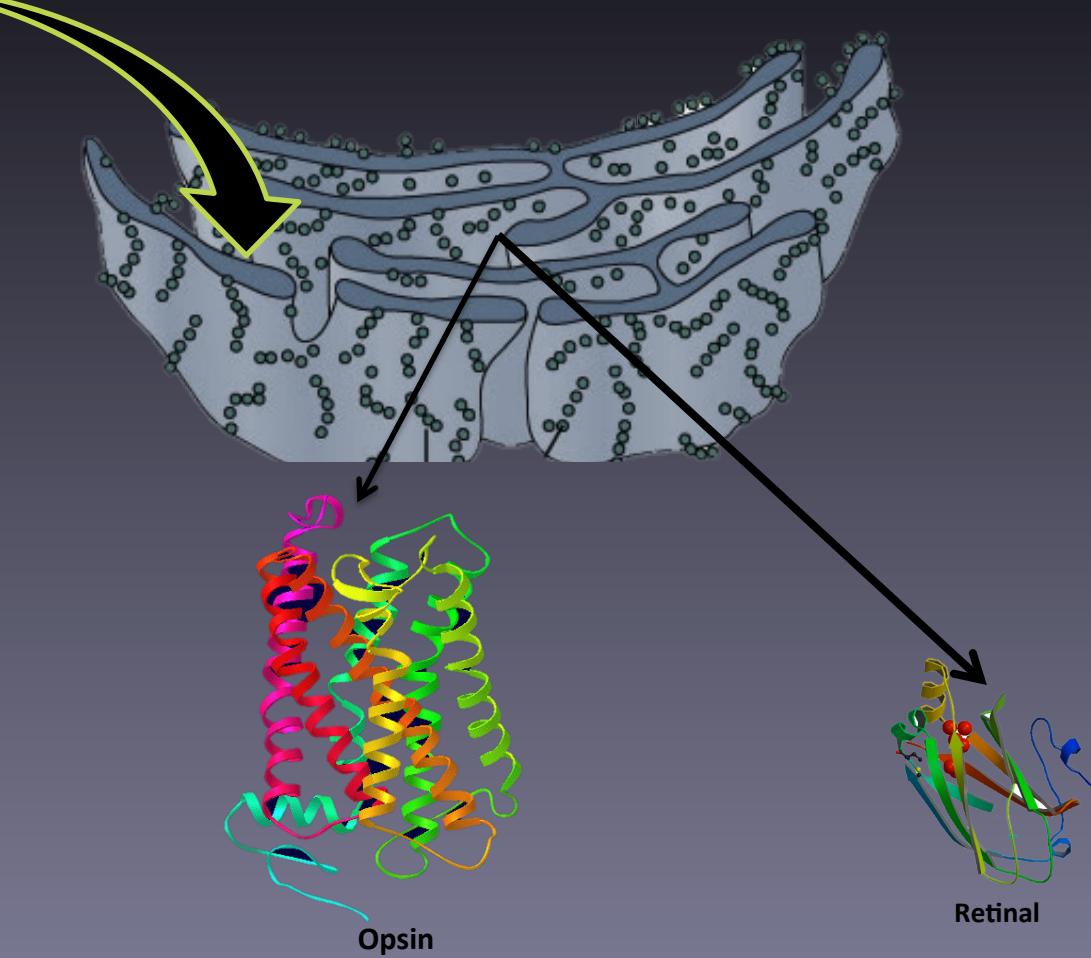
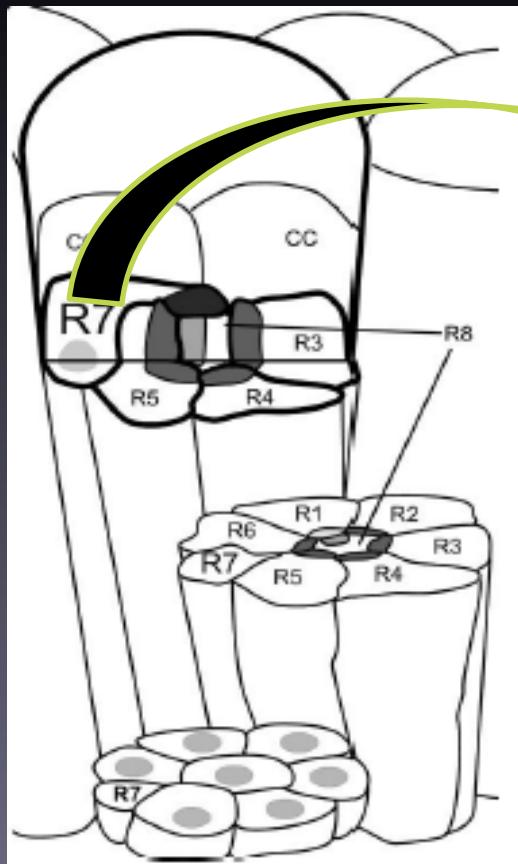
Rhodopsin impairment from Vitamin A deprivation

Hypothesis

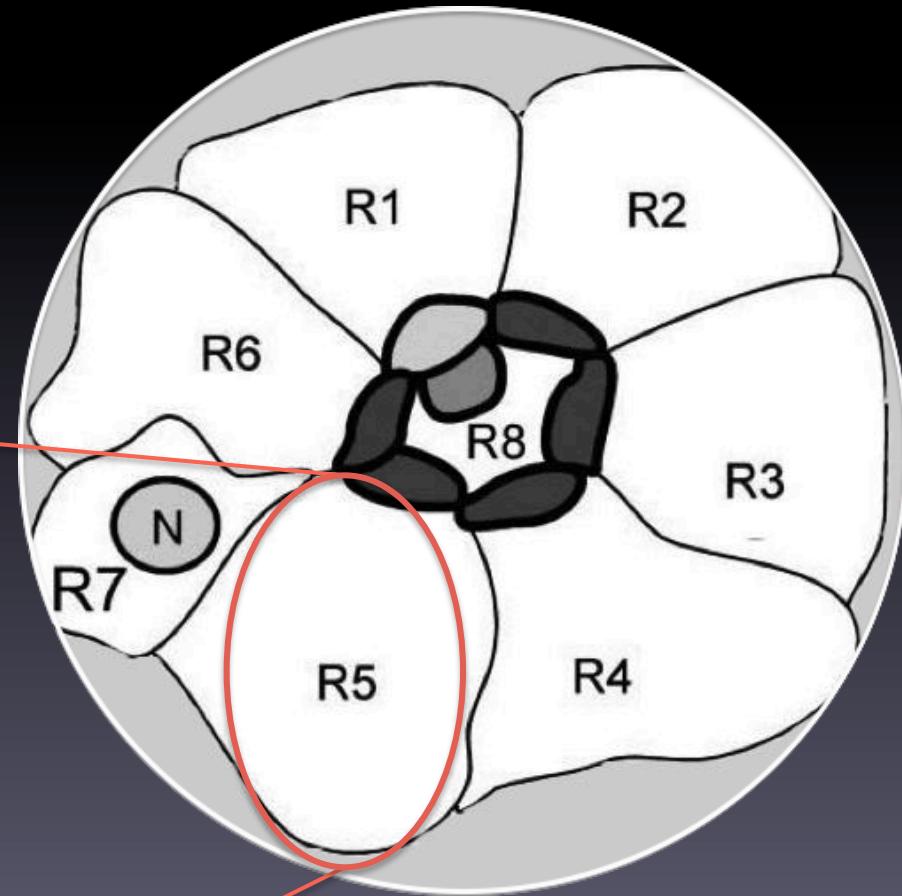
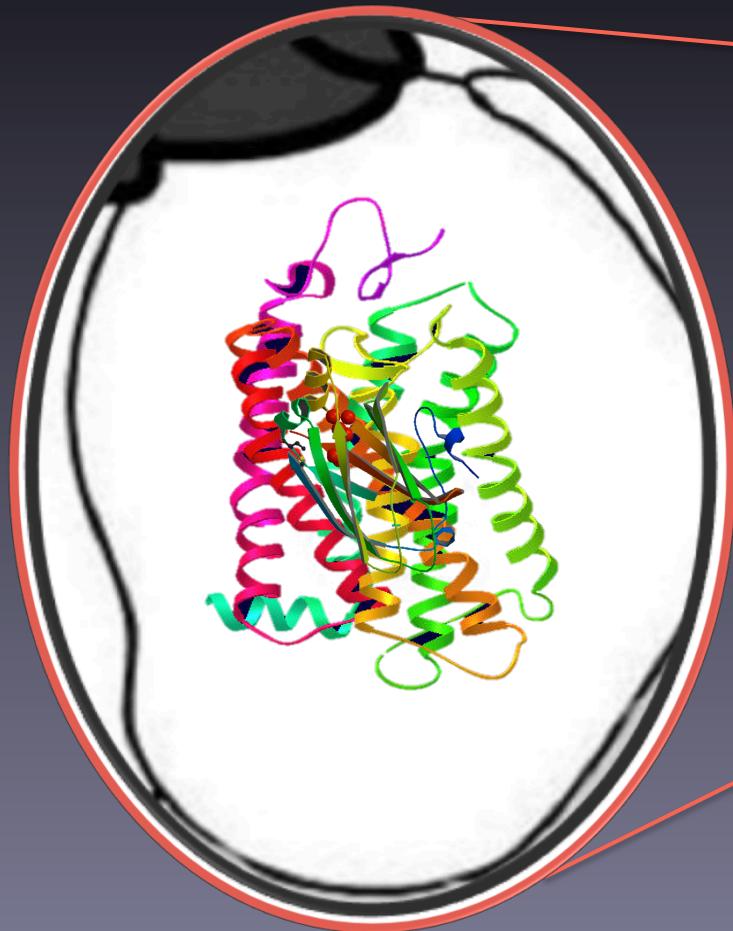


* Crucial role of visual system in behaviors related to disease transmission

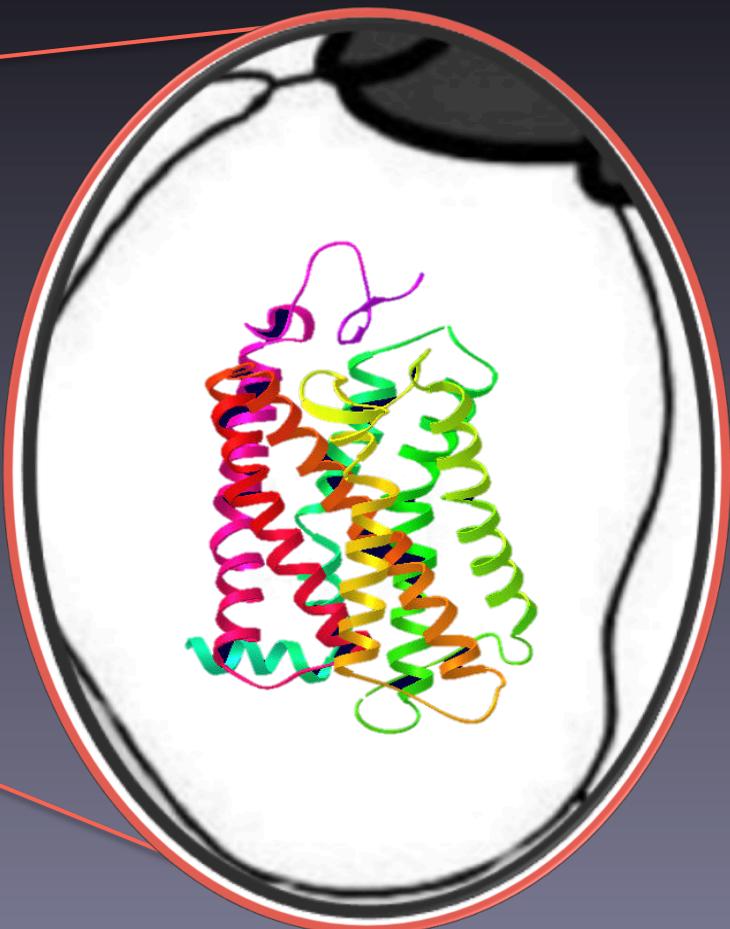
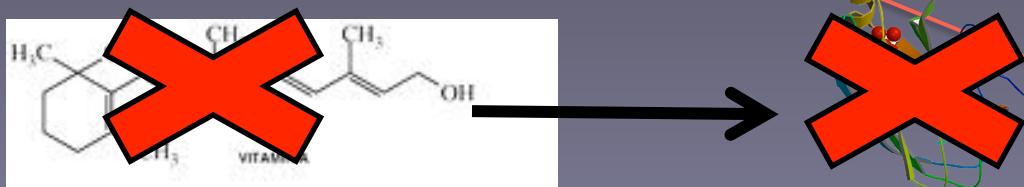
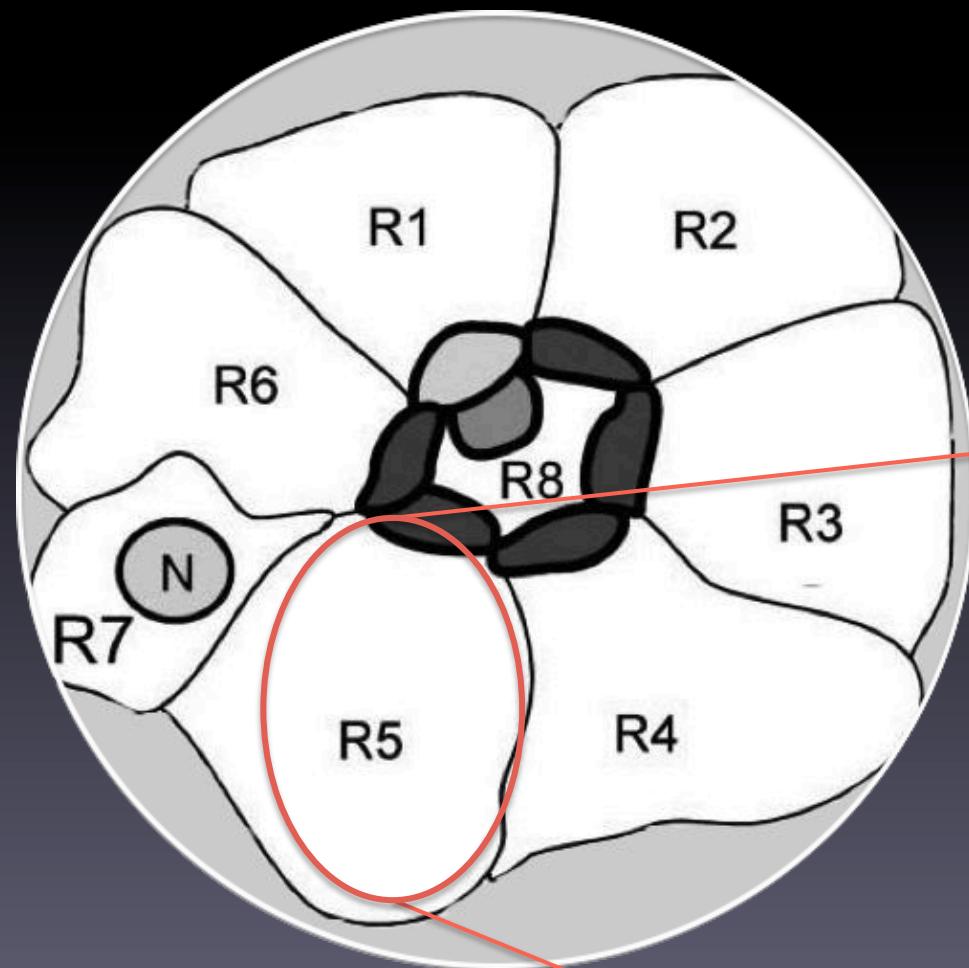
Opsin and Retinal Within the Photoreceptor Cell



Wild Type



Vitamin A deprived



Method of vitamin A deprivation

Feed heart powder



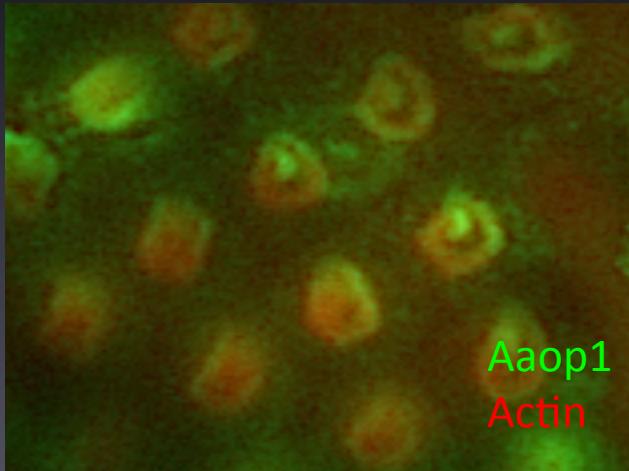
Collect 2-7 days following eclosion



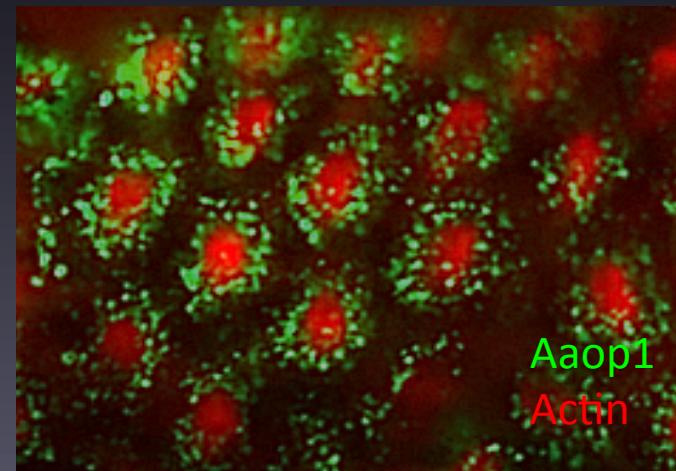
Western blot, ERG, whole mount

Vitamin A Deprivation Altered Aaop1 Localization

Whole mount IF Images- Light Adapted



Liver Powder (wild type)



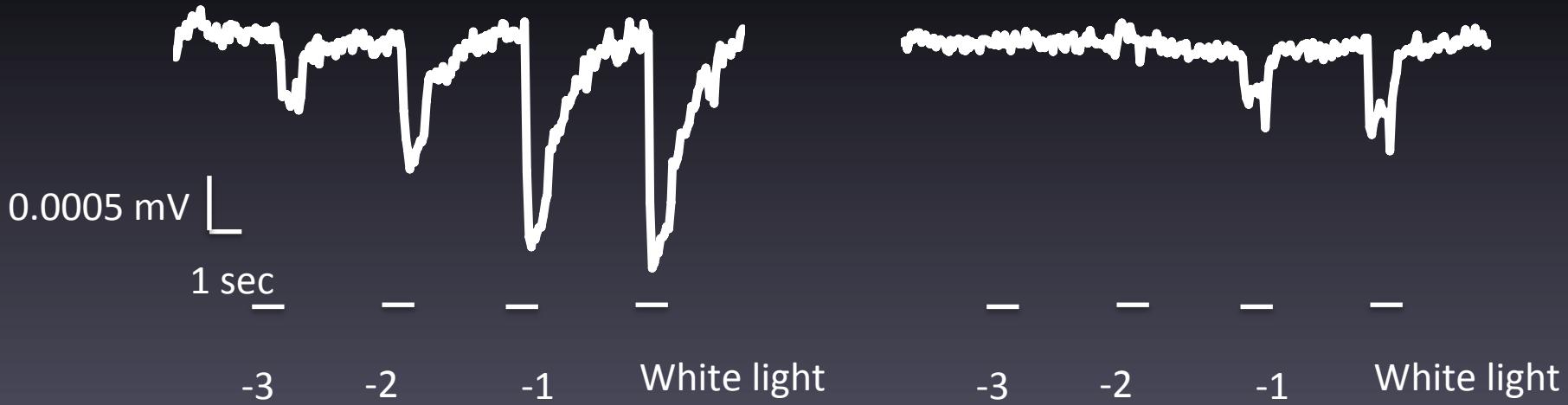
Heart Powder (Vitamin A Deprived)

- Characteristic of red-eyed
- Op1 in rhabdomere and cytoplasm
- Dark adaptation needed for verification

- Op1 only in cytoplasm
- Vesicular

Vitamin A Deprivation Altered Response to Light

ERG



Liver Powder (wild type)

- Proportional response to all light signals
- Clean response
- Standard recovery

Heart Powder
(Vitamin A Deprived)

- More light required for initial response
- Staggered response
- Irregular recovery

Experimental Overview

Light-induced
degradation

Vitamin A
deprivation

RNA interference
knockdown of op 1

Rhodopsin
impairment

Decreased
vector
competence



RNAi knockdown of op1

Hypothesis



* Crucial role of visual system in behaviors related to disease transmission

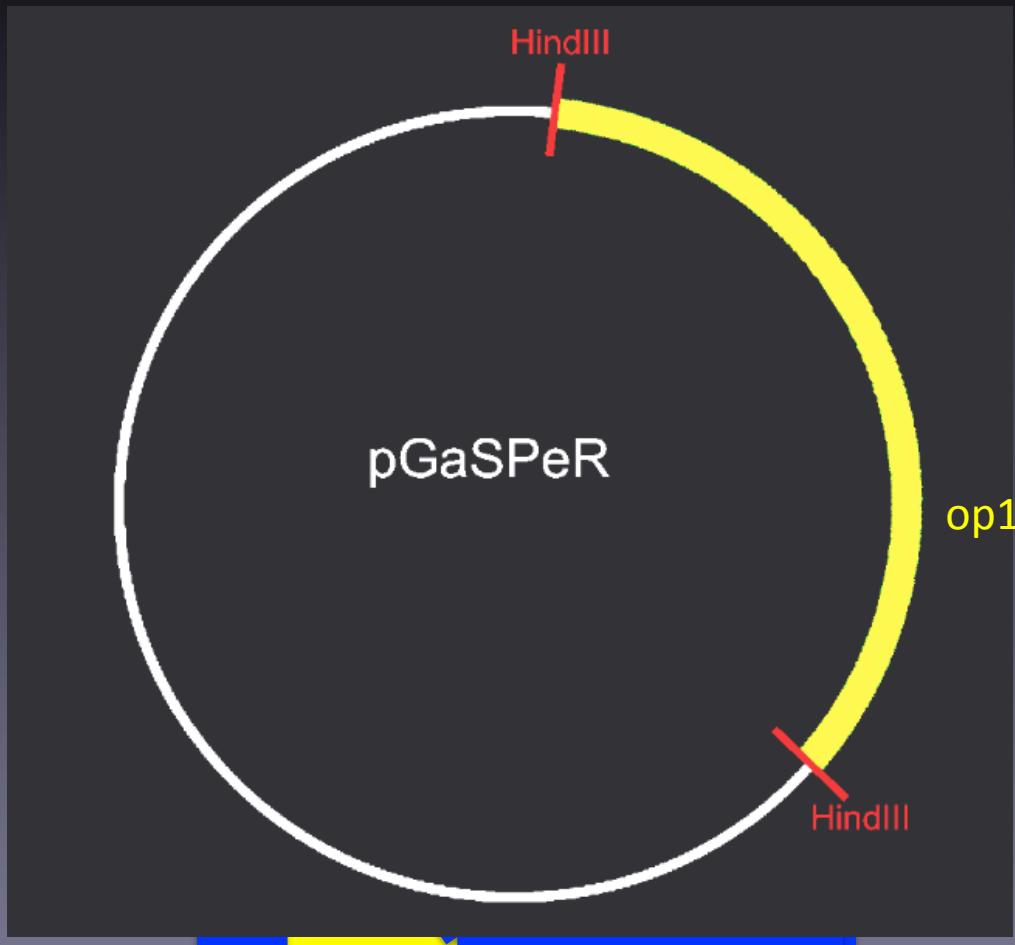
Methods for RNAi knockdown of op1

Generate RNAi

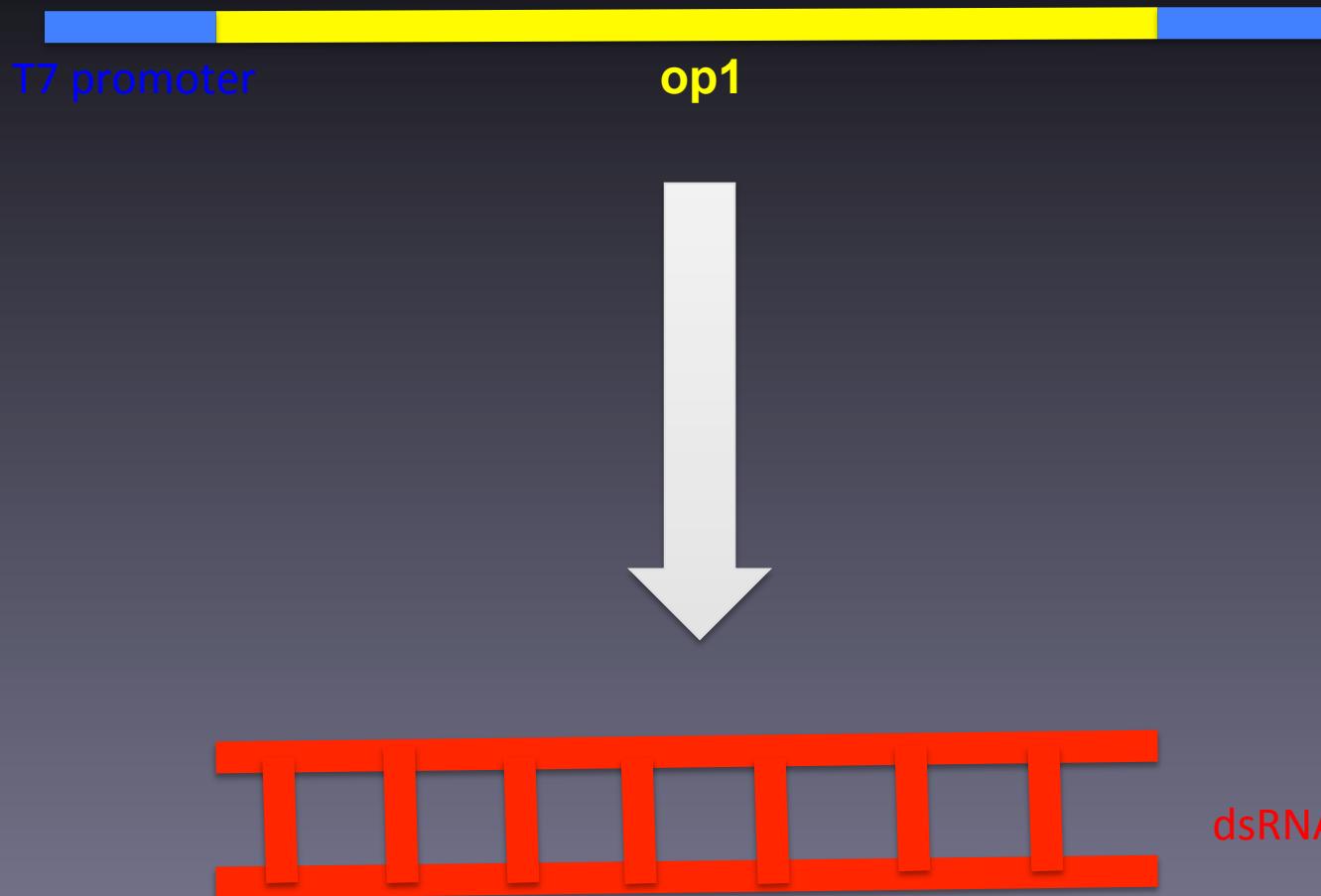
Feed mosquitoes

Western blot, ERG, whole mount

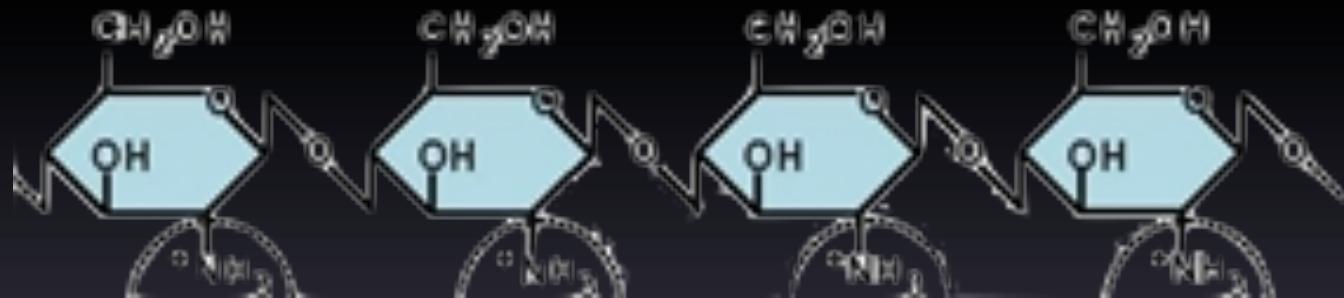
Generating double-stranded RNA



Generating double-stranded RNA



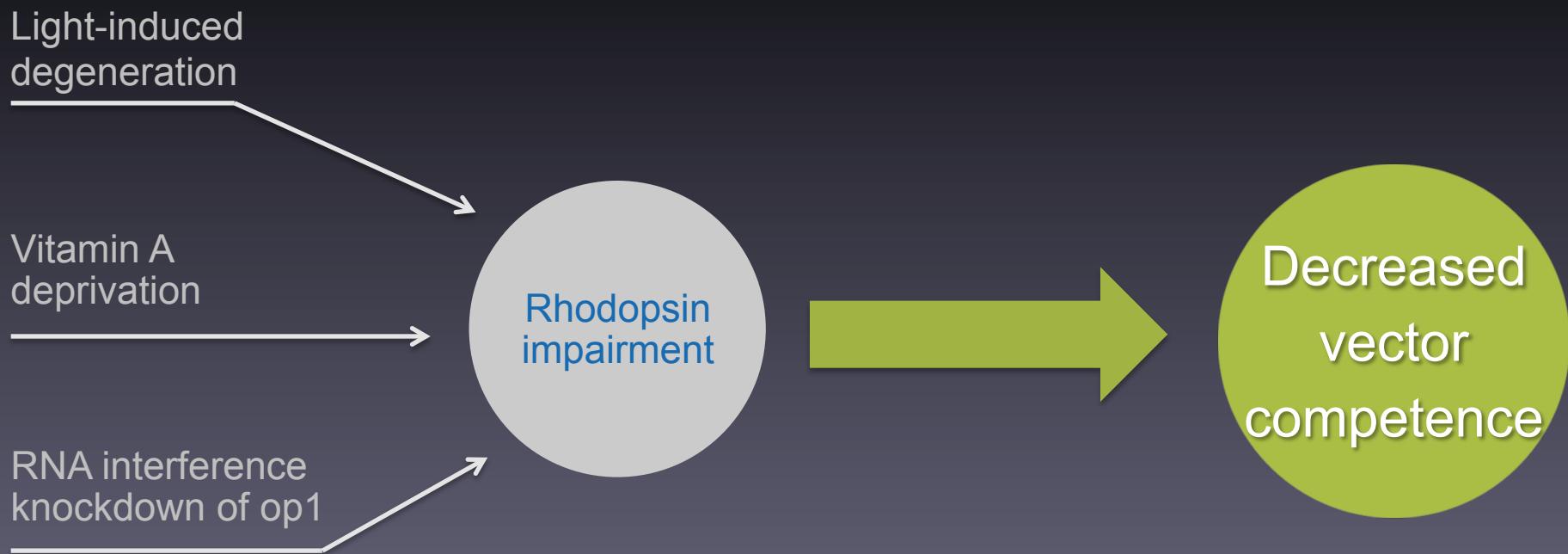
Chitosan



Progress in RNAi knockdown of op1

- Isolated op1
- Generated 16.7-16.9 ng/ μ L dsRNA per reaction
- Synthesized dsRNA/chitosan nanoparticles
- Began feeding 3rd instar larvae

Experimental Overview

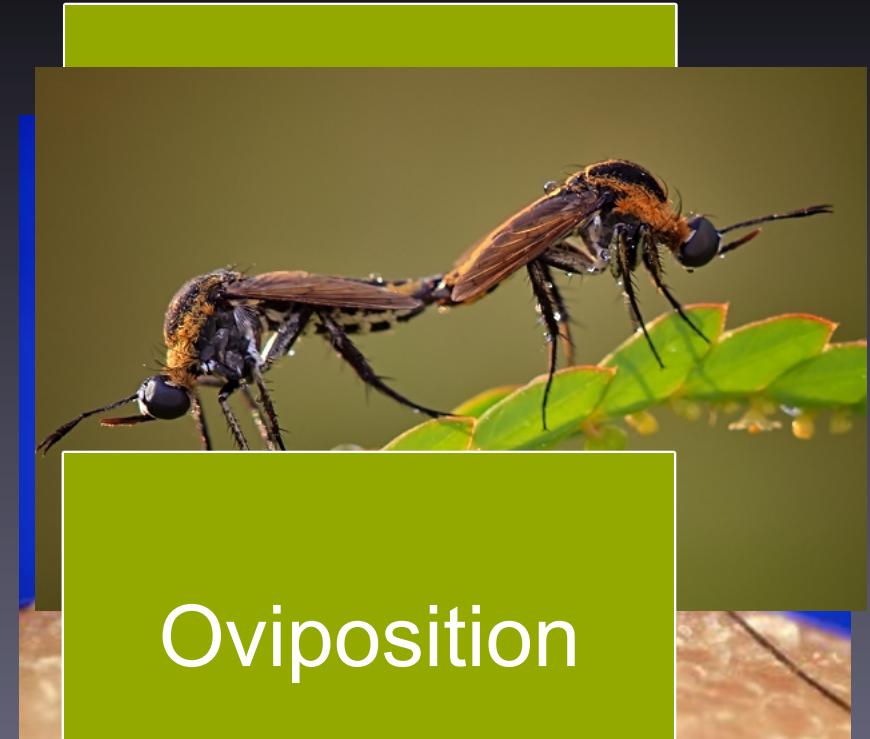


Behavioral Assays

Blood



Oviposition



Designing Assays

METHODS

- Visual Recording (infrared camera)
- Five replicates
- Setting controls

Behavior	Time Period	Set-up	Observation Parameters
Blood Feeding	10 minutes	Mated, non-blood fed female per tube	Time to find host, duration of meal, flight pattern
Sugar Feeding	3 hours	Bucket of 5-10 mosquitoes, flower as sugar source	Freq. of feeding, duration, time to find sugar source
Mating	1 hour	One male and female per tube	Duration of mating, frequency, time to find each other/mate
Oviposition	3 hours	Bucket with cup of water, blood-fed mated female	Where they lay eggs, how long it takes, number of eggs

Overall Significance

- Hypothesis

Quantify behavioral effects



Elucidate role and function of vision



Connect to behavioral changes

- Determining targets
- Negatively affect vector competence for disease control

Acknowledgements

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