University of Missouri-Columbia

Undergraduate Research Science Symposium

Friday, August 2, 1991

Mark Twain Ballroom, Memorial Union

1:30 - 4:00 pm

N-Linked Glycosylation of Opsin is Required for Rhabdomere Maintenance in *Drosophila*.

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The study of the deployment and regulation of opsin, the transmembrane protein central to vision, has been pivotal to invertebrate and vertebrate visual research in recent years.[1] In the compound eyes of *Drosophila*, opsin is contained in a membranous organelle called a rhabdomere. Glycosylation (the adding of a complex sugar) is common in membrane proteins. Over the last several years, scientists have debated whether or not *Drosophila* opsin is glycosylated at the asparagine residue at position #20 of the protein. Recent immunological evidence has indicated that opsin in the rhabdomere is not.[2]

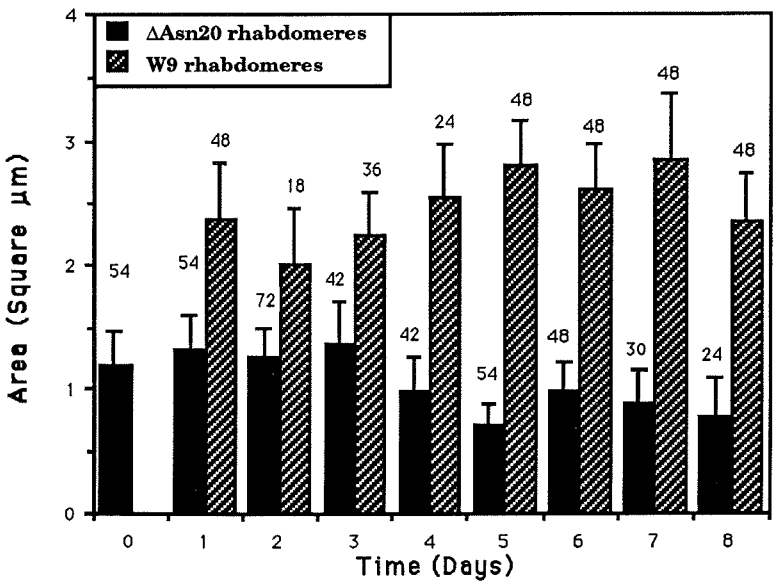
Dr. O'Tousa, at Notre Dame, used germline transfection to create a *Drosophila* mutant called *ΔAsn20*. In this mutant, the asparagine residue at position #20 of opsin is replaced with isoleucine, thereby preventing glycosylation at this site.

This project centered on determining what effect non-glycosylation has on the rhabdomeres that contain opsin. *ΔAsn20* flies were fixed and sectioned for electron microscopy. Photomicrographs of the rhabdomeres at the nuclear level (a measure of depth) and near the equator (a landmark of the center). were obtained on the electron microscope. The photomicrographs were then entered into a morphometric computer for analysis. Rhabdomere perimeters were manually traced to obtain a measurement of cross-sectional area. As a quality control on the accuracy of manually tracing the rhabdomeres, two people (J. Scott and Nazhat) performed the data collection. A linear regression was done to compare the data of the two operators. The results of this correlation showed no difference in consistency of tracing the oulines.

The results of operator #1 are shown below. *W9* flies are the positive control (*W9*= transfection without a substitution). Also an internal control was available to us. Receptor cell #7 expresses a different opsin gene than the one that was transfected, thereby making its rhabdomere an internal control. Rhabdomeres in receptor cells one through six were transfected and their data was pooled together.

A comparison of the cells of *ΔAsn20* and *W9* shows that rhabdomeres in receptor cells1-6 of *ΔAsn20* flies decrease in size with age; whereas these rhabdomeres in *W9* continue to increase with age. Qualitatively, it is noticeable that *ΔAsn20* rhabdomeres have a disconfiguration of their membrane. No difference was detected in the rhabdomeres of receptor cell #7 between *W9* and *ΔAsn20*.

The pattern of degeneration that we observed in *ΔAsn20* is similar to a mutant called *ora* (outer rhabdomeres absent); which is a missense mutation of opsin that results in rhabdomeres that degenerate over time. Since, opsin mRNA levels in *ΔAsn20* are not different from the wild type[3], but the rhabdomeres degenerate with time, we suspect that opsin is being produced, but not deployed into the visual membrane. Considering that opsin is not glycosylated in the rhabdomere,[1] it appears that glycosylation affects either the final synthesis or the deployment of opsin into the rhabdomere.



References:

1). Stark W.S., Christianson J.S., Maier L., Chen D.M. (1991) In: R. E. Anderson, J. G. Hollyfield and M. M. LaVail (ed) Retinal Degenerations. CRC Press. In press.

2). deCouet H. G., Tanimura T. (1987) European Journal of Cell Biology. 44: 50-56.

3). O'Tousa, J.E. (1991) Visual Neuroscience, in press.