Crag keeps the light 'fantastic' for photoreceptors

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The ability of the eye of a fruit fly (*Drosophila melanogaster*) to respond to light depends on a delicate ballet that keeps the supply of light sensors called rhodopsin constant as photoreceptors turn on and off in response to light exposures, said researchers from Baylor College of Medicine and the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital in an article that appears online in the journal *PLOS Biology*.

The gene Crag is key for the trafficking of rhodopsin because it is a guanine exchange factor that activates a protein called Rab11, said Dr. Hugo Bellen, professor of molecular and human genetics and director of the Program in Developmental Biology at BCM.

"When a photon hits the membrane, it activates a protein called rhodopsin," said Bellen, also a Howard Hughes Medical Institute investigator. "Exposure to another wavelength of light converts the metarhodopsin back into rhodopsin. However, the molecular switch can be broken through various causes, and the protein is then stuck in the active form, metarhodopsin."

That can have severe consequences for the photoreceptor. Hence, metarhodopsin needs to be cleared and freshly made rhodopsin needs to replenish the pool to maintain photoreceptor integrity, said Bo Xiong, a graduate student in Bellen's laboratory.

The work led by Xiong suggests that photons increase the calcium influx which in turn activates a protein called calmodulin, which then activates Crag. Crag activates the Rab11 protein that allows transport of vesicles (little bubbles) that are loaded with freshly made rhodopsin. The secretion of the rhodopsin-loaded vesicle into the membrane resets the mechanism so that the photoreceptors can once again capture light.

However, if the Crag is mutated, Rab11 is not activated and the newly synthesized rhodopsin accumulates in the cytoplasm of the cell, resulting in toxicity and leading to the death of the photoreceptors.

Interestingly, flies with this Crag mutation do not suffer photoreceptor degeneration when they are left in the dark.

"If they are kept in constant darkness, there is no photoreceptor damage," said Xiong. If you keep the flies in alternating light and dark for 14 days, the photoreceptors degenerate.

"What happens is a complete breakdown of vesicular trafficking. The whole cytoplasm becomes loaded with rhodopsin and aberrant vesicles and structures. The cell tries to get rid of it these aberrant structures via lysosomes and autophagosomes (the natural clean-up mechanism of cells). As a consequence, the photoreceptor light-sensing membrane becomes smaller as it is not replenished with freshly made rhodopsin."

Crag is a member of the DENN family proteins, which are critical for vesicular trafficking. (Tiny vesicles or bubble-like structures carry proteins from one compartment of the cell to the other in a process called trafficking.)

Xiong and colleagues discovered that Crag modifies Rab11. Indeed, overexpressed Rab11 in the Crag mutant photoreceptor reduced the photoreceptor degeneration. It is likely that this mechanism is conserved in vertebrates as the human ortholog of Crag rescues the fly Crag mutant phenotypes.

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